Retinal Pigment Epithelial Lipofuscin and Melanin and Choroidal Melanin in Human Eyes

John J. Weirer,*† Francois C. Delori,*† Glenn L. Wing,† and Karintra A. Fitch*

Optical measurements of the pigments of the retinal pigment epithelium (RPE) and choroid were made on 38 human autopsy eyes of both blacks and whites, varying in age between 2 wk and 90 yr old. Lipofuscin in melanin-bleached RPE was measured as fluorescence at 470 nm following excitation at 365 nm and was found to be proportional to fluorescence measured at 560 nm in unbleached tissue. Transmission measurements of RPE and choroidal melanin were converted and expressed as optical density units. The choroidal melanin content increased from the periphery to the posterior pole. RPE melanin concentration decreased from the periphery to the posterior pole with an increase in the macula. Conversely, the amount of RPE lipofuscin increased from the periphery to the posterior pole with a consistent dip at the fovea. There was an inverse relationship between RPE lipofuscin concentration and RPE melanin concentration. The RPE melanin content was similar between whites and blacks. Lipofuscin concentration was significantly greater \( (P = 0.002) \) in the RPE of whites compared to blacks; whereas blacks had a significantly greater \( (P = 0.005) \) choroidal melanin content than whites. The amounts of both choroidal and RPE melanin showed a trend of decreasing content with aging, whereas the amount RPE lipofuscin tended to increase \( (\text{whites} > \text{blacks}) \). Per fundus area, the amount of choroidal melanin was always greater than that in the RPE. There was a statistically significant \( (P = 0.001) \) increase in RPE height with age, most marked in eyes of whites after age 50 and correlated with the increase in lipofuscin concentration. Invest Ophthalmol Vis Sci 27:145–152, 1986

The human retinal pigment epithelium (RPE) contains two classes of pigment, melanin and lipofuscin, that are quite different in terms of development and function.1 The RPE melanosomes are synthesized in utero and remain virtually unchanged thereafter. Rarely, premelanosomes are found in normal retina, suggesting a possible slow turnover of melanosomes in mature RPE, although not to a significant degree.2 Mature melanosomes appear to be degraded by incorporation within lysosomes to form melanolysosomes and melanolipofuscin; the process, however, is slow and incomplete because of the inherent stability of the melanin granules.3

The exact biologic role of melanin beyond light absorption is still controversial, but several important properties include scavaging free radicals and excited molecules, electron transfer, and affinity for drugs and metals.4 In contrast to melanin, lipofuscin in the RPE develops after birth and continues to increase with age.5

Lipofuscin is thought to represent lysosomal residual bodies containing end-products of oxidative damage to lipids6 and in the RPE is related to photoreceptor phagocytosis and digestion. Light damage mediated through photosensitized oxidations has been postulated to result in lipid peroxides7 and probably in the accumulation of RPE lipofuscin.8 This suggests that there could possibly be a relation between melanin concentration and lipofuscin accumulation in the RPE.

We have previously reported preliminary studies on the intracellular and topographical distribution of melanin and lipofuscin in Caucasian RPE as a function of age.9 An inverse relationship between melanin and lipofuscin was found in human RPE. The present study extends that work to include blacks, as well as choroidal melanin measurements in both whites and blacks.

Materials and Methods

Subjects

Optical measurements of melanin and lipofuscin concentration were made on 38 eyes that had no known ocular pathology. The donors were 19 whites from 2 wk old to 88 yr old (average age of 42) and 16 American blacks from 6.5 yr to 90 yr (average age of 52). Measurements on both eyes in three subjects were averaged and counted as one. All eyes were obtained less than 12 hr after death. Eyes were fixed in formalin and pro-

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cessed through ethanol dehydration to xylene to paraffin embedding. Pupil–optic nerve sections, 8 μm thick, were cut through the fovea, deparaffinized, and mounted unstained on slides in ultra-violet-inert immersion oil.

Measurement Sites

To obtain general topographic information on melanin and lipofuscin distribution, measurements were first made at regular spacing between the fovea and the periphery on several eyes. From these results, five sites per eye were selected for RPE measurements on all eyes: the two equators, the fovea, and the two parafoveal sites. The latter are located at half the distance from fovea to disc, on either side of the fovea. The parafoveal and equatorial measurements were averaged. Three sites were selected for choroidal melanin measurement: the two equators and the fovea. The two equators were averaged. The far periphery was not included in this study because of the marked variability of the RPE. The far peripheral retina frequently had changes such as pavingstone degeneration that would have invalidated the study.

RPE Melanin

Measurements of melanin concentration in the RPE were made using a Zeiss Photomicroscope III (Carl Zeiss, Inc.; Oberlochen, West Germany) with microspectrophotometric capabilities. Transmission measurements of the 8-μm thick sections were made at 800X magnification using a rectangular aperture of 30 × 3 microns. The effective spectral range of the measuring light, determined by the tungsten lamp of the microscope and detection sensitivity of the PMT, was 500–600 nm. The system was calibrated to 100% transmission at a location adjacent to the section. Transmission measurements were made by aligning the sampling area to the basal and apical side of the RPE cell. These measurements were repeated at each site for five adjacent areas and averaged to reduce variability. The apical and basal transmissions (T) were converted in extinction coefficient of melanin by (−½) × log10 T, and expressed in density units per micron. This extinction coefficient is proportional to the melanin concentration, and is used here as the relative melanin concentration expressed in density units per micron. RPE heights at each site were also measured. Total optical density of the RPE at each site for light traveling in the apical–basal direction was calculated as the product of the RPE height and the mean of the apical and basal extinction coefficients.

RPE Lipofuscin

Lipofuscin concentrations were measured by fluorescence measurement on the same sections and at the same sites as for melanin measurement. However, the melanin was first bleached using potassium permanganate and oxalic acid before fluorescence measurement. Transmission measurements of bleached RPE showed no residual absorption by melanin, ensuring thereby that fluorescence from lipofuscin is not affected by melanin absorption.

Lipofuscin fluorescence measurements were made using as excitation the HBO-100 high-pressure mercury lamp of the Zeiss Photometer in conjunction with a 365-nm interference filter (bandwidth 10 nm). The excitation beam was restricted to a 30-μm circular area on the specimen. The emitted fluorescence of lipofuscin was detected through a narrow band filter centered at 470 nm and sampled using the same 30 × 3-μm rectangular aperture.

At each use, fluorescence measurements were calibrated using a single specific ZnS–CdS grain, to adjust the fluorescence reading to 100 units. As for melanin, fluorescence measurements were made in 5 neighboring apical and basal areas at each site and the results averaged to yield the relative concentration of lipofuscin. Total lipofuscin content was calculated as the product of RPE cell height and the average of apical and basal fluorescence intensities.

Reproducibility of the lipofuscin fluorescence measurements was found to be ±5% for the same spot measured over months on a melanin-bleached specimen from an 88-yr-old white male. Repeated measurements of individual specimens showed that 30 readings of 0.1 sec did not decrease fluorescence at any spot, so 5 readings were taken at each spot and averaged to give the fluorescence intensity at that spot.

The excitation and emission bands used in this and a previous study were selected to match the excitation and emission maxima reported by Chio, and also because, in our spectrophotometric system, this combination yielded the most efficient fluorescence detection in melanin-bleached tissue. Our use of 470 nm as the most efficient wavelength results from the combined effects of the emission spectra of melanin-bleached lipofuscin and the spectral sensitivity characteristics of our monochromator and photomultiplier (S–5 photocathode, detection sensitivity of the system at 470 nm is 1.2 times that at 560 nm). It should be noted that Eldred, using appropriate spectrophotometric corrections, showed that lipofuscin fluoresces between 440 nm, and 700 nm, with a broad and flat maximum between 540 nm and 640 nm.

As a check on the validity of measuring lipofuscin fluorescence at 470 nm, we compared fluorescence measured at 470 nm and at a wavelength within the broad emission peak of lipofuscin, eg 560 nm, on 5 eyes (20 sites) of unbleached tissue. We found that fluorescence measured at 470 nm was proportional to
that measured at 560 nm (linear regression $r^2 = 0.84$, $P < 0.0001$ and intercept not significantly different from zero). Similar results were found by comparing the two wavelengths on melanin-bleached tissues ($P < 0.007$). We also compared fluorescence at 470 nm using melanin-bleached tissue vs fluorescence at 560 nm using unbleached tissue. In 20 eyes, using young and old eyes of both races, there was a positive correlation between the fluorescence measured at 470 nm on melanin-bleached tissue and fluorescence on an adjacent unbleached tissue section at 560 nm ($r^2 = 0.5$, $P < 0.0001$).

**Choroidal Melanin**

Choroidal melanin concentrations were measured on an adjacent section to that used for RPE optical measurements. Transmission measurements in the same manner as for RPE melanin were made. However, the sampling aperture was 300 μm long and had a height $H$, adjusted to cover half of the choroidal thickness. Since the choroid in vivo is a vascular compartment, with variable degrees of extracellular fluid, it is not possible from histological sections to determine choroidal volume, and hence choroidal melanin concentrations. Rather, transmission measurements were converted in optical density by $(- \frac{H}{8}) \times \log_{10} T$ for both the inner choroid (RPE side) and the outer choroid (scleral side). Total optical density of the choroid was obtained by adding inner and outer choroid densities.

**Morphometric Studies**

Ocular sections were studied at 2,000× magnification under oil immersion to corroborate the optical measurements. At this magnification, discrete lipofuscin granules can be counted, and melanosomes differentiated from melanolipofuscin. On representative RPE sections, the optical measurements of lipofuscin fluorescence and melanin density correlated with lipofuscin and melanosome granule counts ($P < 0.001$). This positive correlation was verified in various regions of the eye in young and old patients in both blacks and whites.

**Results**

**Topographical Pigment Distribution**

The topographical distribution of the pigments of the retinal pigment epithelium and choroid in human postmortem eyes demonstrated a consistent pattern as typified by Figure 1. As previously shown,5,12 RPE lipofuscin content increases from the equator to the posterior pole, with a consistent dip in RPE lipofuscin content in the macula. In contrast, the RPE melanin content decreased from the equator to the posterior pole, with a consistent peak of RPE melanin content at the macula. Choroidal melanin content increases gradually from the equator to the posterior pole, with the highest degree of choroidal pigmentation being in the submacular choroid.

**RPE Melanin and Lipofuscin Concentration**

The results of the RPE melanin and lipofuscin concentration measurements are summarized in Table 1. Melanin and lipofuscin are distributed differently within the RPE cell: melanin has a higher concentration in the apical aspect of the cell, whereas lipofuscin is more concentrated basally. This polarity of the lipofuscin and melanin intracellular distributions was more pronounced in younger eyes than older eyes.
Table 1. Relative lipofuscin and melanin concentration in the RPE

<table>
<thead>
<tr>
<th>Site</th>
<th>Whites</th>
<th>Blacks</th>
<th>Racial difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N = 19</td>
<td>N = 16</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lipofuscin*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macula</td>
<td>44 ± 16</td>
<td>35 ± 12</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Apical</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>57 ± 24</td>
<td>41 ± 14</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Paramacula</td>
<td>54 ± 19</td>
<td>50 ± 13</td>
<td>NS</td>
</tr>
<tr>
<td>Apical</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>69 ± 28</td>
<td>64 ± 19</td>
<td>NS</td>
</tr>
<tr>
<td>Equator</td>
<td>41 ± 19</td>
<td>26 ± 10</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>Apical</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>52 ± 20</td>
<td>42 ± 13</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Melanin†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macula</td>
<td>31 ± 13</td>
<td>30 ± 10</td>
<td>NS</td>
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<tr>
<td>Basal</td>
<td>24 ± 14</td>
<td>26 ± 12</td>
<td>NS</td>
</tr>
<tr>
<td>Paramacula</td>
<td>22 ± 8</td>
<td>19 ± 6</td>
<td>NS</td>
</tr>
<tr>
<td>Apical</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Basal</td>
<td>17 ± 10</td>
<td>15 ± 7</td>
<td>NS</td>
</tr>
<tr>
<td>Equator</td>
<td>45 ± 18</td>
<td>45 ± 15</td>
<td>NS</td>
</tr>
<tr>
<td>Apical</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>32 ± 17</td>
<td>29 ± 12</td>
<td>NS</td>
</tr>
<tr>
<td>Cell height‡</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Macula</td>
<td>10.3 ± 2.8</td>
<td>10.2 ± 1.3</td>
<td>NS</td>
</tr>
<tr>
<td>Paramacula</td>
<td>8.8 ± 2.1</td>
<td>9.2 ± 1.5</td>
<td>NS</td>
</tr>
<tr>
<td>Equator</td>
<td>7.0 ± 1.8</td>
<td>6.5 ± 1.0</td>
<td>NS</td>
</tr>
</tbody>
</table>

* Lipofuscin in arbitrary fluorescence intensity units/micron.
† Melanin in milli-density units/micron.
‡ Cell height in microns.

Lipofuscin concentration was greater, on average, at all sites measured, in whites than in blacks. Because of the large variability though, this lipofuscin difference was statistically significant at the P < 0.05 level only at the macular and equatorial regions. When apical and basal concentration measurements at all sites were grouped, then a significance level of 0.002 was reached for the difference in RPE lipofuscin concentration between whites and blacks.

As expected, lipofuscin increases with age. Linear correlation of lipofuscin concentration with age for the different sites showed significant increase in whites (r² = +0.45 to +0.69, P = 0.0001) but only a trend to increase for blacks. The rate of change is greatest in the first two decades of life and shows only moderate increase in the third through fifth decades. After age 50, a more rapid increase in lipofuscin was again noted, much more marked in whites than blacks. This age-related increase in RPE lipofuscin is not linear but shows a sigmoidal pattern as our laboratory has previously reported.5

The RPE melanin concentration at all sites was on average the same in blacks and in whites (Table 1). RPE melanin concentrations showed a trend to decrease with age in whites and blacks. This decrease was most pronounced in whites after age 50.

It is to be stressed that our optical measurements do not differentiate between melanin and melanolipofuscin (complex) granules. It is very obvious, when viewed at 2,000× magnification, that by the age of 50 most of the melanin is incorporated into melanolipofuscin granules. This may account for some loss of cellular

Fig. 2. RPE lipofuscin concentration expressed as a function of RPE melanin concentration, measured apically or basally at the same sites. Included are both blacks and whites.
melanin and lipofuscin distribution polarity mentioned above.

RPE cells were taller in the macular region and diminished in height as one progressed from the macula to the equator (Table 1). There was a statistically significant increase in RPE height with age at the different sites (linear correlation coefficient \( r^2 = +0.28 \) to \( +0.50, P = 0.001 \)), most marked in white eyes after age 50. RPE cell height correlated significantly with RPE lipofuscin concentration \( (r^2 = +0.34, P < 0.0001 \) for all sites).

**RPE Lipofuscin and Melanin Correlation**

The relationship between lipofuscin and melanin apical and basal concentrations is shown in Figure 2 for all sites in all eyes measured in this study. An inverse relationship exists for RPE melanin and lipofuscin. Linear correlation of both concentrations was significant \( (r^2 = +0.23, P = 0.0001 \) for all sites). It is to be noted that this inverse relationship holds both when the RPE pigments are compared on a site basis (Fig. 2 and Table 1) and on a topographical basis (Fig. 1).

**Choroidal Melanin Content**

Regional choroidal melanin content is shown in Table 2. Blacks have a significantly greater total choroidal melanin content than whites \( (P = 0.005 \). Choroidal melanin content is on average 2.35 ± 0.97 times higher in the outer choroid half (adjacent to the sclera) than in the inner choroid half (adjacent to the RPE). No statistically significant change in total choroidal melanin density with age was found, although there is a trend for the ratio of outer/inner choroidal melanin density to increase with age.

**Total Optical Density of Melanin**

Since the melanin of the RPE and choroid are superimposed upon each other in vivo when exposed to light, it is useful to know their optical density in relation to each other. Table 3 gives, for black and white subjects separately, the average values of the total optical density of melanin in both the RPE and choroid. For each eye, the optical density of RPE melanin is obtained by averaging apical and basal melanin concentrations and multiplying by cell height. The choroidal values are obtained by summing the outer and inner optical density values.

**Selected Disease States**

The present study excluded any eyes with known ocular pathology. In order to explore further the significance of lipofuscin accumulation, several diseased eyes were studied. Figure 3 shows the topographical distribution of lipofuscin in a 70-yr-old white man with central areolar atrophy, an entity generally considered to be a variant of senile macular degeneration (SMD). The lipofuscin concentration at any site is several times higher than normal for this age and is higher than ever found in a normal eye. The RPE cells at the posterior pole have already degenerated. The remaining RPE cells were swollen and contained diminished RPE melanin. A second eye with senile macular degeneration, but with intact RPE in the posterior pole was also found to have increased lipofuscin but not as marked as in Figure 3.

Eyes from two individuals who died in their third and fourth decades from neoplastic disease were also studied. Both individuals received extensive antimetabolite therapy, and in both cases the RPE lipofuscin concentration was approximately twice normal.

**Discussion**

This study used optical methods to measure the pigments of the RPE and choroid in human autopsy eyes. Several limitations are inherent in the techniques utilized. The major problem is ensuring that fluorescence measurements at 470 nm following melanin bleaching is representative of lipofuscin. When viewed with fluorescent microscopy, lipofuscin appears golden-yellow, consistent with an emission peak in the 540 to 640 nm

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### Table 2. Choroidal melanin content (density units)

<table>
<thead>
<tr>
<th>Site</th>
<th>Whites Mean ± SD</th>
<th>Blacks Mean ± SD</th>
<th>Racial difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macula</td>
<td>0.24 ± 0.04</td>
<td>0.55 ± 0.34</td>
<td>( P = 0.003 )</td>
</tr>
<tr>
<td>Equator</td>
<td>0.86 ± 0.3</td>
<td>0.93 ± 0.44</td>
<td>( P = 0.001 )</td>
</tr>
</tbody>
</table>

### Table 3. Total optical density of melanin (density units)

<table>
<thead>
<tr>
<th>Site</th>
<th>Whites Mean ± SD</th>
<th>Blacks Mean ± SD</th>
<th>Racial difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macula</td>
<td>0.29 ± 0.11</td>
<td>0.29 ± 0.10</td>
<td>NS</td>
</tr>
<tr>
<td>Paramacula</td>
<td>0.17 ± 0.05</td>
<td>0.16 ± 0.06</td>
<td>NS</td>
</tr>
<tr>
<td>Equator</td>
<td>0.27 ± 0.10</td>
<td>0.24 ± 0.11</td>
<td>NS</td>
</tr>
</tbody>
</table>
range. Our measurements were performed at 470 nm in order to be consistent with our previous study. Furthermore, 470 nm was considered the emission peak for extracted lipofuscin. Recently, though, the 470-nm emission maximum of extracts of lipofuscin-ladden cells was shown possibly to result from instrumental bias. In order to link our histologic measurements made at 470 nm to those at 560 nm, we compared values of 5 eyes at both wavelengths and found a positive correlation ($r^2 = 0.84, P = 0.0001$). Also, to determine the effects of melanin bleaching on the tissue we compared fluorescence measurements at 470 nm using melanin bleached tissue with fluorescence measured at 560 nm on unbleached tissue and found a positive correlation ($r^2 = 0.5, P < 0.001$).

The fluorescence measurements were calibrated using a single specific ZnS–CdS grain, to adjust the fluorescence reading to 100 units. Lipofuscin fluorescence measurements were made relative to this standard, with the assumption that lipofuscin correlates linearly with fluorescence. Internal quenching most likely causes deviation from linearity. Furthermore, the assumption was made that all fluorescence was derived from lipofuscin. Using fluorescence microscopy at 2000× magnification, all of the fluorescence appeared to be derived from rather uniform lipofuscin granules. Since the photoreceptors are not fluorescent under these conditions, phagosomes are probably not being included in the measurements, but this can not be proven. The fluorescent granules in infant eyes are not only smaller than those found in adult eyes, but also have a light green autofluorescence in contrast to the golden yellow fluorescence of adult eyes. Feeney suggests that these smaller fluorescent bodies could represent secondary rather than tertiary lysosomes (lipofuscin). What the contribution of secondary lysosomes are to our fluorescent measurements is not known. Finally, the optical methods we used for measuring melanin in the RPE can not differentiate melanosomes from complex granules (melanolysosomes and melanolipofuscin). With fluorescence microscopy, the melanolipofuscin granules are easily identified, having a fluorescent rim and a nonfluorescent melanin core. Although not specifically quantified, the melanosomes were noted to decrease with age and the melanolipofuscin granules increased, such that by age 50 melanolipofuscin granules typically outnumbered melanosomes.

This study verified our previous study and those of others, showing an increase in the lipofuscin content with age. As noted previously, the increase in lipofuscin with age is not linear but shows a sigmoidal pattern. The increase is greatest in the first two decades of life and shows only moderate increase in the third through sixth decades. Beginning with the sixth decade a more rapid increase in lipofuscin was again noted, much more pronounced in whites than blacks. This rapid accumulation of lipofuscin in the first two decades of life is related to the increased exposure of the retina to shorter UV wavelengths (295 to 400 nm), since the crystalline lens develops a yellow pigment that absorbs the shorter wavelengths on a time scale that matches the RPE lipofuscin buildup.

RPE melanin decreases from the equator to the posterior pole, as noted by others, with an increase in the macular region. Interestingly, the topographical distribution of melanin is the inverse of the topographical distribution of lipofuscin (Fig. 1). Indeed, if the concentration of lipofuscin to melanin is compared at each site measured (Fig. 2), this same inverse relation holds. It is tempting to relate the increase in lipofuscin in whites during the sixth decade to the decrease in RPE melanin noted at this time.

Although there is a large variability of melanin from site to site in the RPE and from individual to individual, on average the RPE melanin content was the same in both blacks and whites (Tables 1 and 3). On the other hand, choroidal melanin, on average, was approximately twice as large in blacks as in whites (Tables 2 and 3). This is consistent with a previous study of ocular melanin. These tissue differences in melanin reflect the fact that melanin is derived from two distinct regions embryologically, the neural crest and neuroepithelial cells. The neurocrest is the origin for melanocytes that migrate and provide pigment for such sites as skin, hair, and uvea. Such pigmentation shows marked racial variability. Since the neuroepithelium provides melanin for pigmented structures of the cen-

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**Fig. 3.** Topographical distribution of RPE lipofuscin concentration in a 70-yr-old white man with central areolar atrophy. Note that lipofuscin fluorescence is absent centrally because of atrophy of the RPE. Compare the RPE lipofuscin concentration in this diseased eye with lipofuscin concentration of a normal eye as shown in Figure 1.
nal nervous system such as the ganglion substantia nigra, it is not surprising that the RPE, which is derived from neuroepithelium, does not show racial differences in pigmentation.

The inverse relation between RPE melanin and lipofuscin is intriguing and may suggest a protective mechanism in the formation of lipofuscin. Lipofuscin formation is a complex process, most likely involving lipid peroxidation.2,20 Many factors such as light, oxygen, and nutrients probably play a role. Melanin could provide photoprotection not only by direct absorption of light but also by serving as a scavenger of light-induced free radicals.3 It is unlikely, though, that this scavenger effect is the sole mechanism, since whites have a much greater RPE lipofuscin content in spite of the fact that both whites and blacks have an equal content of RPE melanin.

Light damage would be related to the total accumulated light exposure during one's life. The difference in RPE lipofuscin between whites and blacks is probably related to the differences in their choroidal pigmentation. A photon of light that was not absorbed by the photoreceptor-RPE complex would have a greater possibility of being reflected by the deeper layers of the fundus and having a second pass through the photoreceptors in whites than in blacks. Indeed, fundus reflectance (white light) in whites is approximately 5% versus 1% in blacks.19 Thus, the ratio of total photoreceptor light exposure between whites and blacks will be 105/101. This ratio would be increased by the additional contributions from intraocular light scatter and light penetrance through the iris and sclera. Whether this small light exposure ratio can account for differences in RPE lipofuscin between blacks and whites is an unanswered question. Indeed, we currently believe that the dip in lipofuscin noted at the macula reflects the photoprotection afforded by not only the increased melanin in the RPE and choroid in the macula (Fig. 1) but also by the neurosensory macular pigment.21,22 (As we previously discussed,3 though, differences in phagosome production in the RPE of rod-rich vs cone-rich areas may contribute to this dip and cannot be discounted.) Furthermore, fundus pigmentation was also found to be an important parameter in the incidence of senile macular degeneration in a study of a Caucasian population.23,24

Finally, the question of whether lipofuscin accumulation is toxic to the RPE has not been decisively answered, although inferential evidence strongly suggests that it is.20 If lipofuscin is formed within lysosomes after phagic sequestration of damaged or aged membranes and organelles and represents undigestible residue, then lipofuscin is analogous to the accumulation of undigestible residues in lysosomal storage diseases, since in both cases the appropriate catabolic enzymes are missing for complete degradation.25 Feeney-Burns15 has shown that the percent of cytoplasmic space decreases with increasing lipofuscin. We have shown that RPE cell height increases with age and is correlated with the increase in RPE lipofuscin concentration. Similar findings have been noted by others in the rat RPE.26 We hypothesize that, just as in lysosomal storage disease, the accumulation of undigestible residue (lipofuscin) leads to compromise of cellular function. Supportive evidence comes from examination of diseased eyes in which lipofuscin accumulation is excessive. Figure 3 demonstrates the topographical lipofuscin profile in an eye with a variant of senile macular degeneration. It is very tempting to speculate that these RPE cells became so engorged with lipofuscin that they "ruptured" or degenerated from cytoplasmic crowding. Notwithstanding these speculations, the mechanism of lipofuscin buildup and the role of melanin in possible photoprotection remain complex and not well understood.

Key words: lipofuscin, melanin, retinal pigment epithelium, choroid, aging, pigments

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