Color Matching in Diabetes: Optical Density of the Crystalline Lens and Macular Pigments

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Purpose. To measure the optical density of the crystalline lens and macular pigments in a group of patients with diabetes mellitus and compare the results with those in a group of control subjects.

Methods. Color matches were performed using a Wright tristimulus colorimeter. The reference wavelength used was 490 nm, desaturated with 650 nm. Lens optical density was measured by mixing spectral primaries of wavelengths 420, 515, and 650 nm to match the reference. Wavelengths 420 and 515 nm were chosen, because they are absorbed equally by the macular pigment. To measure macular pigment density, two color matches were performed, one foveal and one 5° extrafoveal. The reference stimulus was matched by mixing spectral primaries of 460, 530, and 650 nm. The ratio of the foveal to extrafoveal color match gives the optical density of the macular pigment. Thirty-four diabetic patients and 34 control subjects performed the lens density color match, and of these, 26 diabetic patients and 30 control subjects performed the macular pigment density color matches.

Results. There is a significant increase in the optical density of the lens in diabetes with age in comparison to the control subjects ($P < 0.001$), with a duration dependence of 0.02 log units/year. The mean macular pigment density in the diabetic patients was 0.13 ± 0.20 log units and in the control subjects 0.32 ± 0.24 log units ($P = 0.0015$). Patients with grade 2 maculopathy had significantly lower pigment density than those with no maculopathy ($P = 0.016$).

Conclusions. The ocular media of diabetic persons are abnormal, with increased lens and reduced macular pigment optical density. The relationship between reduced macular pigment levels with increasing severity of maculopathy may implicate oxidative stress as a causative factor. (*Invest Ophthalmol Vis Sci.* 2002;43:281–289)

The absorption spectrum of the human crystalline lens has been studied in vivo and after death.[1–5] There is a degree of interindividual variation among people of the same age and an increase in the relative absorption of short wavelengths with age.[3] The optical changes that occur in the diabetic lens before the onset of cataract have been studied previously, by using lens autofluorescence[6–10] and psychophysical thresholds.[11] Autofluorescence of the lens can be induced by illumination with 420-nm light—the autofluorescence being at 530 nm. The degree of autofluorescence is calculated from digitized images and the result expressed in arbitrary units of pixel gray scale difference[8] or equivalent fluorescein concentration.[9] These studies show a significant increase in the autofluorescence of the diabetic lens compared with that of age-matched normal lens. Autofluorescence is further increased in diabetic persons with nephropathy compared with those without.[7]

There is only one published study in which a psychophysical method was used to assess relative light loss in the ocular media in diabetic persons.[11] Absolute thresholds to flashes of 420- and 550-nm light presented at 15° eccentricity were determined after 40 minutes of dark adaptation. These wavelengths were chosen to have an equal absorption by rhodopsin. The difference between the thresholds gives the relative light loss in the ocular media in units of optical density. The normal group showed a small increase with age less than 60 years, increasing thereafter in agreement with the model developed by Pokorny and Smith.[5] The diabetic group had an accelerated increase in differential lens absorption that was parallel to the increase in the normal group after the age of 60 years. The data were analyzed to determine a separate effect of disease duration on the diabetic lens, giving a gradient of 0.018 log units/year. Moreland used the data to derive equations to estimate the age of lens-matched normal persons.[12] These have been used to estimate the contribution of the lens to the results of psychophysical tests.[13 14]

Lutein and zeaxanthin are concentrated in the photoreceptor axon layer and the inner plexiform layer of the macula,[15,16] forming a prereceptoral optical filter.[17,18] The absorption spectra of these pigments have been well characterized, peaking at 460 nm.[18] Interobserver optical density of the macular pigment can vary over 1 log unit, and this variation causes differences in color perception.[15,19,20] Some studies have shown that macular pigment density appears to be age-independent in normal eyes[21–24] but a small age-dependent decline has been reported recently.[25,26]

Initially the macular pigment was thought to have an optical function in the reduction of chromatic aberration.[27] More recently, a potentially more important role as an antioxidant has been described[28]—the carotenoids protecting the macula from short wavelength light or against light-induced oxidative damage. This has been of particular interest in age-related macular degeneration,[29] where study has shown an association between high levels/intake of antioxidants and reduced risk of development of the disease.[30]

In this study, color matching was used to investigate the differential optical density of both the crystalline lens and macular pigments in a group of patients with diabetes mellitus, and the results were compared with those of a group of normal volunteers. The color of two adjacent fields of view appears the same if the underlying cone excitations are the same, even if the spectral distribution of the light in the adjacent fields is different. Color matching is insensitive to the absolute numbers of receptors stimulated,[31] unlike flicker photometry,[32] but is sensitive to the absorption spectra of the cones and of the ocular media. The cone excitation ratio to monochromatic light is, however, independent of absorption in the ocular

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media, because light incident on each cone type has been affected equally by prereceptoral filtering. A recent study of the effects of laser photocoagulation in diabetic persons showed complete loss of photoreceptors in treated areas, but receptor morphology appeared normal in nonphotocoagulated areas,[33] an important prerequisite for the comparison of color match results to assess the ocular media of diabetic persons and control subjects.

Patients and Methods

Patients

All patients gave written consent to be involved in the study. The tenets of the Declaration of Helsinki were observed, and the study had the approval of the Research and Ethics Committee of St. Mary's Hospital, Imperial College of Science, Technology and Medicine, United Kingdom. Thirty-four diabetic persons and 34 control subjects participated in the study to measure the lens optical density, and of these, 26 diabetic persons and 30 control subjects performed further color matches to assess the optical density of the macular pigments. The age range of the patients was 25 to 72 years, and the duration of disease ranged from 2 to 38 years (mean duration, 13.8 ± 10.3). The diabetic group consisted of 24 persons with type II and 10 with I diabetes. Twenty-two had had no laser treatment, four had undergone panretinal photocoagulation, five had had macular photocoagulation, and three had had both forms of treatment. Visual acuity was assessed using the Early-Treatment Diabetic Retinopathy Study (ETDRS) log minimum angle of resolution (logMAR) acuity chart; the mean acuity of the patient group was 0.04 ± 0.08 log units. None of the diabetic persons had cortical, posterior subcapsular, or significant nuclear cataract. The level of retinopathy was assessed using the modified Airlie House classification by dilated fundoscopy using a 90-D biomicroscope lens and slit lamp and a 60-D lens to grade maculopathy. The mean level of retinopathy was 3.0 ± 1.6 and the mean maculopathy grade was 0.63 ± 0.80.

The 26 patients performing the macular pigment color match consisted of 19 who had type II diabetes, and the remaining 7 had type I diabetes. Two of these patients had undergone laser treatment to the macula, two had received panretinal photocoagulation only, and two had undergone both macula laser and panretinal photocoagulation. The mean level of retinopathy was 2.8 ± 1.6 and the mean maculopathy grade was 0.54 ± 0.76. The mean logMAR visual acuity was 0.03 ± 0.08. The control group were selected on the basis of normal visual acuity and color vision, the age range being 20 to 67 years. Previous studies have shown an association of macular pigment density with iris color,[34] smoking,[35] and gender.[36] Comparison of these parameters across the two groups showed no statistical differences ($P = 0.59$ for smoking, $P = 0.75$ for iris color, and $P = 0.46$ for sex distribution).

Methods

All color matches were performed using a tristimulus colorimeter that produces a 1°, 20-minute bipartite field.[37] The lower hemifield consisted of a reference wavelength and a desaturation wavelength, and the upper hemifield contained two matching wavelengths (different for the two experiments) and the desaturation wavelength. When the relative radiance of the wavelengths in the upper hemifield are adjusted such that the color and brightness of the two fields appears the same, the cone excitations in the two retinal areas illuminated are the same. For the reference wavelength, the ratio of cone excitation is independent of prereceptoral filtering. The optical density of the crystalline lens can be estimated using matching wavelengths that are equally absorbed by the macular pigments,[21] and the macular pigment density can be derived from the ratio of two color matches, one foveal and the other extrafoveal, made with matching wavelengths that are absorbed by the pigment.[19] Color matching has been used to derive a spectral absorption curve for the macular pigment[19] that is in agreement with the spectral absorption characteristics found using other methods.[18,38] Color matches performed using different field sizes have been used to generate a spatial profile, showing an approximately exponential decay of pigment density with eccentricity.[39]

Throughout the study, each color match was performed 10 times by each observer to allow an estimate of the experimental error. Steady head position was maintained using a dental bite bar, and each subject was dark adapted for 10 minutes to allow cone adaptation.

The subjects underwent a period of training on the colorimeter, performing the Rayleigh match, in which primaries of 530 and 650 nm were matched to a reference wavelength of 590 nm. The color match to assess lens optical density consisted of the reference wavelength 490 nm, desaturated with 650 nm, matched by mixing spectral primaries of 420, 515, and 650 nm. Wavelengths of 420 and 515 nm for the lens density measure were chosen after a series of control experiments in which different wavelengths were used on a small group of young control subjects.[40] Comparison of postmortem transmission between lens alone and whole media shows very little difference at the wavelengths used.[3] The cornea absorbs mainly in the ultraviolet,[41] the absorption being age independent[42]; the aqueous and vitreous can be considered as having the absorption spectrum of water.[43] The value in the color match gives a measure of light loss in the lens by both absorption and scattering. Any forward-scattered light that uniformly illuminates the bipartite fields cancels at the point of color match, and scattering of light in the eye is largely wavelength independent in normal eyes.[44] In this study we assumed that the effects of absorption dominate the measurement. The relative optical density for the lens is obtained by

$$d_{420} - d_{515} = \log E_{420} - \log E_{515} - \log K \quad (1)$$

where E is the radiance of the matching wavelength, K is a constant that depends on the short- (S) and medium- (M) wavelength cone absorption spectra and the optical density (d) is determined by $d = -\log T$, where T is the transmission of the ocular media at the wavelengths used.

To obtain the macular pigment density, each subject performed two color matches: The first was performed with foveal fixation, and the second 5° extrafoveally. The matching wavelengths used were 460, 530, and 650 nm. The reference stimulus (lower hemifield) was identical with that used in the lens optical density experiment. Eccentric presentation was achieved using a small (<0.1°) red fixation marker. To avoid Troxler's phenomenon, a rotating sector disc was used to introduce a slow flicker in the bipartite field (5 Hz). Of the matching wavelengths, 460-nm light is strongly absorbed by the macular pigment, whereas the absorption of 530 nm is slight. The decrease in macular pigment density with increasing eccentricity has been assessed, and by 5° eccentricity the pigment density is approximately 5% of its peak value.[23,39,45] Another advantage of a 5° extrafoveal match is that the light path is not significantly longer than for foveal fixation, and consequently there is no confounding factor of different path lengths through the remaining ocular media. The macular pigment density is calculated from the ratio of the foveal to extrafoveal color-match ratios as

$$d_{460} - d_{530} = \log\left(\frac{E_{460}}{E_{530}}\right) - \log\left(\frac{E'_{460}}{E'_{530}}\right) \quad (2)$$

where E is the matching radiance for foveal fixation, E' is the matching radiance for the extrafoveal match, and d is the optical density. Results are expressed as mean ± SD.

Results

The mean age of the diabetic group was 48.1 ± 11.6 years and of the control group, 36.7 ± 15.1 years ($P = 0.001$). Given the known increase of lens optical density with age[3,4,11] and the suggested decrease of macular pigment optical density with

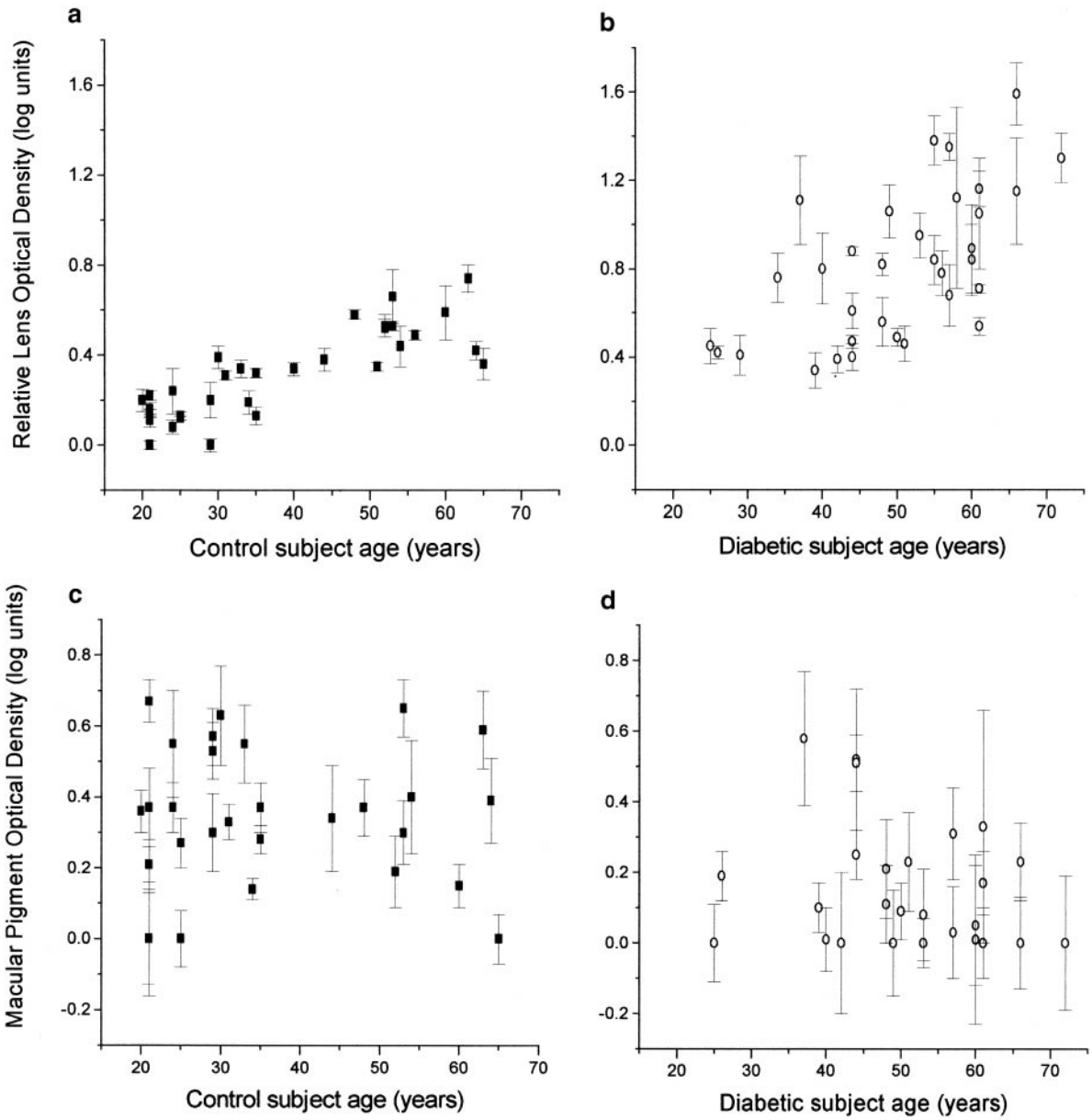

FIGURE 1. Differential optical density of the lens in the control (**a**) and the diabetic (**b**) groups. Macular pigment optical density in the control (**c**) and diabetic (**d**) groups.

age,[25,26] a comparison of means of these measures between the two groups would not be appropriate. Excluding control subjects less than 30 years of age provided an age-similar control group of 16 subjects of mean age 47.1 ± 12.6 years ($P = 0.38$ in comparison with the diabetic group).

For the purposes of establishing age-dependence relationships in the data, all control subjects were used, but for comparison of means with the diabetic persons, the age-similar control group was used.

The results for the Rayleigh color match showed no age dependence in control subjects or diabetic persons and no statistically significant difference between the diabetic persons in comparison with the age-similar control group or the whole control group. The mean color-match ratio for the control group was −0.26 ± 0.06 log units and for the diabetic group, −0.24 ± 0.08 log units ($P = 0.17$). This finding is important for the interindividual comparison of results.

The optical density of the lens in the diabetic group was significantly greater than that of the age-similar control group ($P < 0.001$, Mann-Whitney test). The results for the lens optical density are plotted as a function of age in Figure 1a for the control group and in Figure 1b for the diabetic patients. There is a significant correlation with age in both groups, the rate of increase being 0.0085 log units/year ($R = 0.81$, $P < 0.0001$) in the control subjects and 0.017 log units/year ($R = 0.66$, $P < 0.001$) in the diabetic patients.

Multivariate analysis of the data for the diabetic patients shows significant correlation with subject age ($P = 0.0012$), but not with duration of disease ($P = 0.14$), level of retinopathy ($P = 0.56$), grade of maculopathy ($P = 0.13$), or previous laser treatment ($P = 0.61$). The diabetic group contained a small subgroup of four patients of the same age (44 years). On the assumption that these individuals have similar lens optical densities owing to their age, linear regression gives a duration dependence of 0.021 log units/year of diabetes ($R = 0.86$). The duration dependence was also estimated for the 10 patients with type I diabetes in the study, because the age of onset was known. The lens density at the onset of diabetes was estimated

from the line of best fit to the normal subjects. The gradient of increase from this value to the present measurement was calculated, and the gradient of the line of best fit to the normal subjects was then subtracted, providing an estimate of the duration effect. The mean value obtained for the 10 patients was 0.019 ± 0.009 log units/year.

An equation for the estimation of the age of a lens-matched normal subject was also derived (see Appendix), which yields a linear relationship of

$$A_n = A_d + 2.364D \quad (3)$$

where A_n is the age of a lens-matched normal subject, with the restriction A_n less than 60 years, A_d is the age of the diabetic subject, and D is duration of diabetes.

Six diabetic patients and three control subjects had measured macular pigment densities of less than zero, although in all cases the experimental error included zero. The negative values represent sampling error around zero, and the macular pigment densities for these subjects were taken as zero. Analysis of the data with these points as zero, in comparison with analysis of the raw data obtained (including the negative values), showed no effect on the overall results.

The mean macular pigment density in the diabetic group was 0.13 ± 0.20 log units and 0.36 ± 0.18 log units in the age-similar control group ($P = 0.0015$; Figs. 1c, 1d). Linear regression did not show a significant age dependence in either the control group or the diabetic group ($R = -0.17$, $P = 0.36$ and $R = -0.16$, $P = 0.43$, respectively). In the absence of an age-dependence relationship in the data, comparison of the mean of the entire control group and the diabetic group can be performed, and this also showed a statistically significant reduction in pigment density in the diabetic group ($P = 0.0005$). There was no correlation between pigment density and duration of diabetes ($P = 0.20$) or previous laser treatment ($P = 0.37$). Patients with more severe grades of maculopathy had less macular pigment optical density (Fig. 2). Multiple nonparametric tests between the different grades of maculopathy were performed with the probability required for significance reduced to 0.017 (three tests) using the Bonferroni method. At this level of significance the diabetic subjects with grade 2 maculopathy had a significantly reduced pigment density in comparison with the diabetic patients with no maculopathy ($P = 0.016$). A similar analysis was performed for the level of retinopathy with the probability corrected for the number of tests performed, but no significant relationships were found.

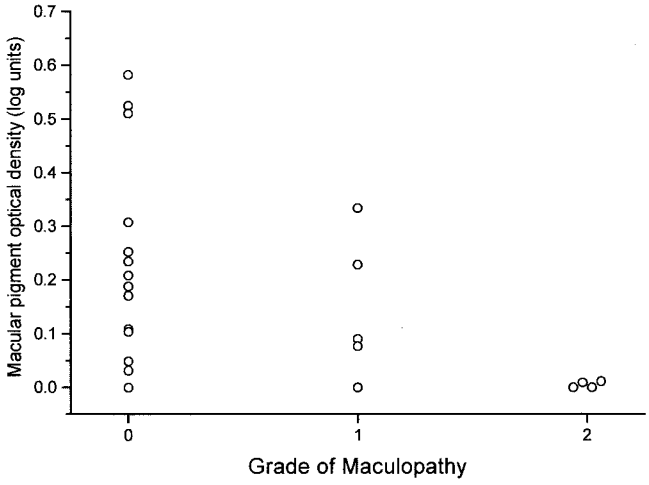

FIGURE 2. Macular pigment density in the diabetic group against grade of maculopathy. The data points for the patients with grade 2 maculopathy have been shifted laterally for clarity.

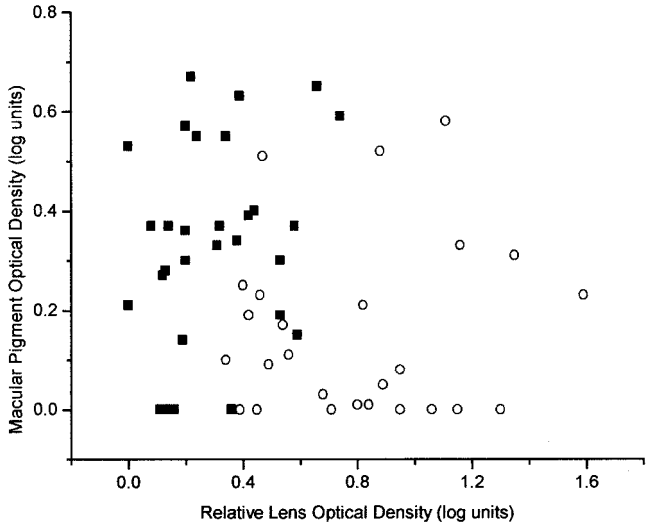

FIGURE 3. Plot of differential lens optical density against macular pigment optical density for both groups. (■) Control and (○) diabetic subjects. Linear regression parameters for the control ($R = 0.25$, $P = 0$ 0.17) and the diabetic ($R = 0.10$, $P = 0.65$) groups.

A plot of the measured lens optical density against the macular pigment density is shown in Figure 3. The results of the lens optical density are independent of the macular pigment density in both control and diabetic groups ($R = 0.25$, $P = 0.17$ and $R = 0.10$, $P = 0.62$ respectively).

DISCUSSION

The results of the Rayleigh match in this study showed no difference between the diabetic patients and the control group, a finding in agreement with Elsner et al.,[46] who found that at low illuminance levels the Rayleigh match was normal in diabetes and that the optical density of the M and L cone photopigments was normal. At higher levels of illumination, the diabetic patients exhibited bleaching abnormalities, a finding that was not investigated in this study.

The experiments designed to measure the lens and macular pigment densities relied on the underlying assumption that the cone absorption spectra are the same in both the diabetic and control groups. If the optical density of a cone photopigment changes, there is a corresponding change in the absorption spectrum—the bandwidth broadening for increasing optical density and narrowing for decreasing optical density.[47] In a disease state leading to cone dysfunction, it is possible that the photopigment density could be reduced, because of absence of pigment production or of disc replacement in the diseased receptor outer segment. This could lead to a change in the color-match ratios that in turn would give an apparent change in the optical density of the prereceptoral filters in the diabetic patients. Although M-cone photopigment density is probably normal in diabetes, there is evidence of S cone pathway dysfunction.[48,49] Later studies suggest that this localizes to the postreceptoral pathway[50,51] and the dysfunction thus may not reflect an S-cone change. However, a model was developed (see Appendix) to investigate the effect of cone dysfunction on the color-match ratios and the derived media optical densities, based on a consideration of the cone excitations and the effect of reduced photopigment concentration and decreased outer segment length.

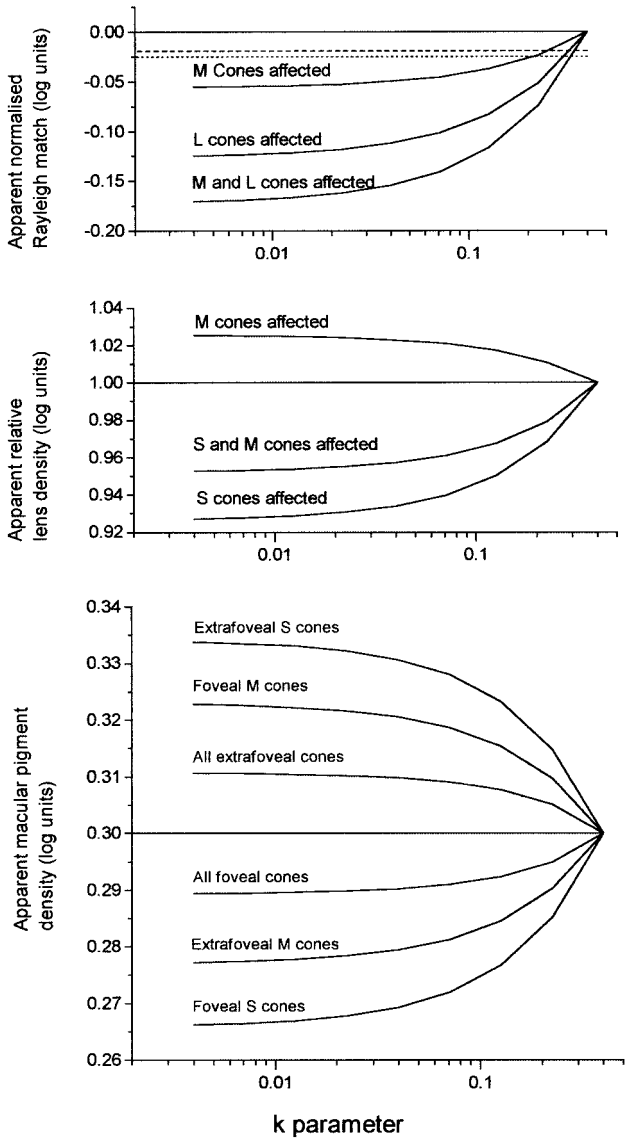

FIGURE 4. Color-matching model. The effect of a reduction in the product of photopigment concentration and outer segment length on color match ratios used in the study. *Top*: decrease in the Rayleigh match ratio with decreasing *k*. *Dashed line*: one-tailed 95% confidence interval for the control subjects; *dotted line*: one-tailed 95% confidence interval for the diabetic patients. Both of these lie well within the bounds of the model, indicating that the M and L cone pigments are probably unaffected by early disease. *Middle*: effect of a reduction in *k* on the apparent lens density. *Bottom*: apparent macular pigment density occurring for reduction in *k* for either the foveal or extrafoveal cones involved in the color match. Overall, the change in apparent lens density and macular pigment density was small and cannot account for the differences found between the control and diabetic subjects.

The results are shown in Figure 4 which shows that, overall, the effect of changes in the cones on the Rayleigh match, apparent lens density, and apparent macular pigment density is small, even for a 2 log unit (100-fold) decrease in the product of cone photopigment concentration and outer segment length.

The one-tailed 95% confidence intervals for Rayleigh match in the control subjects and the diabetic patients are plotted in Figure 4 (top), indicating that the spread of data in this experiment is well within the bounds of the change predicted by the model (i.e., M and L cone optical densities are not significantly affected by diabetes).

The estimated lens optical density would be decreased in the case of S cone photopigment loss (Fig. 4, middle), which is opposite to the effect seen in the results of the 420- and 515-nm color match.

The model was also used to investigate apparent changes in macular pigment optical density that may occur due to cone dysfunction in foveal and extrafoveal locations. A 2-log-unit reduction in S cone photopigment and outer segment length product in the fovea, maintaining normal S cones in the extrafoveal location, would result in an underestimation of the macular pigment density by 0.05 log units (Fig. 4, bottom). This does not explain the reduction measured in the diabetic patients. An apparent reduction in macular pigment density caused by S-cone change would also be accompanied by an underestimation of the optical density of the crystalline lens, as all the patients who performed the macular pigment color matches had also performed the lens density match. Our results for the optical density of the lens agree closely with those obtained by Lutze and Bresnick.[11] The two methods used in the present study to derive a diabetes duration-dependence relationship gave values of 0.019 log units/year and 0.021 log units/year, which agree with the result of 0.018 log units/year derived by Lutze and Bresnick. Estimating the age of a lens-matched normal subject using equation 3 agrees well with the equations from Moreland,[12] who combined the Lutze and Bresnick data with the two-component model of Pokorny et al.[5]

The similarity of the results in the two studies is particularly important, considering the different experimental methods used. Lutze and Bresnick[11] measured dark-adapted absolute thresholds to two wavelengths equally absorbed by rhodopsin. They conducted further experiments to verify that rods were the receptors involved in detection rather than cones. Our method uses a color threshold at low photopic luminance, which relies on cones. The similarity of the results obtained in the present work in comparison with those obtained previously suggests that they represent a true ocular media change rather than an apparent change caused by receptoral abnormality in the foveal cones of the diabetic patients.

The results of autofluorescence studies have shown an increase in the lens absorption in diabetes.[6–10] The origin of the increased light loss in the short-wavelength end of the spectrum is not entirely clear, but it has been suggested that it is due to an accumulation of advanced glycosylated end products (AGEs).[8,52] Autofluorescence studies have shown that glycosylated collagen absorbs at 370 nm and emits at 440 nm,[53] and that autofluorescence occurs at these wavelengths in "browned" lenses.[54,55] Browned lenses also autofluoresce at other wavelengths,[56] with a significant emission at 520 nm.[9,10] In diabetes, the long-term exposure of the delicate lenticular environment to hyperglycemia is likely to lead to an increased accumulation of AGEs, with accompanying optical effects. AGEs have been implicated in the pathogenesis of complications of diabetes[57,58] and specifically in the formation of cataract.[59–62] Glycosylation of lens α-crystallin has been measured in excised diabetic and normal lenses.[63] The diabetic lenses had a threefold increase in α-crystallin glycosylation in comparison with normal lenses, although there was no significant difference in the degree of lens browning between the two groups in this study. The authors suggest that differences in lens browning between diabetic patients and control subjects may be due to glycosylation of proteins other than crystallins. Studies of bovine lens have shown that nonenzymatic glycosylation occurs both in the crystallins[64] and the membrane proteins.[65] Further study may be able to identify the relative contribution to overall lens browning resulting from the glycosylation of different proteins.

Lutein and zeaxanthin are the only carotenoids present in the lens[66] and are concentrated in the lens epithelium and cortex.[67] A high dietary intake of carotenoids has been linked with a reduced incidence of nuclear cataract[68] (although this study provided only weak support for the association), and a reduced need for cataract extraction is seen in women[69] and men[70] in the United States with high carotenoid intake. The use of vitamin supplements containing vitamin C and E for longer than 10 years may also lower the risk of cataract,[71] suggesting a protective role of these antioxidants. In our study there was no association between lens optical density and macular pigment optical density for either the control group or the diabetic patients (Fig. 3). The wavelengths used to measure lens density are equally absorbed by lutein and zeaxanthin, and consequently the result of this color match would be unaffected by both macular and lenticular carotenoid concentrations.

Macular pigment optical density showed no dependence on age in our study, a finding that is in agreement with some previous studies in normal subjects,[21–23] although a small age-dependent effect has also been reported.[25,26] The possibility of an age-related decline in macular pigment density is not resolved at present.

There are several mechanisms by which macular pigment levels could be reduced in diabetes. First, there may be a genetic influence. There is a wide variation in macular pigment density in the patients with no maculopathy (Fig. 2), which makes a strong genetic influence on macular pigment density in diabetic patients unlikely. Macular pigment density has been measured in monozygotic twins,[72] with results suggesting that pigment levels are not entirely genetically determined. Second, the diabetic diet could be deficient in lutein and zeaxanthin or absorption from the gut could be reduced. Granado et al.[73] studied the serum levels of antioxidants in a group of European insulin-dependent diabetic patients, first-degree relatives, and control subjects. They found no significant difference in serum levels of lutein and zeaxanthin between groups, although the diabetic group had lower levels of retinol and higher levels of β-carotene, α-carotene, and β-cryptoxanthin than did first-degree relatives without diabetes. However, Ford et al.[74] found a significant reduction in the serum levels of macular carotenoids in patients with newly diagnosed and established diabetes patients in the United States in comparison with normal subjects. These findings may relate to different diets in the two study populations. Study of the absorption of carotenoids in diabetic persons would help to resolve this issue.

Finally, the pigment density could become low as a result of a reduced rate of incorporation into retinal tissue or an increased rate of removal from the retina. Thickening of basement membranes of retinal capillaries in diabetes,[75–78] the increased affinity of oxygen for glycosylated hemoglobin,[79] the existence of a redox shift due to the effects of hyperglycemia on glycolysis and sorbitol metabolism,[80] and the presence of abnormal vasculature in the parafovea of diabetic persons[81] imply that diabetic retinas are under continuous oxidative stress. An analysis of retinal tissues from primate and human eyes for oxidation products of lutein and zeaxanthin showed that, indeed, these pigments appear to play a role as antioxidants.[28]

Analysis of the data in this study has shown that the only factor with a statistically significant correlation with lower levels of macular pigment among the diabetic patients was grade of maculopathy. Because the grade of maculopathy provides an indication of the severity of microvascular disease in the macula, this may imply that lower pigment levels are found in diabetic maculae that are under greater oxidative stress. The macular pigment density in an individual is likely to represent an equilibrium value of rate of incorporation into retinal tissue combined with rate of removal from tissue, including conversion to other compounds. A low macular pigment level could result from either reduced incorporation into retinal tissue or from increased rate of removal. It is possible that increasing antioxidant protection to the diabetic retina may reduce the probability of development of microvascular complications. In the San Luis Valley Diabetes study of antioxidants in diabetes, the effect of dietary and supplement intakes of vitamin C, vitamin E, and β-carotene on progression of diabetic retinopathy was examined in patients with type II diabetes.[82] There was no observed protective effect of these nutrients against diabetic retinopathy, and indeed among those patients not taking insulin, increased vitamin E intake was associated with an increased risk for severity of retinopathy, as was increased intake of β-carotene in patients taking insulin. This study highlights the complexity of the relationship between antioxidants and diabetic retinopathy, but the effect of dietary intake of lutein and zeaxanthin on grade of maculopathy was not specifically assessed nor were serum levels of any antioxidant measured.

Hammond et al.,[83] showed that macular pigment levels could be modulated by diet in some but not all subjects. Of 11 participants, 8 showed increased serum levels and macular pigment density, 2 showed an increase in serum level but not in macular pigment density, and 1 showed no response in either parameter. The increase in macular pigment optical density persisted to the posttest time point (range, 1–6 months after cessation of the diet).

Our study showed that diabetic patients have increased differential lens optical density and reduced levels of macular pigment. The lenticular changes probably result from an accumulation of AGEs. Lens density may also be affected by oxidative stress in diabetic persons, although the color match used in the study cannot provide any information regarding the concentration of carotenoids in the lens. The results of a study of antioxidant levels in the diabetic lens, including lutein and zeaxanthin, would be very interesting indeed.

Reduced macular pigment density may result from increased oxidative stress in the diabetic macula. Further study is indicated to pinpoint the cause more precisely. A controlled trial of dietary modification with serial measurement of serum lutein and zeaxanthin and macular pigment optical density would be of interest in patients with diabetes. If pigment density does not increase after a dietary supplement, the incorporation of the carotenoids into retinal tissues would be at fault. Conversely, demonstration of an increase in pigment optical density would indicate that an increased rate of elimination of pigment from the tissues is the likely reason for the observed low value.

Acknowledgments

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APPENDIX

Modeling Color-Match Ratios

Color matching is dependent on the transmission coefficient of the ocular media and also on the cone absorption spectra. The fraction of light absorbed by the photoreceptors is determined by the equation[47]

$$f = 1 - e^{-k\alpha} \qquad (A1)$$

where k is the product of photopigment concentration and length of the outer segment and α is absorption spectrum of the photopigment. This is known as the correction for self-screening. In a pathologic state causing damage to the photoreceptors, k is likely to be reduced. Although receptor morphology appears to be normal in nonphotocoagulated retina in diabetes,[33] the product k could still be reduced.

When a color match has been achieved, a consideration of the S and M cone excitation ratio and transmission through the ocular media leads to the expression (equivalent to equation 1)

$$\frac{E_1}{E_2} = \frac{T_2}{T_1}\left(\frac{\dfrac{m_2 s_3}{m_3} - s_2}{s_1 - \dfrac{m_1 s_3}{m_3}}\right) \qquad (A2)$$

where E is the matching radiance, T is the media transmission, and m and s are the spectral absorption coefficients, with subscripts 1 and 2 indicating the shorter and longer matching wavelengths, respectively, and subscript 3 the reference wavelength.

The color-match relationship is more accurately described by replacing the cone absorption spectra with the more precise term after correction for self-screening. This gives

$$\frac{E_1}{E_2} = \frac{T_2}{T_1}\left[\frac{(1 - e^{-k_m m_2})(1 - e^{-k_s s_3})/(1 - e^{-k_m m_3}) - (1 - e^{-k_s s_2})}{(1 - e^{-k_s s_1}) - (1 - e^{-k_m m_1})(1 - e^{-k_s s_3})/(1 - e^{-k_m m_3})}\right] \qquad (A3)$$

Equation A3 can be used as a model to investigate the effect of cone dysfunction on apparent lesser density by altering the values of the parameters k_s and k_m. k for a healthy cone is taken as 0.4,[84] and the Pokorny and Smith[85] fundamentals are used for the cone absorption spectra. A similar equation considering the M and L cone absorptions can be used as a model of the Rayleigh match.

To investigate the effect of cone dysfunction on the macular pigment density, two equations are used, incorporating the macular pigment and lens transmissions in a foveal model and solely the lens transmission in the extrafoveal model. The log

ratio of the matching radiances then gives the measure of macular pigment density and altering the parameter k for changes in M or S cone self-screening at either or both locations models the effect of differential cone dysfunction on the apparent macular pigment density.

Estimation of Lens-Matched Normal

A diabetic person of age A_d and duration of diabetes D has a lens density L_d of

$$L_d = m_n A_d + m_d D + c \quad (A4)$$

where m_n is the normal increase in lens density per year, m_d is the increase due to diabetes per year, and c is lens density at birth. A nondiabetic subject of age A_n has a lens density of L_n determined by

$$L_n = m_n A_n + c' \quad (A5)$$

Assuming there is no difference in the lens densities at birth between those patients who are destined to become diabetic and those who are not, the age of a normal person with a lens density matched to a diabetic person is thus

$$A_n = A_d + \frac{m_d}{m_n} D \quad (A6)$$

In this study $m_d = 0.020$ and $m_n = 0.00846$ log units/year, which yields the result

$$A_n = A_d + 2.364D \quad (A7)$$