Foveal Cone Photopigment Distribution: Small Alterations Associated with Macular Pigment Distribution

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PURPOSE. To map the photopigment distribution of central foveal cones in healthy adult subjects before potential onset of age-related macular degeneration. To compare alterations in cone photopigment distribution to those of macular pigment and examine those loci for subretinal changes.

METHODS. Eleven healthy subjects (age range, 31–59 years) underwent reflectometry with a scanning laser ophthalmoscope. The difference in cone photopigment density in the fovea was mapped for the long-wavelength- and middle-wavelength-sensitive cones, using 594-nm light. Macular pigment was mapped with 488-nm and 514-nm light. Subretinal changes were investigated with infrared imaging (850–860 nm).

RESULTS. Most subjects had small alterations in the regularity of their foveal cone photopigment distribution. Alterations were spatially related to macular pigment alterations but not to the presence of subretinal defects. Subjects were classified into three groups according to the type of alterations in the regularity of pigment distributions: central peak of photopigment and macular pigment, small foveal alterations, and broad distribution with missing central peak of photopigment or macular pigment. The resultant groups differed significantly in age, 43, 46, and 59 years, for groups 1, 2, and 3, respectively (P < 0.05).

CONCLUSIONS. Small alterations in the distributions of foveal cone photopigment or macular pigment were found that varied among the subjects. Larger alterations in older subjects may indicate changes in foveal architecture with age, including potential vulnerability of central cones before the onset of clinically significant changes in the retinal pigment epithelium.

Vision losses associated with aging are still poorly understood, even though age is a risk factor for diseases such as age-related macular degeneration (ARMD), macular hole, and glaucoma. Individual differences in the function of foveal cones can increase with age, even though the average data show little change. Individual differences in the prognostics for continued foveal vision vary even more widely in disease, especially with exudative manifestations of ARMD, such as pigment epithelial detachments.

The anatomic substrate for best visual acuity or color vision depends on the health of long and tightly packed cone photoreceptors in the fovea, the retinal pigment epithelial cells that provide metabolic support for cones, and the neural cells and their metabolic support in the inner retina subserving these cones. Maintaining tight packing of photoreceptors is necessary for good acuity. Small alterations in the distribution of foveal cones are not amenable to study with previous methods. In the central 1° of young subjects, factors other than packing limit visual acuity. The central foveal cones, which are narrow and rodlike, have been less studied than the much larger cones found more peripherally. With some ex vivo methods, the most central cones cannot be easily measured, whereas in other methods the largest individual differences are reported for the central 1°, or decreased density of foveal cones is attributed to age-related changes.

By use of in vivo measurements of the optical density of cone photopigment, photoreceptor damage is detectable early in many diseases, often before other signs appear. Central foveal cones may be more susceptible to damage associated with aging or eye disease caused by the absence of room for support cells, the paucity of retinal capillaries near the fovea, or distance from the choroidal blood supply to the cone myoids.

To elucidate the differences among subjects in the distribution of functioning cone photoreceptors in the fovea, we measured the difference in photopigment density using retinal densitometry specifically designed for foveal cones. The main assumption was that the change in the spatial distribution of light returning from the fundus before and after exposure to a bright light is caused by photopigment bleaching. The photopigment density difference at a given retinal location was...
calculated from the difference in the logarithm of aligned bleached versus dark-adapted images. This measurement depended on the following factors: percentage of retina covered by cones, cone length, photopigment concentration per unit of optical path length, cone directionality, and photopigment extinction spectra. Our method is strongly resistant to scattered light, regardless of the source. A narrow illumination beam is focused on one small spot of the retina at a time, and only light returning to the detector during this time contributes to the measurement for this location. In the foveal center, the cones are tightly packed and surrounded by interphotoreceptor matrix. In young, healthy people, the coverage of the foveal surface is good, so that well-functioning cones should capture a high percentage of the light. In older people, missing or poorly functioning cones may capture less light, which causes increased stray light at that locus.

The distribution of photopigment density difference across the fovea is correlated with histologic measurements of cone topography. More important, as shown by an in vivo comparison in the same healthy subjects, cone packing density measured with ocular speckle interferometry correlates with cone photopigment density difference measured with retinal densitometry. The change with age in the distribution of photopigment density difference has not been measured with retinal densitometry. Regarding density difference averaged over the entire fovea, there is one report of decreased optical density with increasing age, whereas other reports indicate decreased optical density only in the age range of ARMD. Although cone photopigment density may be decreased and photopigment kinetics slowed in an older person, there is no measurable relation to visual threshold.

The interpretation of retinal densitometry measurements has been furthered by using complementary methods, such as color matching, on the same subjects. In color matching, a psychophysical technique, the photopigment optical density depends on the photopigment extinction spectra, concentration, and optical path length in functioning cones, but does not depend on the number of photoreceptors per unit area or stray light from anterior segment sources. Color matching with a range of light levels sufficient to test photopigment at full and dilute concentrations provides estimates of the optical density of both long- and medium-wavelength-sensitive cone types, and the range of individual differences. These cone photopigments are readily measured in the fovea using the light levels and the single wavelength of the present retinal densitometry technique.

When healthy subjects were tested with color matching and retinal densitometry, the results agreed well for the central 2°, and mirrored individual differences. However, outside the central 2°, color matching produced proportionally higher optical densities than did retinal densitometry. Taken together, these results indicate that changes with eccentricity in the central fovea, where the coverage by foveal cones is high, are largely determined by the length of cones. Furthermore, when the distribution of cone photopigment from retinal densitometry is measured in the same young, healthy subjects as the cone spacing measured by stellar speckle interferometry, the amount of photopigment per cone is nearly constant or increases slightly with eccentricity across the central 2°. Thus, when the photopigment is decreased within the central 2°, it is likely that there has been a change in the photopigment coverage or in the length of cone outer segments.

Cone photopigment optical density, when measured with color matching, decreases little with aging, except at the foveal center. Thus, there are differences in the relative changes across the foveal and parafoveal regions with aging. Previous reports on difference in cone photopigment density and aging, including our reported trend of decrease with age in seven subjects, used fewer subjects than color-matching studies. Regeneration averaged over the central 4° is slowed with age, in agreement with retinal densitometry. The color-matching method is sensitive and reliable. However, it is difficult to test extremely small loci, because older subjects can have difficulty in discriminating small targets or in maintaining fixation accurately. Measurements are an average, heavily weighted by the outermost locations of the test field. Thus, small alterations in photopigment density distribution are not detected or localized accurately with color matching.

Foveal cones not only contain photopigment in their outer segments, but also are associated with macular pigments. At least two types of retinal tissue contain macular pigments: cone axons and the inner plexiform layer. These are in distinct layers that change across the fovea in the amount of radial displacement relative to their cone outer segments. Thus, if cones are missing or irregularly spaced, there could be an alteration, not only in the photopigment distribution, but also in the macular pigment distribution, with the macular pigment alteration slightly displaced.

To elucidate the differences among subjects in the distribution of macular pigment across the fovea, we used a reflectometry technique that is objective and resistant to stray light. The main assumption is that the major difference in the distribution of light returning from the macula at 488 nm versus 514 nm is caused by macular pigment absorption, when the cone photopigment is bleached. The measurement is little affected by changes in other ocular pigments, such as melanin, which can be striking in measurements that include short (450-488 nm) and long (610-670 nm) wavelengths. By measuring macular pigment and cone photopigment, the inner and outer portions of cone photoreceptors can both be studied at a given retinal location.

It is difficult to distinguish early, subtle changes in retinal and subretinal tissues that are indications of disease rather than aging. Drusen and other subretinal deposits, (e.g., in Bruch’s membrane), are the clinical hallmark of ARMD. However, the extent of subretinal disease is often difficult to observe clinically, and aspects of photoreceptor function that indicate irreversible cell damage are not visualized with clinical methods. The in vivo detection and localization of small or subtle subretinal changes is improved with infrared imaging with a scanning laser ophthalmoscope (SLO). The in vivo detection of loss of choriocapillaris or potential outer retinal ischemic changes is challenging and is not addressed here. Although the presence of subretinal drusen in donor tissue can be associated with decreased photoreceptors, the in vivo relation of photoreceptor function to subretinal changes is not understood.

In the present study, we compared measurements of cone photopigment and macular pigment distributions in healthy subjects before their seventh decade. First, we examined the alterations during adulthood in these distributions from the ideal shape, which peaks at the foveal center and smoothly decreases with increasing eccentricity. Second, we investi-
Asian, and the other 10 were white with eyes of widely varying color vision.

To investigate potential changes during middle age, before the transition to early ARMD, with clinical methods including slit lamp biomicroscopy, indirect ophthalmoscopy, and stereo fundus photography. Our study excluded subjects with decreased visual acuity or subretinal disease in the fovea, including large (>63 μm) or confluent drusen in the fovea and no clumped hyperpigmentation in the macula.

METHODS

Subjects

To investigate potential changes during middle age, before the major effects of aging or eye disease, eleven healthy subjects aged 31 to 59 years were recruited (Table 1). Subjects had normal color vision and at least 20/20 visual acuity. One was Asian, and the other 10 were white with eyes of widely varying pigmentation. No subject had a refractive error of more than 6 diopters spherical correction, and the large individual differences in spherical equivalent were unrelated to the main findings for photopigment, macular pigment, or subretinal structures. None had diagnosed systemic or eye disease at the time of the test.

After the purpose, procedure, and consequences of the study were explained, each subject signed a consent form and followed a protocol approved by the Institutional Review Board of the Schepens Eye Research Institute, Boston, Massachusetts. The research was conducted according to the tenets of the Declaration of Helsinki. Each subject had an examination or on a subsequent day. A pupil diameter of at least 6 mm was achieved by using 1 to 2 drops of 0.5% to 1% tropicamide (Mydriacyl; Alcon Ophthalmic, Fort Worth, TX).

Apparatus

The research SLO and image acquisition system, designed for quantitative imaging,17 were used for all three types of data collection: retinal densitometry, macular pigment measurements, and infrared imaging. Several differences of this instrument from the commercial models are important for quantitative results. There are eight laser sources, stable in power. Three are unique: a 594-nm HeNe (Research Electro-optics, Boulder, CO), an 830-nm laser diode with beam shaping (Liconix, Santa Clara, CA), and an infrared laser (Chr:Li:SaF; EyeScan, Schwartz Electro-optics, Concord, MA) tuned to 860 nm. The power of the illumination beam can be adjusted rapidly over a continuous range. Quantitative measurements are calibrated before each experiment, using a photometer (DR-2550; EG&G, San Diego, CA). Each visible wavelength laser was adjusted using neutral density filters in combination with an analog adjustment of the acousto-optic modulator for that laser. The infrared lasers have three log-unit-neutral density wedges for continuous control of intensity. The argon wavelengths were made monochromatic by use of interchangeable, six-cavity interference filters, centered on the laser line of 488 or 514 nm. The laser beam path lengths were at least 1 m long before the entrance aperture of the instrument, permitting beam shaping, collimation, and spatial filtering.

The detection pathway and image acquisition system were both linear with respect to incident light. Head positioning was performed with a slit lamp stand and circuitry independent of instrumental control or image acquisition, so that precise alignment of the subject was independent of other controls. The image acquisition computer and the SLO control computer operated separately, allowing images to be acquired rapidly without interruption.

Procedure

To avoid bleaching from the clinical examination, subjects participated in the imaging experiments before the clinical examination or on a subsequent day. A pupil diameter of at least 6 mm was achieved by using 1 to 2 drops of 0.5% to 1% tropicamide (Mydriacyl; Alcon Ophthalmic, Fort Worth, TX).

Gated whether alterations are spatially colocalized with changes to the retinal and subretinal structures.

Table 1. Individual Data for Age, Sex, Eye Color, Spherical Equivalent, Group, and Photopigment and Macular Pigment Distributions

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (y)</th>
<th>Sex</th>
<th>Eye Color</th>
<th>Spherical Equivalent (Diopters)</th>
<th>Group</th>
<th>Symbol in Figures 2 and 3</th>
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<tr>
<td>1</td>
<td>31</td>
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<td>Brown</td>
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<td>1*</td>
<td>Crosses</td>
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<tr>
<td>2</td>
<td>42</td>
<td>M</td>
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<td>+1.00</td>
<td>1</td>
<td>Large circles</td>
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<tr>
<td>3</td>
<td>44</td>
<td>M</td>
<td>Hazel</td>
<td>-5.75</td>
<td>1</td>
<td>Triangles</td>
</tr>
<tr>
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<td>54</td>
<td>M</td>
<td>Hazel/green</td>
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<td>1</td>
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<tr>
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<td>-1.5</td>
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<tr>
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<tr>
<td>11</td>
<td>59</td>
<td>F</td>
<td>Brown</td>
<td>+0.00</td>
<td>3</td>
<td>Large circles</td>
</tr>
</tbody>
</table>

Group 1, foveal peak of photopigment and macular pigment; group 2, at least one alteration in the regularity of foveal photopigment and macular pigment; group 3, severe or large central alteration of foveal photopigment or macular pigment.

* Alteration observed near central fovea in both cone photopigment and macular pigment, much smaller than those in group 2, and otherwise peaked distribution.

† Large central alteration in macular pigment, but photopigment somewhat peaked in fovea.
Subjects were dark adapted for 15 minutes, allowing the cone photopigment to regenerate. Infrared imaging was performed during a portion of the dark-adaptation period. Then retinal densitometry was performed. The macular pigment measurements immediately followed retinal densitometry.

Images were acquired digitally with 512 × 480 pixels and 8 bits of gray scale (FG100 AT; Imaging Technology, Bedford, MA). Four successive images were acquired at video rates, and two or more were averaged to reduce noise. The large field size was used for all measurements: 28.6° × 23.0° with 3.50 min arc per pixel for a model emmetropic eye in horizontal and vertical directions. For the infrared images, a small field size was also used: 15.2° × 13.6° with 1.75 min arc per pixel.

All sessions were viewed in real time and videotaped, so that reflectance artifacts and media changes such as vitreous floaters could be localized and minimized. Focusing and motion parallax provided depth cues that aided in localizing and identifying fundus features, so that thickened structures were distinguished from flat, depigmented ones. Focusing and motion parallax also provided information about the layer of origin of artifacts such as the foveal reflex, which is typically very small and round and changes directions predictably during eye movements.

### Infrared and Visible Wavelength Imaging to Localize Subretinal and Retinal Disease

Infrared imaging was used to quantify and localize subretinal disease, as described previously. It is more sensitive than other methods in the detection of pathologic structures such as drusen. The images were digitized at 830 nm or 860 nm. Direct mode imaging was performed by using a small confocal aperture, which results in collection of light returning from the fundus at a high angle, that is, directly backscattered light. Drusen appear brighter than surrounding fundus features. In direct mode imaging is performed with an annular aperture, resulting in the collection of light that primarily has been multiply scattered. Drusen appear thickened, casting shadows. Two field sizes were tested, so that four infrared images were available to examine for subretinal structures. Data analysis consisted of determining the location and type of drusen from all of the images, although in some subjects the subretinal changes were extremely subtle.

Visible wavelength imaging was also used to quantify and localize subretinal disease. We analyzed the retinal densitometry and macular pigment images under bleached photopigment conditions, 594 nm, and 514 and 488 nm, respectively, for the presence of drusen, indicated by bright, circular patches. These wavelengths also provide excellent visualization of exudates and motled pigmentation in patients with these conditions. A final 594-nm imaging condition was added to maximize the visualization of retinal vascular features and superficial retinal features, such as epi-retinal membranes, that cause measurement errors in patients.

### Retinal Densitometry Procedure for Cone Photopigment Distribution

To map the foveal cone photopigment distribution, we used the method developed previously. After bleaching, images were digitized for macular pigment measurements, using an 800-μm aperture. A higher video gain was selected for the shorter wavelength illumination, because of lens and pigmentation differences among subjects. The power was then adjusted for each subject to provide a high-quality, dark image of the macular region, which maximizes the number of gray-scale steps on a logarithmic scale for the macular pigment, similar to the cone photopigment technique. The resultant powers of 488 nm and 514 nm were 5.3 to 5.4 and 5.4 to 5.5 log scotopic td and 4.5 to 4.6 and 5.2 to 5.3 log photopic td, respectively. This was sufficient to maintain a bleached state of rod and cone photopigments, which was evident in the uniform absence of density difference measured outside the central 6°.

The macular pigment density difference (MDD) was computed digitally from aligned images as MDD = log(image at 514 nm) − log(image at 488 nm · ε), where ε is the ratio of 514-nm to 488-nm illumination in a 10 × 10-pixel region of interest at 7° to 9° eccentricity. This provides a density difference of 0 log unit at an eccentricity at which cone photopigment density difference and macular pigment density are unmeasurable. The distributions, when shown as topographic surfaces in pseudocolor, were similar to the photopigment data, in that the peaks were emphasized (not shown). Small differences across the fovea were best shown by the en face method and radial averages.

### Imaging Macular Pigment Distribution

To map the macular pigment distribution, we used a method developed previously. After bleaching, images were digitized for macular pigment measurements, using an 800-μm aperture. A higher video gain was selected for the shorter wavelength illumination, because of lens and pigmentation differences among subjects. The power was then adjusted for each subject to provide a high-quality, dark image of the macular region, which maximizes the number of gray-scale steps on a logarithmic scale for the macular pigment, similar to the cone photopigment technique. The resultant powers of 488 nm and 514 nm were 5.3 to 5.4 and 5.4 to 5.5 log scotopic td and 4.5 to 4.6 and 5.2 to 5.3 log photopic td, respectively. This was sufficient to maintain a bleached state of rod and cone photopigments, which was evident in the uniform absence of density difference measured outside the central 6°.

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RESULTS
Cone Photopigment Distribution and Macular Pigment Distribution

The difference in cone photopigment density decreased with increasing retinal eccentricity in all subjects, if the entire macula was considered. Beyond approximately 6° eccentricity, cone photopigment was too low to be reliably measured, as shown previously. When only the foveal region was considered, the distributions of cone photopigment and macular pigment formed a continuum of features that differed from previous measurements in young subjects, and the ideal photopigment shape that is predicted by histology. To represent these small alterations in the expected distribution of foveal cone photopigment better, not previously characterized, the subjects were classified in three groups (Table 1). Each subject’s distributions of cone photopigment and macular pigment were consistent, except for those in subject 8 of group 3.

Group 1 consisted of healthy subjects with shallow foveal peaks of cone photopigment density difference and macular pigment. The topographic map of a sample subject (subject 2) emphasizes the foveal peak of cone photopigment, with a steadily decreasing amount of photopigment in all directions (Fig. 1A). The en face map of cone photopigment distribution in the same subject shows that there were no alterations in the smooth distribution in the foveal region (Fig. 1B), although the subject had vitreous floaters in the initial images. Similarly, the en face map of macular pigment in this subject has a foveal peak (Fig. 1C). The radial profiles show foveal peaks in photopigment distribution at 1° eccentricity (Fig. 1D). Figure 1 shows a comparison of foveal cone photopigment and macular pigment distributions in three representative subjects, one per group. (A, B, C) Subject 2 from group 1, which comprised healthy subjects with foveal cone distribution less peaked than in some young subjects. (D, E, F) Subject 6 of group 2, which comprised subjects with small foveal defects in photopigment. (G, H, I) Subject 10 of group 3, which comprised older subjects with a central lack of foveal cone photopigment or macular pigment.
Subjects were classified according to their photopigment and macular pigment data (Table 1), rather than on the basis of age or gender. The groups differed significantly in age (analysis of variance; $P < 0.05$). The average age was 43, 46, and 59 years in groups 1, 2, and 3, respectively. Group 3 subjects, those with large, central alterations in photopigment or macular pigment distribution, were older than all of the other subjects in the sample. All women ($n = 5$) had alterations, but only the older men did. The youngest women ($n = 2$) had the smallest alterations, and the oldest woman the largest alteration. All group 2 subjects were women and younger than the oldest man in group 1. In group 3 the oldest subject, a man, had the greatest peak in photopigment. Thus, the differences among subjects in cone photopigment distribution and the corresponding ones in macular pigment distribution were probably related to changes in the structure or function of the central fovea that occur with age and possibly gender. It is unknown whether small alterations became larger with time, or larger alterations developed by other means to the extent that the smaller ones were no longer detectable.

The distributions of photopigment and macular pigment were consistent with the individual images used in the measurements at 594 nm or 488 nm and 514 nm, respectively, and did not arise from image-processing artifacts. Group 1 subjects had round, darkened regions with distinctly darker centers, particularly apparent in the 488-nm images. In contrast, group 3 subjects had darkened regions of greater diameter. These regions were more uniformly dark, without distinct centers. This cannot be attributed to spherical refractive error, because across the region. In contrast to group 2, the absence of a central peak in group 3 does not result from a low measurement at one location and a normal measurement at adjacent locations.
the fovea were well visualized, with retinal imaging conditions, in one man. These drusen and the punctate defects were unknown to the subject before testing. An example of the final 594-nm images, which provided the highest contrast of retinal features, is shown for subject 6 in group 2 (Fig. 4A).

On examination of all 830-nm or 860-nm images for subretinal features, all subjects in this study had subretinal deposits in the fovea, but most of these were smaller and more subtle than could be detected clinically (Table 3). These deposits lay beneath the photoreceptor layer. An example of the direct-mode infrared images is shown for the smaller field size, to emphasize the foveal region, in subject 6 in group 2 (Fig. 4B). Although subjects with large or confluent druse were excluded from our study, one subject had a large, thickened druse outside the foveal region, well localized on infrared imaging but not detectable clinically. Placoid patches of subretinal deposits\(^{34}\) were seen in all subjects older than 52 years (group 3: 3 men, 1 woman). Punctate defects that were seen in the fovea with retinal imaging were seen with infrared imaging also, but only in the direct mode and when the superficial layers were in focus. Examination of the deeper layers revealed no punctate defects with any imaging mode or field size, although subretinal defects such as breaks in Bruch's membrane were readily detectable with this method.\(^{35}\) The largest retinal punctate defect was best seen at 488 nm and 514 nm in subject 5 in group 2 (not shown). The defect was well localized and superior to the foveal center, but there was no subretinal feature at this location more striking than at any other, even at two times the magnification.

**Comparison of the Distributions of Cone Photopigment and Macular Pigment with Retinal and Subretinal Features**

We compared the loci of alterations in the distributions of foveal cone photopigment and macular pigment to the loci of retinal and subretinal defects. All five subjects who had a punctate defect in retinal imaging also had a corresponding alteration in photopigment and macular pigment distributions. The shape of the retinal defects corresponded better to the photopigment distribution than to the macular pigment distribution, consistent with the containment of the macular pigment in the displaced axons of the cones in the central fovea. The subjects with punctate retinal defects included one subject in group 1, all subjects in group 2, and one subject in group 3. There was no corresponding subretinal defect.
The four group 3 subjects, who were also the older subjects, had central alterations in the distributions of cone photopigment or macular pigment at locations without subretinal defects other than small drusen. Some of the younger subjects in groups 1 and 2 had subretinal changes similar to or more marked than those in group 3 subjects, yet had more peaked photopigment and macular pigment distributions. Thus, the distributions of foveal cone photopigment and macular pigment differed among subjects before development of clinically significant subretinal changes, in a manner consistent with age and gender. In some subjects the alterations corresponded to the presence of punctate retinal defects.
DISCUSSION

Both foveal cone photopigment and macular pigment were readily measurable in healthy subjects before their seventh decade. The distributions of photopigment and macular pigment differed among subjects. Differences in the heights of the foveal peaks of the two types of pigments and alterations in the smoothness of the distributions were found. Retinal or subretinal defects could not be observed clinically that corresponded with variations in the pigment distributions, and those observed with laboratory methods were either small and subtle or were located outside the fovea. These findings placed the subjects in a continuum with three broadly defined groups, groups 1, 2, and 3, that differed significantly with age.

The present photopigment and macular pigment results may be combined with previous cone photopigment data to characterize the full range of potential results. Group 0 represents the ideal distribution, based on histology, of a sharp foveal peak of photopigment and macular pigment, decreasing smoothly in all directions from the peak. Young healthy people typically have steep foveal peaks of difference in photopigment density, and a large amount of cone photopigment. Figure 5A shows the data of a 22-year-old healthy man, replotted from Eisner et al. Group 1 consists of healthy subjects with foveal peaks of cone photopigment density difference (Figs. 1A, 1B) and macular pigment (Fig. 1C) that are shallower than the peaks measured for group 0. Group 2 consists of subjects with shallow peaks of cone photopigment (Figs. 1D, 1E) and macular pigment (Fig. 1F), with one or more foveal alterations in the smoothness of the distributions. Group 3 consists of subjects with measurable cone photopigment (Figs. 1G, 1H) and macular pigment (Fig. 1I), but with large, round, central alterations in one or both distributions. Group 4 consists of patients with clinically significant disease that affects the photoreceptor-retinal pigment epithelial complex, but with measurable cone photopigment or macular pigment. An example is shown in Figure 5B, a 62-year-old male patient with ARMD. This patient did not have exudation or marked atrophy in the test eye, but had exudation in the fellow eye. There was measurable foveal cone photopigment, but markedly less than in most other subjects; the distribution had no distinct peak at the foveal center. Group 5 consists of patients with clinically significant disease and no measurable foveal cone photopigment (not shown).

Previous results concerning changes in foveal cone photopigment with aging differ among studies and methods, with differences reported primarily in subjects beyond the seventh decade.
The present study describes differences between subjects with better resolution than previous studies; some of the measured alterations of cone photopigment distribution in the fovea were less than 100 μm wide. Because our study excluded aged subjects and those with clinical evidence of subretinal disease in the fovea, decreased visual acuity, large drusen, or clumped hyperpigmentation that could be seen on infrared imaging, the small alterations found in the distributions of photopigment and macular pigment could not be attributed to clinically significant subretinal changes. The present retinal densitometry results agreed well with previous color-matching results. The subjects all had measurable cone photopigment when the entire central 4° was considered, and older subjects had less photopigment in the central 1° than expected from more eccentric locations. The color-matching results indicate a decreased optical path or lower concentration of photopigment, but our results indicate that the cones not only may have been altered in some older eyes, but that they were more scarce in the central fovea. In retinal densitometry, photopigment optical density depends strongly on the number of cones per unit area,24 anterior segment stray light, and the factors affecting color matching. Anterior segment stray light cannot cause the observed punctate, hypopigmented retinal defects, and thus was not a factor in the present results. However, the photopigment results alone did not distinguish between reduced photopigment per cone and fewer cones per unit area.

Our data are best explained by a change with aging in the foveal architecture, particularly the foveal center. Histologic measurements of cone coverage differ most among healthy subjects in the central 1°. Our data are consistent with three related hypotheses. The first is that, with increased age or chance of insult, there is an increased probability that the foveal cones have died in the area of the defects to a greater extent than elsewhere. Group 3 subjects had lost a significant proportion in the central 1°. Patchy loss, rather than a loss across a large retinal region, suggests spatially discrete mechanisms—for example, decreased choroidal or retinal circulation, particularly near the foveal avascular zone. Our data are inconsistent with photoreceptors that have normal coverage but produce little photopigment, because the macular pigment was also decreased.

The second hypothesis is that the structure of the fovea and positions of the photoreceptors may not be static in adults. Cone and rod distributions change during childhood, with the cones migrating into the central fovea and becoming thin and tightly packed several years after birth.42 The interphotoreceptor matrix in rods changes in composition along the axis of the outer segments with exposure to light.43 There is a repositioning of retinal tissues in macular holes. The retina is pulled away centrifugally from the foveal center to form a hole but often moves centripetally to a more central location after surgery, with an improvement in visual acuity consistent with foveal cone function.44 Thus, cone packing and the adhesiveness of the interphotoreceptor matrix could change with disease or, over short duration, with location, age, or gender. In Group 2 subjects, the photoreceptors at locations with punctate alterations in photopigment distribution may not have been as closely and regularly packed as at other locations or in other subjects. A hypothesis of a somewhat fluid, partially adhesive, foveal architecture is consistent with the finding of large, central alterations in cone photopigment and macular pigment distributions in the oldest subjects. Ex vivo studies of human donor eyes indicate significant loss of rod cell bodies with aging, greater than the loss of cone cell bodies,45 but disagree about the distribution.45 A reverse migration of some cones outward may fill in the spaces.

A third hypothesis is that the photoreceptors do not die or undergo migration outward but that there is a broadening in the foveal curvature with age, similar to that shown previously with reflectometry.47 This is consistent with the differences between groups 1 and 3 in cone photopigment and macular pigment distributions. The punctate alterations in distribution are not readily explained by an overall broadening but could be explained by a local shape change. A broadening of the foveal curvature with aging is consistent with the cellular architecture's also changing, particularly the long, tightly packed foveal cones with their displaced axons and bipolar cells, resulting in a flattened fovea and a thinned photoreceptor layer.

Our data imply changes to vision in older people. First, if there is less cone photopigment in the central fovea, then correspondingly fewer quanta are caught there. In the dark, the central fovea may be less sensitive in older people than in younger ones. An older person may choose to look slightly to one side of the central fovea, similar to when using rods at night, to use a region of sensitivity more similar to that in the younger person. Spreading the remaining cones evenly serves to make the quantal catch uniform across locations, producing a uniform background against which objects could be discriminated, rather than obscured by scotoma boundaries. Second, reduced cone photopigment in the central fovea of the oldest subjects is also consistent with an array of cone photoreceptors that is less packed than in younger subjects. This implies decreased spatial resolution in older people. However, ocular media may limit resolution and visual acuity more than the redistribution of foveal cones. Third, small alterations in photopigment may correspond to discrete changes in sensitivity. The present equipment quantifies sensitivity for a wide variety of locations and experimental conditions,48,49 but fixation is too unstable for accurate localization of small defects.30 Threshold measures depend on many factors but not photopigment regeneration or concentration in young51 or older52 subjects.

A change in foveal architecture may be a part of the aging process in which the foveal structures are repositioned and the vitreous detaches from the fovea, so that a macular hole does not result. It is unlikely that the changes found were the result of significant reduction of the supporting retinal pigment epithelial cells. The central macula has a fairly constant density with aging,52 and we did not find localized subretinal defects colocated with most pigment alterations.

In conclusion, there were corresponding foveal alterations in distributions of the cone photopigments and the macular pigments that occurred to a greater extent in older people and women. The alterations were consistent with a change in foveal architecture that resulted in a thinned photoreceptor layer in the central fovea. These photoreceptor changes occurred without corresponding, localized changes to the retinal pigment epithelial cells.

References
25. Kilbride PE, Hutman LP, Fishman M, Read JS. Foveal cone pigment
22. Johnson LV, Hageman GS, Blanks JC. Interphotoreceptor matrix
18. Eisner AE, Burns SA, Webb RH. Mapping cone pigment optical
17. Eisner AE, Burns SA, Hughes GW, Webb RH. Reflectometry with a
19. Curcio CA, Millican CL, Allen KA, Kalina RE. Aging of the human
16. Eisner AE, Burns SA, Delori FC, Webb RH. Quantitative reflectometry
15. Curcio CA, Sloan KR, Kalina RE, Hendrickson AE. Human photo-
14. Farber DB, Flannery JG, Lolley RN, Bok D. Distribution patterns of
13. Eisner AE, Burns SA, Lobes LA Jr, Doft BH. Cone photopigment abnor-
12. Burns SA, Eisner AE, Lobes LA Jr, Doft BH. Cone photopigment abnor-
11. Farber DB, Flannery JG, Lolley RN, Bok D. Distribution patterns of
10. Curcio CA, Sloan KR, Kalina RE, Hendrickson AE. Human photo-
9. Panda-Jonas S, Jonas JB, Jakobczyk-Mija M. Retinal cone topogra-
7. Marcos S, Navarro R. Determination of the foveal cone spacing by
6. Oesterberg G. Topography of the layer of rods and cones in the

25. Kilbride PE, Hutman LP, Fishman M, Read JS. Foveal cone pigment
23. Oesterberg G. Topography of the layer of rods and cones in the
22. Johnson LV, Hageman GS, Blanks JC. Interphotoreceptor matrix
7. Marcos S, Navarro R. Determination of the foveal cone spacing by
6. Oesterberg G. Topography of the layer of rods and cones in the