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Macular Pigment and Lutein Supplementation in Choroideremia

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Choroideremia is an incurable X-linked retinal degeneration caused by mutations in the gene encoding Rab escort protein-1. A group of clinically defined and genotyped patients were studied to determine: (1) the degree of rod and cone dysfunction and structural abnormality in the central retina and the level of macular pigment; and (2) the response of macular pigment and foveal vision to a 6 month trial of supplementation with oral lutein (at 20 mg per day). Rod and cone-mediated function was measured with dark-adapted static perimetry; in vivo retinal structure was determined with optical coherence tomography; and macular pigment optical density was measured with heterochromatic flicker photometry. In this cohort of patients (ages 15–65 years), both rod- and cone-mediated central function declined with age as did central retinal thickness. Macular pigment levels did not differ between patients and male control subjects. Supplementation of oral lutein in a subset of patients led to an increase in serum lutein and macular pigment levels; absolute foveal sensitivity did not change. It is concluded that macular pigment density can be augmented by oral intake of lutein in patients with choroideremia. There was no short-term change in the central vision of the patients on the supplement, but long-term influences of lutein supplementation on disease natural history warrant further study.

Key words: carotenoids; choroideremia; cone; lutein; macular pigment; perimetry; REP-1; retinal degeneration; rod.

1. Introduction

Choroideremia (CHM) is an X-linked progressive retinal degeneration affecting photoreceptors, retinal pigment epithelial (RPE) cells and choroid (MacDonald et al., 1997; Syed et al., 2001). The molecular basis for CHM is mutation in the gene encoding Rab escort protein-1 (REP-1) (Cremers et al., 1990; Merry et al., 1992; van Bokhoven et al., 1994). To date, mainly loss of function mutations have been identified in CHM patients (MacDonald et al., 1998). Facility of clinical diagnosis and greater understanding of molecular cause in CHM, however, have not yet led to the treatment of this serious retinal disease. As in many inherited retinal degenerations, CHM patients lose peripheral vision until only a limited central island of function remains; this island eventually is also lost to the disease (McCulloch and McCulloch, 1948; Kärnä, 1986).

Prompted by the intriguing report of short-term improvement in central vision after lutein supplementation in inherited retinal degenerations (Dagnelie, Zorge and McDonald, 2000), a series of studies of macular pigment (MP) and lutein supplementation in patients with retinitis pigmentosa (RP) or Usher syndrome were performed recently (Aleman et al., 2001). Augmenting MP, the yellowish carotenoid complex principally composed of lutein and zeaxanthin, may ameliorate central retinal dysfunction/degeneration through a number of proposed actions, such as filtering shorter wavelengths of light and preventing photochemical damage, possibly quenching singlet oxygen, and stabilizing microtubules (Bone et al., 1997; Landrum et al., 1997; Hammond, Wooten and Snodderly, 1998; Russell, 1998; Cho, Hung and Seddon, 1999; Schalch, Dayhaw-Barker and Barker, 1999; Bernstein et al., 2001; Crabtree et al., 2001; Hammond, Wooten and Curran-Celentano, 2001; Yemelyanov, Katz and Bernstein, 2001). Lower risk for age-related macular degeneration has been associated with higher dietary intake and serum levels of non-vitamin A carotenoids (Seddon et al., 1994; Snodderly, 1995; Beatty et al., 1999; Bone et al., 2000) and this has led to the speculation of wider value to other central photoreceptor-RPE disease (Dagnelie et al., 2000). In patients with RP or Usher syndrome, as a group, MP was normal; and it could be augmented in many but not all of the patients by supplemental lutein taken orally over a 6 month

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period. Central vision was unchanged in these patients to short-term lutein supplementation (Aleman et al., 2001).

The present study extends this work to CHM, a molecularly and clinically more homogeneous retinal degeneration. First, details of central retinal rod and cone-mediated function and in vivo retinal structure were investigated in a group of genotyped CHM patients; then was questioned if MP optical density and serum carotenoids were normal in CHM; lastly, a subset of patients were studied over a 6 month period while they were supplemented with lutein to determine if baseline serum carotenoid, MP and vision could be modified.

2. Materials and Methods

Subjects and Mutation Analysis

Patients with CHM ($n = 13$) and subjects with normal vision ($n = 40$) participated in the study. All patients were examined and diagnosed clinically. Mutations in the CHM gene were identified with single strand conformation polymorphism (SSCP) analysis and direct sequencing (Nesslinger et al., 1996) as described previously (MacDonald et al., 1998). Informed consent was given by all subjects; institutional approval was obtained and the tenets of the Declaration of Helsinki were followed.

Evaluation of the Macula in CHM: Function, Structure and Macular Pigment

Central rod- and cone-mediated function was measured with two-color (500 and 650 nm stimuli, 1·7° diameter, 200 msec duration) dark-adapted static perimetry. Sensitivity was determined at fixation and at 2° intervals along the horizontal meridian across the central 20° (Jacobson et al., 1986, 2000). These profiles were analysed for photoreceptor-mediation; rod- or cone-mediated sensitivities were averaged across the central 20° and plotted against the age of the patient. For the central rod averages, only those profiles were included in which all detectable sensitivities using the 500 nm stimulus were rod-mediated ($n = 9$); for central cone averages, only those profiles were included in which all detectable sensitivities with the 650 nm stimulus were cone-mediated ($n = 13$).

Macular structure was quantified by the in vivo technique of optical coherence tomography (OCT); the principles of OCT (Huang et al., 1991; Hee et al., 1995) and the present techniques (Jacobson et al., 1998, 2000) have been published. Horizontally and vertically oriented scans crossing fixation were obtained in all subjects. Retinal thickness at fixation was measured with a computer algorithm (Hee et al., 1996) and averaged results from at least three central scans were used for the data analyses.

Macular pigment optical density was measured with heterochromatic flicker photometry (Werner, Donnelly and Kleigl, 1987; Hammond, Wooten and Snodderly, 1997; Bone et al., 2000) using an LED-based MP densitometer (Wooten et al., 1999; Hammond and Caruso-Avery, 2000). Details of this methodology in patients with retinal degeneration have recently been provided (Aleman et al., 2001). As in this earlier study of inherited retinal disease, 5° eccentricity was used as a reference in many patients to minimize secondary effects due to degeneration by limiting testing to the more healthy retina; all patients were able to perform matches reliably at this location.

Supplementation with Lutein

A subset of seven patients with CHM participated in a 6 month pilot trial of oral lutein supplementation. There was no placebo control group and no attempt was made to mask the patient as to the content of the supplement. Dietary information was provided by patients at the baseline through the Health Habits and History Questionnaire (HHHQ) developed by the National Cancer Institute (Block et al., 1986); and the data were analysed using the HHHQ Diet System Analysis Software (Block et al., 1994). Following two baseline visits (separated by no more than 1 month), subjects supplemented their diet with a commercially available form of lutein at 20 mg per day (TWIN Laboratories Inc., NY, U.S.A.). Subjects were instructed to take the lutein supplement with dinner, presuming this meal contained the most fat and would enhance absorption of the supplement (Khachick, Beecher and Smith, 1995). A further visit occurred 6 months after starting the supplement. Baseline and follow-up visits included a clinical examination, fasting (overnight) venous blood sample for serum carotenoids, and measurements of MP density and absolute sensitivity at the fovea with a 650 nm target (Aleman et al., 2001). In all but one patient, both eyes were able to be studied.

Statistical Analyses

SAS (version 8·00) statistical software was used to perform data analyses. Mean values from the two baseline visits were used in describing the study groups and in calculating the change after supplementation with lutein. The average of the measurements from each eye was also used to establish person-specific characteristics. *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 (Zeger and Liang, 1986). Inter-session variability was assessed with signed and absolute differences of measurements between the first and second baseline visit. Inter-session 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 the eyes. Therefore, analyses of inter-session variability treated the data from each eye as independent observations. Means of inter-session differences and person-specific variables were compared with independent *t*-tests with adjustment for unequal variance when indicated. The Wilcoxon rank sum test was used to compare highly skewed distributions. Proportions were compared using chi-square tests with the exact computation of the *P* values.

3. Results

Clinical and molecular details of the CHM patients in this study are listed (Table I). The identified mutations in the CHM gene would be predicted to lead to a non-functional REP-1. Visual acuity was 20/30 or better in one or both eyes in all but two patients. The extent of kinetic visual field measured with the V-4e test target was within normal limits in two of three patients between ages 15 and 22 years. In the second through fourth decades of life, most patients had central and peripheral islands separated by mid-peripheral scotomas. Later ages showed only a small central island of vision. For this kinetic perimetry target, there was increasing loss of visual field extent with patient age (Spearman correlation coefficient, $r = -0{\cdot}85$; $P < 0{\cdot}0001$).

Macular Function and in vivo Structure

What are the function and structure of the central retina in patients with CHM? Representative results of two color dark-adapted perimetry across the central 20° of visual field in three CHM patients indicate that there can be substantial rod-mediated function near fixation although this decreases with increasing eccentricity (Fig. 1(A)). The average sensitivity of rod-mediated loci in the central field (excluding the value at fixation) was calculated and plotted against age (Fig. 1(B)). Average rod sensitivity declined with increasing age (Spearman correlation coefficient, $r = -0{\cdot}78$; $P = 0{\cdot}01$). Long-/middle-wavelength (L/M) cone function peaked at fixation and decreased with eccentricity (Fig. 1(C)). Similar to central rod-mediated function, average L/M cone-mediated function declined with age (Spearman correlation coefficient, $r = -0{\cdot}94$; $P < 0{\cdot}0001$). The slopes of these rod- and cone-mediated functions vs age were not significantly different (slope difference estimate, $-0{\cdot}005$; 95% confidence interval, $-0{\cdot}02$ to $0{\cdot}01$; $P = 0{\cdot}52$) suggesting a relatively equal reduction of these parameters with increasing age in the central field of this group of CHM patients.

Foveal structure in the CHM patients was assessed in vivo with OCT (Fig. 2). Cross-sectional retinal images in four representative patients illustrate the range of central retinal thicknesses observed (Fig. 2(A)–(D)). It was first inquired whether, on average, the foveal thickness in the CHM patients differed from that in a group of normal men. The average thickness in the patients (mean ± S.D.+ = $182{\cdot}5 \pm 59{\cdot}3$ μm, $n = 11$) and normals (mean ± S.D. = $166{\cdot}9 \pm 14{\cdot}7$ μm, $n = 13$) were similar; there was no statistically significant difference between the two groups (*t*-test; $P = 0{\cdot}41$). Inspection of the cross-sectional images in the CHM patients, however, suggested greater complexity than revealed by the averaged data. There was an apparent relationship to age. When OCT thickness in the central fovea was plotted against age of the patient (Fig. 2(E)), there was a strong correlation (Spearman correlation coefficient, $r = -0{\cdot}86$; $P < 0{\cdot}0001$) indicating a decline with the age of the patient. There was no such relationship in normals ($r = -0{\cdot}23$; $P = 0{\cdot}44$). In this sample of CHM patients, there was also greater than normal retinal thickness in some of the younger individuals; with more advanced disease stage and age, thickness became equal to normal, and finally it was reduced below normal (Fig. 2(E)). These surprising foveal thickness data among the patients accounted for the similarity between patients and normals when only averaged results were compared.

A measure of central cone function (dark-adapted sensitivity, 650 nm target, at fixation) was also plotted for the CHM patients (Fig. 2(F)) and a decline with age was found (Spearman correlation coefficient, $r = -0{\cdot}87$; $P = 0{\cdot}0001$), suggesting a relationship between the central-most structure and cone-mediated sensitivity.

Macular Pigment in Patients with Choroideremia

In anticipation of the lutein supplementation trial in CHM patients, the authors asked a number of questions about MP in CHM. Does MP level in CHM differ from normal? Representative spatial profiles of MP optical density in two normal male subjects and three CHM patients (P1, P5, and P8) are shown (Fig. 3). MP peaked within the central 1° and declined with retinal eccentricity in all normal subjects and patients; from the examples shown it is evident that there was a range of measurable MP densities. As a group, the mean MP density (measured with the conventional 1° diameter stimulus) was comparable between the CHM patients (mean ± S.D. = $0{\cdot}23 \pm 0{\cdot}18$, $n = 11$) and normal male subjects ($0{\cdot}27 \pm 0{\cdot}08$, $n = 13$), and there were no statistically significant differences. Mean MP density levels in both CHM patients and normal male subjects in the current study were within the normal range of other published data (that included both sexes) using the same instrumentation and target (Wooten et al., 1999; Hammond and Caruso-Avery, 2000; Aleman et al., 2001).

Are MP measurements made in CHM patients more variable on successive sessions than those

TABLE 1

Clinical and molecular characteristics of the patients

Patient number	Age at first visit (years)	Position	Mutation	Predicted effect	Eye	Visual acuity*	Refraction	Kinetic visual field Extent (V-4e)‡	Lutein trial participant (age at baseline)
1	15	Intron 9	1274 + 1 G to A	anomalous splicing and truncated gene product	RE	20/20	−0·75	96·6	N
					LE	20/20	−0·25		
2†	18	Exon 6	829 C to T	R267X	RE	20/20	−2·50 − 0·25 × 045	74·5	Y (30)
					LE	20/20	−3·00 − 1·00 × 145		
3	22	Exon 6	829 C to T	R267X	RE	20/20	+0·75 − 0·50 × 090	89·6	Y (22)
					LE	20/20	+0·50 − 0·75 ×090		
4	23	Exon 5	579_587 del 9 ins 8	E183fsX196	RE	20/20	−0·50 − 0·75 × 095	28·9	Y (29)
					LE	20/20	−0·50 − 0·25 × 085		
5†	23	Exon 6	829 C to T	R267X	RE	20/20	−2·25 − 1·00 × 068	69·0	Y (36)
					LE	20/20	−2·75 − 0·50 × 102		
6	31		§		RE	20/20	−5·00 − 1·00 × 090	63·4	Y (36)
					LE	20/20	−4·75 − 0·75 × 085		
7	33	Exon 6	838 C to T	R270X	RE	20/30	−9·00 − 1·25 × 180	5·0	Y (45)
					LE	20/25	−9·25 − 1·25 × 045		
8	34	Intron 2	146 + 1 G to A	anomalous splicing and truncated gene product	RE	20/20	−1·75− 0·50 × 100	59·4	Y (34)
					LE	20/50	−2·25		
9	34	Exon 14	1693 A to T	R555X	RE	20/400	−2·00 − 1·00 × 060	27·5	N
					LE	20/30	+0·50 − 1·25 × 135		
10†	46	Exon 13	1614_1618 del TGTT	H529fsX535	RE	20/50	−7·50	0·2	N
					LE	20/50	−7·50		
11	48	Exon 6	787 C to T	R253X	RE	20/40	−6·50 − 0·50 × 090	17·6	N
					LE	20/25	−6·75 − 0·50 × 090		
12	58	Exon 6	838 C to T	R270X	RE	20/25	+0·50 − 2·00 × 090	0·3	N
					LE	20/20	+0·25 − 1·00 ×090		
13†	65	Exon 13	1614_1618 del TGTT	H529fsX535	RE	20/40	+4·25 − 0·50 × 110	5·2	N
					LE	1/200	+4·25 − 0·50 × 035		

* Best corrected visual acuity.
† Patients 2 and 5 are siblings; Patients 13 and 10 are uncle and nephew, respectively.
‡ Average of both eyes, expressed as a percentage of normal mean of V-4e target; 2 S.D. below normal equals 90 %.
§ No mutation detected to date.
RE, right eye; LE, left eye; bp, base pair; del, deletion; ins, insertion.

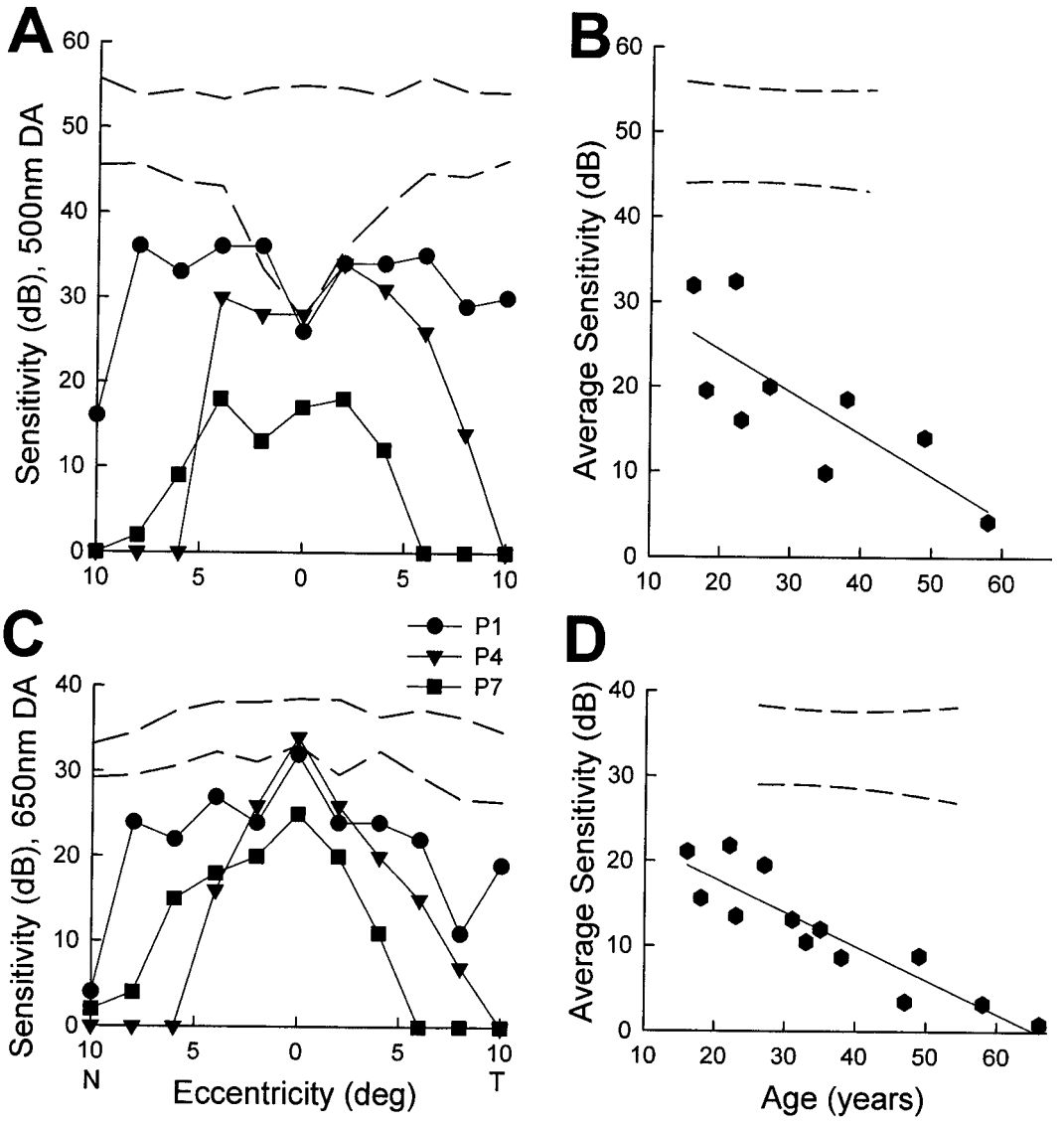

FIG. 1. Central rod- and cone-mediated visual function in choroideremia as a function of age. (A,C) Horizontal profiles in the dark-adapted state (500 nm stimulus, A; 650 nm stimulus, C) from three representative patients at different disease stages. Connected symbols are patient data. Broken lines define ± 2S.D. from the mean normal for each test condition (Jacobson et al., 1991). (B, D) Average sensitivity derived from the profiles exemplified in (A) and (C) plotted against age of the subject at the time of testing. Solid lines are the linear regression fit to the patient data and dashed lines define the 95 % prediction interval of a group of normals (of both sexes) for each test condition (B, $n = 21$, age range, 14–42 years; D, $n = 5$, age range, 25–51 years). Normal data in (C) and (D) were obtained in the dark during the cone plateau phase following a bright adapting exposure (Jacobson et al., 1991).

made in normals? Inter-session variation in MP, as illustrated by the profiles in normal subjects and patients of Fig. 3, was quantified by determining mean absolute differences between MP densities measured on two visits separated by less than 1 month. At the four retinal eccentricities tested, the mean differences (S.D.) for each stimulus in CHM patients were as follows: 0·17°: 0·04 (0·05); 0·5°: 0·05 (0·06); 1·0°: 0·05 (0·05); and 2·0°: 0·05 (0·05). For the normal male subjects, the differences were: 0·17°: 0·03 (0·01); 0·5°: 0·02 (0·01); 1·0°: 0·03 (0·02); and 2·0°: 0·04 (0·01). The absolute value of the differences in CHM patients between baseline sessions was within the expected range of normals; a test for difference between patient and normal results at all eccentricities was not significant. To examine the potential issue of systematic learning effects, signed differences between the visits were calculated; there was no substantial increase between the visits for the four stimuli in patients or normals. Mean differences were: 0·00 and 0·00 at 0·17°; 0·00 and −0·01 at 0·5°; 0·02 and 0·01 at 1·0°; and 0·02 and −0·01 at 2·0°, for CHM patient and normal eyes, respectively.

Is there a relationship between MP density (at 0·5° eccentricity) and serum level or dietary intake of lutein in CHM patients? Mean serum lutein and dietary lutein were not significantly different in the

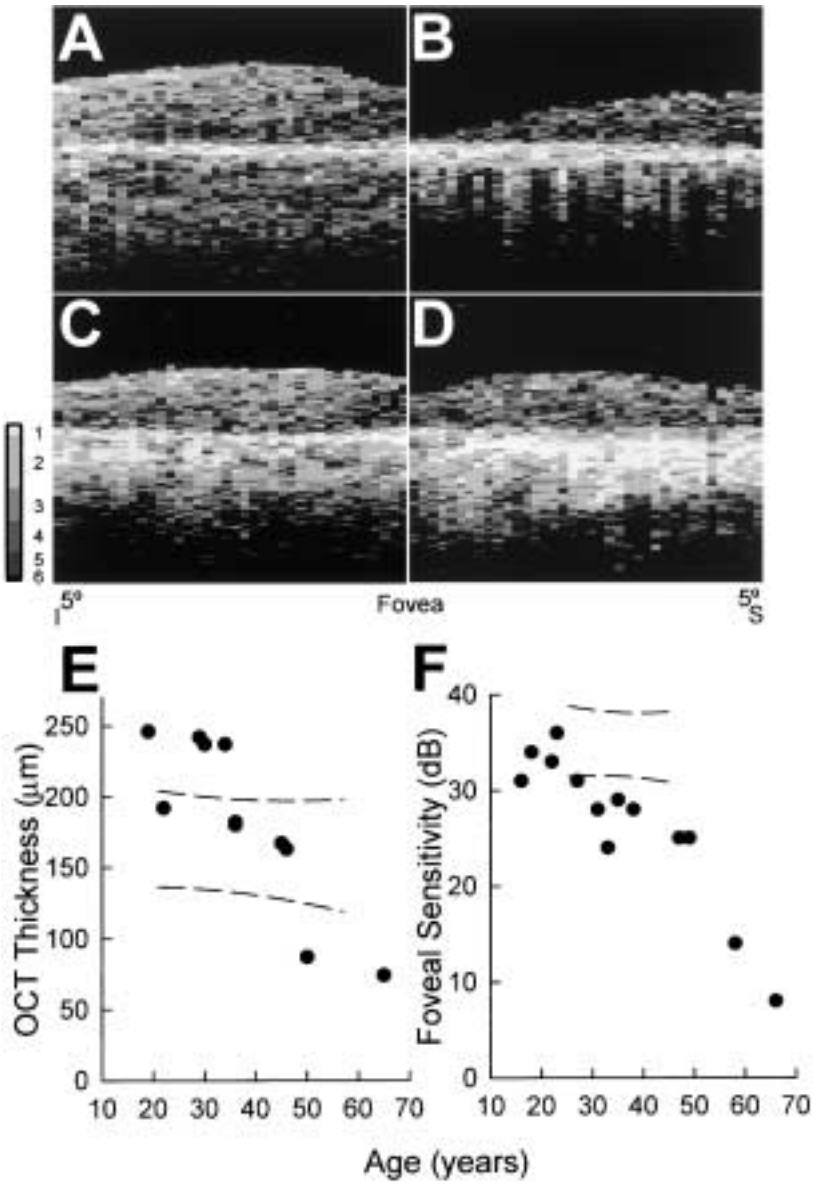

FIG. 2. Central retinal structure and foveal function in choroideremia. (A–D) Vertical OCT scans split at the fovea to permit comparison of central retinal thickness in different patients. OCT images are displayed with the logarithm of reflectivity mapped to a gray scale (lower left). Numbers on the gray scale allow comparison with images using pseudocolor displays (1, white; 2, red; 3, yellow; 4, green; 5, blue; and 6, black; Jacobson et al., 2000; Aleman et al., 2001). (E) OCT thickness as a function of age of the choroideremia patients. (F) Foveal sensitivity (650 nm, 1·7°, dark-adapted) as a function of age in choroideremia. Dashed lines define the 95% prediction interval of a group of normal male subjects for each test condition (E, $n = 13$, age range, 21–57 years; F, $n = 8$, age range, 26–47 years).

patients compared with normal subjects (Wilcoxon rank test, $P = 0{\cdot}41$ for serum lutein, $P = 0{\cdot}18$ for dietary lutein). Neither serum nor dietary lutein correlated with MP density in CHM patients (Spearman correlation coefficient, $r = -0{\cdot}12$, $P = 0{\cdot}64$; and $r = 0{\cdot}09$, $P = 0{\cdot}72$); in normal subjects there was a significant correlation of MP density level with serum lutein ($r = 0{\cdot}52$, $P = 0{\cdot}007$) but MP was not correlated with dietary lutein ($r = 0{\cdot}13$, $P = 0{\cdot}53$).

Does macular disease severity, estimated by functional and structural measures, relate to MP density in CHM patients? Using the absolute threshold to a 650 nm target at fixation as the measure of central retinal function, correlation was found with MP density in CHM patients (Spearman correlation coefficient, $r = 0{\cdot}73$, $P = 0{\cdot}0006$). Using the OCT measurement of central retinal thickness as the structural parameter, there was also a strong relationship with MP density ($r = 0{\cdot}66$, $P = 0{\cdot}003$). This suggests that the MP level may be related to the stage of disease in the CHM patients.

Effects of Lutein Supplementation

CHM patients ($n = 7$) between the ages of 22 and 45 years participated in a pilot trial of the effects of oral lutein supplementation on MP and central vision.

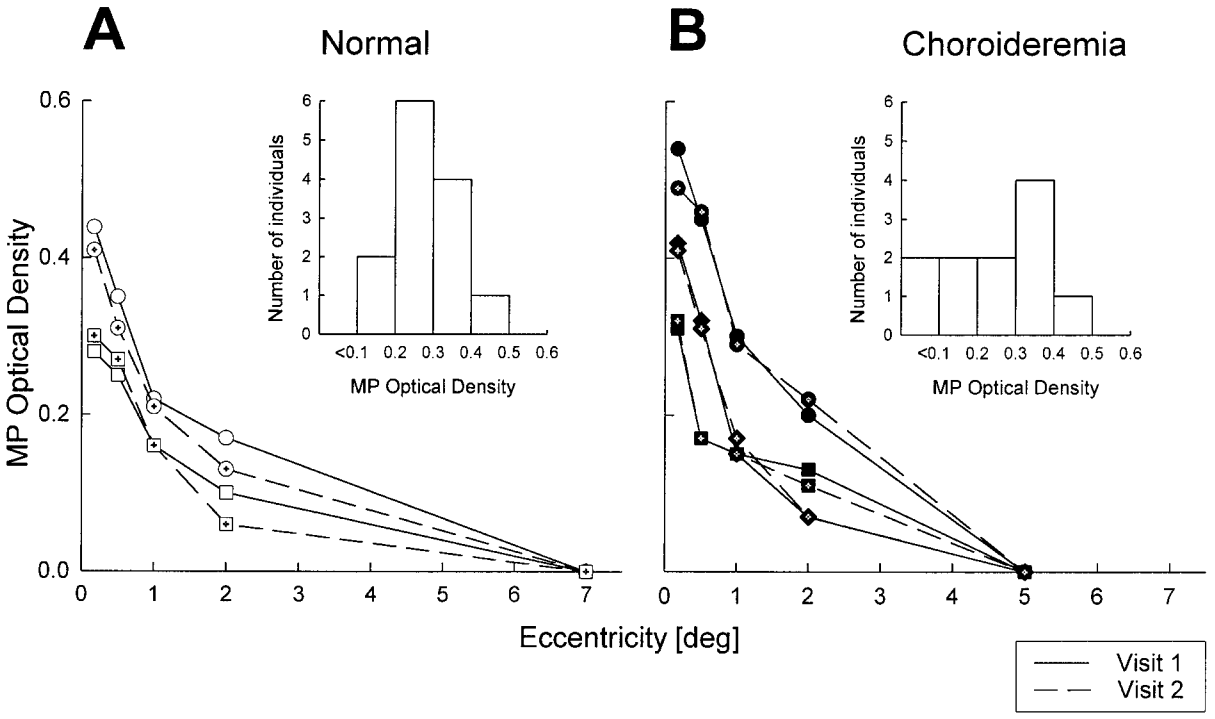

FIG. 3. Spatial macular pigment profiles and their variation in normal subjects and choroideremia patients. (A) Two normal subjects, open symbols, and (B) three patients (P1, P5, and P8), filled symbols, tested on two different visits (symbols connected with dashed lines denote the second visit). Insets are the frequency distributions of macular pigment density for the 0·5° eccentricity target in each group.

Mean MP densities at four eccentricities at baseline and after 6 months of lutein supplementation in four normal male subjects (Fig. 4(A)) and the CHM patients (Fig. 4(B)) are shown. Mean MP at 0·17° retinal eccentricity increased between baseline and post-supplementation by 0·09 in normal subjects ($P = 0·0026$) and by 0·08 in the patients ($P < 0·0001$). Patients also showed significant increases in MP of 0·07 at 0·5° ($P = 0·0007$); 0·06 at 1·0° ($P = 0·005$); and 0·04 at 2·0° ($P = 0·0009$). Normal subjects showed no statistically significant differences at 0·5°, 1·0° and 2·0° eccentricities ($P = 0·56$, 0·35 and 0·66, respectively).

The distribution of change between the two baseline MP values for the most central targets (0·17° and 0·5° eccentricities) for each patient eye was then compared with the distribution of change between baseline and 6 months post-supplementation (Fig. 4(C) and (D)). Overlap was present between the distributions of MP differences at baseline versus post-supplementation, but there was a significant shift towards higher MP density for each target ($P < 0·0001$ for 0·17° target, and $P = 0·017$ for 0·5° target). Comparison of serum lutein levels at baseline versus post-supplementation showed a shift in the distribution towards higher levels ($P = 0·03$).

The observation from the spatial profiles (Fig. 4(A) and (B)) that normal subjects tended to change only at the 0·17° eccentricity while CHM patients showed a response at this and other eccentricities led to the attempt try to define further the response to supplementation in normal subjects vs patients. We plotted the difference in MP density in each eye between average baseline and after supplementation at 0·17° vs 0·5° eccentricities (Fig. 4(F)).

Vertical and horizontal lines in Fig. 4(F) define the 95th percentile for differences within patients between the two baseline values. For the normal subjects, 3/8 (37·5 %) eyes showed no change in MP with supplementation at either central target (Fig. 4(F), lower left quadrant). When there was a response, it was an increase in MP density only at 0·17° (5/8 or 62·5 %; Fig. 4(F), upper left quadrant). For the patients, 6/13 (46·1 %) eyes showed no increase in MP with either target. When there was a response (7/13 or 53·9 %), it could be an increase in MP with the 0·17° (4/13), the 0·5° (2/13) target or both (1/13). If response to lutein supplementation is defined by this statistical criterion using the two central or peak MP measures, then both normal and patient groups had non-responders and responders.

Were there any predictive factors that would help determine which patients would respond to lutein supplementation with increased MP and which would not? If a retinal responder was defined as an individual with at least one eye showing a statistically significant increment in MP level (for one or both central targets) post-supplementation, then five of the seven patients were responders. Accepting that the numbers of patients are small, it was asked whether

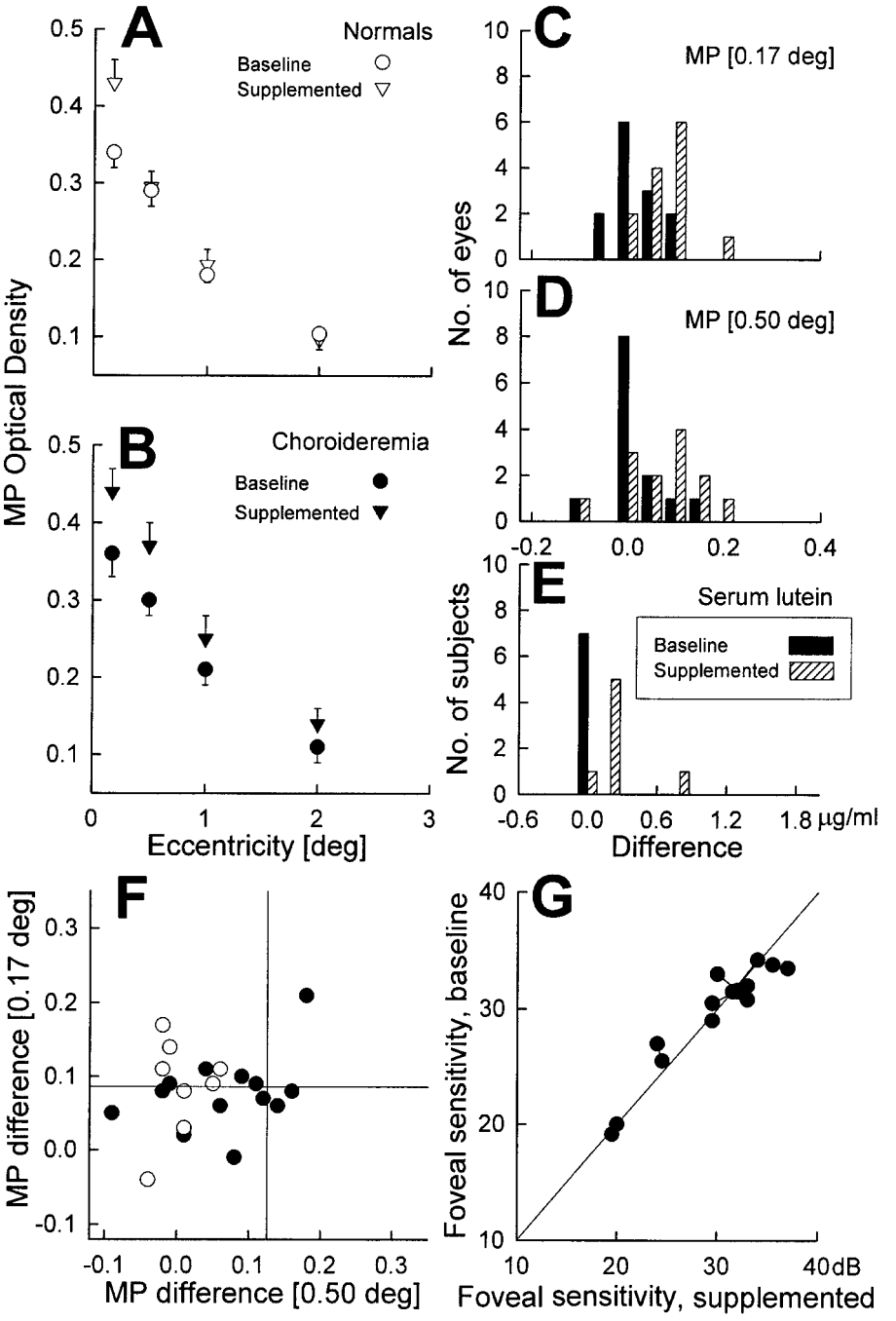

FIG. 4. Effect of lutein supplementation on macular pigment, serum lutein and foveal sensitivity (A,B) Mean macular pigment spatial profiles for normal subjects and patients at baseline (circles) and after 6 months of lutein supplementation (triangles). Error bars, S.E.M. (C–E) Distribution of differences in macular pigment (0·17°and 0·5° eccentricities) and serum lutein (µg ml^{-1}) between baseline visits (black bars) and between mean baseline and 6 month post-supplementation values (hatched). (F) Change in macular pigment at 0·17° vs 0·5° eccentricities after supplementation. Baseline inter-session variability (95 % confidence limits) is defined by the horizontal and vertical lines. Normal subjects, open circles; CHM patients, filled circles. (G) Foveal sensitivity (650 nm, 1·7°, dark-adapted) in patients at baseline and after 6 months of lutein supplementation. The diagonal line represents no change. Lines connecting symbols are from the two eyes of the same patient, when performed.

the two groups differed in any obvious way? Responder vs non-responder groups did not differ in average age (32 vs 35 years); mean baseline serum lutein values were similar (0·15 vs 0·21 µg ml^{-1}); and mean serum lutein increases were not dramatically different (200 % vs 175 %, respectively). Considering central disease severity, responding and non-responding eyes showed no major difference in average foveal sensitivity (29·1 vs 29·2 dB) or average retinal thickness (223 vs 198 µm).

An important question is whether there was any difference in absolute threshold (650 nm stimulus) at fixation between baseline and post-supplementation. Foveal thresholds were little changed between visits at

baseline and after 6 months of lutein supplementation (Fig. 4(G)). Pre- and post-supplementation results were highly correlated ($r = 0{\cdot}96$, $P < 0{\cdot}0001$); a test for difference was not significant ($P = 0{\cdot}50$).

4. Discussion

CHM has been recognized as clinically distinct from other retinal degenerations for more than half a century (reviewed in McCulloch and McCulloch, 1948; Kärnäi, 1986); and, for the past decade, the molecular cause has been known to be mutations in the gene encoding REP-1, part of the complex membrane trafficking pathways of cells (reviewed in Alory and Balch, 2001). As the molecular pathology of CHM is better understood, longstanding questions about disease expression may be answered. For example, the early funduscopic feature in CHM of abnormal melanin pigmentation in retinal pigmented epithelium (RPE) and choroid in hemizygotes and heterozygotes (McCulloch and McCulloch, 1948) could result from defective transport of melanosomes in RPE and choroidal melanocytes secondary to Rab27a hypoprenylation (Pereira-Leal, Hume and Seabra, 2001). The disease has been suggested to lead from deficient melanosome transport, to light damage at the level of the RPE, and then secondary photoreceptor and choroidal disease (Stenmark and Olkkonen, 2001). REP-1, however, has been localized in photoreceptors (van den Hurk et al., 1997; Bernstein and Wong, 1998; Syed et al., 2001), so albinotic-appearing RPE and choroid may be only part of the expression and CHM could also have a primary effect on photoreceptors. As in many retinal degenerations, the CHM disease sequence is likely to be complex and there may be a cascade of Rab-associated defects due to relative loss of REP-1 vs REP-2 activity (Cremers et al., 1994; Alory and Balch, 2000, 2001; Detter et al., 2000; Pereira-Leal et al., 2001).

As a prelude to a pilot intervention with oral lutein in CHM patients, details of macular function and structure and macular pigment in CHM were sought. How do the results compare with those in the literature? There are considerable published data on central fundus changes and visual acuity in CHM (for example, McCulloch, 1969; Kärnä, 1986; Sieving, Nieffennegger and Berson, 1986; Grover et al., 1998; Roberts et al., 2001). The visual acuity results in the cohort of CHM patients generally concur with those in the literature. Rod- and cone-mediated function across the central retina in CHM has not been specifically explored; a decades-long decline was found in both photoreceptor systems. Foveal retinal thickness by OCT became reduced with age and this paralleled a loss of foveal cone sensitivity. An unexpected observation was a greater than normal foveal thickness in some of the younger CHM patients. These patients all had excellent visual acuity and were not fixating eccentrically. There was no macular edema in the patients. There are histopathological reports in two patients with late stages of CHM of extensive inner retinal gliosis and a preretinal membrane (Ghosh and McCulloch, 1980). A thin preretinal membrane could in theory alter foveal architecture leading to the OCT observations in the younger CHM patients, but this was not evident. It is interesting to note that there is also a lack of normal foveal architecture in forms of albinism, which share with CHM melanin pigmentation disturbances in RPE and choroid (Fulton, Albert and Craft, 1978). Whether there is a developmental component to the OCT observations of the central retina of younger CHM patients is an intriguing but speculative possibility.

Macular pigment levels have not been reported previously in CHM patients. In the cohort of CHM patients, if analysed as a group, MP was found to be within normal limits. It is important to note that the validity of the assumptions implicit in the use of the current heterochromatic flicker photometry technique to estimate MP was not explicitly proven in the patients. Specifically, it was assumed that the difference in sensitivity to the blue and green stimuli at the level of L/M-cone outer segments was invariant across the central retinal locations. Theoretically, outer retinal degeneration could affect the relative abundance and photopigment density of L and M cones differentially across the regions tested, although there has been no such evidence to date. Generation of psychophysical MP absorption spectra in CHM patients remains to be performed in future studies.

Were there measurable effects of oral supplementation of lutein on serum carotenoids and MP in CHM? Supplementation increased the serum levels of lutein above baseline in all CHM patients and this is consistent with the earlier observations in patients with other inherited retinal degenerations and studies in normal subjects (Hammond et al., 1997; Landrum et al., 1997; Aleman et al., 2001; Hininger et al., 2001). Macular pigment levels increased from baseline in the CHM patients as a group. If changes in MP greater than the 95th percentile of changes between baseline sessions are considered to be evidence of an effect of supplementation, then about 54 % of the eyes would be considered as responding whereas 46 % were not responding. Oral lutein intake over 6 months thus did not assure a measurable increase in MP. This was also the experience in RP and Usher syndrome (Aleman et al., 2001) and reflects observations in some groups of normal subjects (Hammond et al., 1997; Landrum et al., 1997). The factors that were examined were not predictive of whether or not there would be a response. Whether other techniques of measuring MP would detect higher percentages of responders to supplementation awaits further study (Bernstein et al., 1998; Berendschot et al., 2000; Delori et al., 2001; Ermakov, McClane and Gellerman, 2001).

No short-term benefit on foveal dark-adapted function was found in this pilot study. This mirrors

the observations that were made in RP and Usher syndrome (Aleman et al., 2001). Considering the progressive loss of macular structure and function and macular pigment in CHM, it would seem worthwhile to determine if there is long-term benefit to central vision of this supplement.

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