Hue Discrimination and S Cone Pathway Sensitivity in Early Diabetic Retinopathy

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Measures of hue discrimination and M (green) and S (blue) cone pathway sensitivities were compared in a group of 24 diabetics with either early background retinopathy or no retinopathy. The Farnsworth-Munsell 100-hue test was used to measure hue discrimination, and a two-color increment threshold technique was used to measure S and M cone pathway sensitivities. The results were compared to the level of diabetic retinopathy, to the degree of macular edema, and to the duration of the disease. No significant correlation was found between the Farnsworth-Munsell 100-hue error scores and the level of retinopathy; S cone pathway sensitivity loss, however, correlated significantly with both the level of retinopathy and the degree of macular edema. Our results indicate that measurements of S cone pathway sensitivity using an increment threshold technique provide a more sensitive method than hue discrimination for detecting color vision deficits in early diabetic retinopathy. Invest Ophthamol Vis Sci 31:1008–1014, 1990

Foveal function is often affected in diabetic patients with early background retinopathy. There are reports of deficits both in contrast sensitivity1–4 and in color vision. A number of studies have demonstrated decreased hue discrimination using the Farnsworth-Munsell 100-hue test (FM 100-hue) and an increase in tritan (blue-yellow) defects.6–8 There are also reports, using spectral sensitivity techniques, of decreased sensitivity in the S (blue) cone pathways in patients with early diabetic retinopathy.9–12 Both decreased hue discrimination using the FM 100-hue test and decreased sensitivity in the S cone pathways have been found in patients who show no evidence of retinopathy on clinical examination.6,8,11,12 Despite these findings, there is disagreement in the literature as to the value of either the FM 100-hue test or spectral sensitivity techniques as screening devices for retinopathy. For example, in a group of patients with background retinopathy, no significant correlation was found between the level of retinopathy and the FM 100-hue error score.2 It also has been suggested that in diabetics reduced S cone pathway sensitivity reflects increased lens density rather than retinopathy level.13

In view of conflicting reports, we chose to study a select group of diabetics who showed evidence of early background retinopathy or no retinopathy on fluorescein angiography, and to compare measures of S cone pathway sensitivity with measures of hue discrimination. One of the drawbacks of existing studies is that the presence and level of retinopathy were often determined without the aid of fluorescein angiography, and to compare measures of S cone pathway sensitivity with measures of hue discrimination. One of the drawbacks of existing studies is that the presence and level of retinopathy were often determined without the aid of fluorescein angiography, and it is possible that microvascular abnormalities or retinal nonperfusion were overlooked. In addition, few studies have restricted their patient population to a group with minimal retinopathy, and only one study, of seven patients, has systematically compared the results of measures of S cone sensitivity to measures of hue discrimination.10

As a first step in determining the possible predictive value of S cone pathway sensitivity and hue discrimination, we measured M (green) and S cone pathway sensitivities using a two-color increment threshold technique and hue discrimination using the FM 100-hue test. The relationships of these measures to the level of diabetic retinopathy, the degree of macular edema, and the duration of the disease were analyzed. The implications of the results regarding the possible anatomic sites and mechanisms of S cone pathway sensitivity loss are discussed. Preliminary reports of this work have been presented at the Non-invasive Assessment of the Visual System meeting14 and at the annual meeting of the Association for Research in Vision and Ophthalmology.15

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Materials and Methods

Subjects

Twenty-four patients with diabetes mellitus requiring insulin therapy participated in the study. All had corrected Snellen visual acuity ≥ 20/30— in the tested eye. The age range was 24–68 yr (mean, 45.8 ± 13.9 yr). The age at onset of the diabetes ranged from 8–54 yr (mean, 27.6 ± 14.7 yr), and the duration on insulin therapy ranged from 7–40 yr (mean, 18.2 ± 9.1 yr). A requirement for inclusion in the study was that patients showed no evidence of background diabetic retinopathy or only early background retinopathy as determined by clinical contact lens examination, color fundus photographs, and fluorescein angiography. None had a history of hypertension or other metabolic disorders. None of the eyes studied showed evidence of significant lens opacities or glaucoma. Fourteen subjects ranging in age from 23–61 yr (mean, 38 ± 11.6 yr) with no known abnormality of the visual system comprised the control group. Informed consent was obtained from all subjects prior to testing.

Farnsworth-Munsell 100-Hue Test

Hue discrimination was evaluated in all patients with the FM 100-hue test administered under standard Illuminant C lighting conditions (MacBeth easel lamp with an illumination of 290 lux). The right eye was tested in each patient. The order of presentation of the boxes was varied randomly, and no time limit was imposed. The subject’s correction for the viewing distance (40 cm) was worn. The error scores were calculated for each patient using the Farnsworth method. They were then compared with the age-similar normal data of Verriest et al16 for monocular FM 100-hue testing without prior binocular testing experience. The total error score was adjusted for age by comparing the error score for each diabetic eye with the 95th percentile of normal scores for that patient’s age. Quadrant analysis was performed on the raw scores to evaluate the tendency towards a tritan defect. Total error scores were partitioned into blue-yellow and red-green partial scores as described in Smith et al.17

Two-Color Increment Threshold Test

Light stimulation for increment threshold testing was provided by a two-channel Maxwellian view system, described previously.18 The filament image of the bulb in the pupil plane was 1.8 × 2.0 mm. Monochromatic light for the test channel was provided by 480-nm and 540-nm interference filters with half bandwidths of approximately 6 nm, and for the background channel by a 600-nm narrow band interference filter. The retinal illumination was calibrated with an EG&G model 550 photometer (Salem, MA) and calculated using the method described by Westheimer.19

A two-color increment threshold procedure described previously18 was used to measure the sensitivity of an M and an S cone pathway. Foveal increment thresholds were obtained for a 480-nm test light (1.2°, 200 msec) superimposed on 14°, 600-nm steady adapting fields. These conditions were selected to allow measurement of Stiles pi 4 (an M cone pathway) and pi 1 (an S cone pathway) mechanisms. The protocol described by Greenstein et al18 was used to determine threshold. After 10 min of dark adaptation, complete increment threshold curves were obtained on each subject using a modified method of limits procedure. The use of the same test light (480 nm) allowed us to measure the relative thresholds of the M and S cone pathways unaffected by differences in preretinal absorption. The contribution of macular pigment density to these threshold measures was estimated by comparing the value of M cone pathway thresholds obtained with the 480-nm test light at an adapting background of 0.96 log Td to the thresholds obtained with a 540-nm test light. According to Bone and Sparrock,20 absorption at 540 nm is minimal even for subjects with high density macular pigment. If M cone sensitivity was decreased as a result of increased macular pigment density rather than as a result of disease, then the decrease should be negligible for data obtained with the 540-nm test light. All thresholds were adjusted for the contribution of macular pigment.

Complete increment threshold curves were obtained on all patients. The sensitivity loss of the M cone (pi 4) pathway and the S cone (pi 1) pathway compared to the normals was calculated by comparing the log decrease in sensitivity at 0.96 log Td to the log decrease at 3.87 log Td. In a previous study18 we have demonstrated that these levels of adaptation provide adequate measures of M cone and S cone pathways, respectively. These levels of adaptation were chosen so that sizable losses in sensitivity of each pathway could be measured and to ensure that the S and M cone pathways were approximately equally adapted.

Assessment of Level of Retinopathy and Degree of Macular Edema

The level of retinopathy and degree of macular edema was assessed for each patient based on contact lens examination, color stereo fundus photographs, and fluorescein angiography. Angiography was performed according to the Early Treatment Diabetic
Retinopathy Study (ETDRS) protocol. The level of retinopathy and degree of macular edema were determined by two independent and experienced graders using a classification scheme based on the modified Airlie House classification of diabetic retinopathy.

**Level of Retinopathy**
- Level 1: normal fundus
- Level 2: one or more microaneurysms only
- Level 3: microaneurysms with one or more other nonproliferative lesions present of mild to moderate degree
- Level 4: microaneurysms with one or more other nonproliferative lesions present of severe degree

**Degree of Macular Edema** (on the basis of fluorescein angiography)
- Grade 0: no macular edema
- Grade 1: questionable macular edema
- Grade 2: macular edema definitely present, but not involving center of macula
- Grade 3: edema definitely present at center of macula, but thickness of retina here less than elsewhere
- Grade 4: edema (thickness) at center of macula is as great or greater than elsewhere

The levels of retinopathy and degree of macular edema recorded in this study represent the averaged results of the two graders. For macular edema, the grades were identical in all cases, but for retinopathy levels they were one level apart in five cases.

**Results**

**Farnsworth-Munsell 100-Hue Test**

The error scores of 33% of the patients exceeded the 95th percentile score for age-matched normals; Spearman Rank correlation coefficient analysis showed no significant correlation between age corrected error scores and level of retinopathy. Since diabetes is said to lead to tritan or blue-yellow defects, we compared the differences between the square roots of the blue-yellow and red-green partial scores (difference score) and the level of retinopathy. These data are shown in Figure 1, in which the age-corrected difference score for the diabetics versus the level of retinopathy is plotted. The age-corrected difference score was obtained by calculating the numerical difference between the patient's score and the difference score in age-similar normals as determined by Smith et al. According to Smith et al, error scores above +2.8 on the ordinate (the upper dashed horizontal line) indicate a significant blue-yellow axis defect, whereas data below −2.8 (the lower dashed horizontal line) indicate a significant red-green axis defect. Thus, positive values indicate a blue-yellow axis, and negative values indicate a red-green axis. There is a trend in the data in the direction of a blue-yellow axis; however, only four patients (24% of patients with signs of retinopathy) have corrected positive difference scores which exceed +2.8. Spearman rank-order correlation coefficient analysis showed no significant correlation between corrected difference scores and the levels of retinopathy.

**Increment Threshold Data**

Figure 2 shows the mean foveal increment threshold data for 7 normals in the 20–40-yr age group (open symbols) compared to those for 7 normals in the 41–61-yr age group (filled symbols). Since the data for the two age groups were very similar, we compared the data from the diabetic eyes to the mean thresholds for all the normals. Figure 3 shows mean foveal increment threshold data and curves for 14 normals. The two curves are the best fit of a function commonly used to describe tvi (threshold versus intensity) data. According to extensive literature, the lower curve represents detection mediated by the M cone pathway, and the upper curve represents detection mediated by the S cone pathway.

Representative foveal increment threshold data for
Fig. 2. Mean foveal increment threshold data for two groups of normal observers for a 480-nm test light on a series of 600-nm adapting fields. The open triangles represent mean thresholds for seven normal observers in the 20-40-yr age group, and the filled triangles are data for seven normal observers in the 41-61-yr age group. Error bars represent ±1 SD.

A diabetic eye also are shown in Figure 3. The data from the diabetic eye (filled symbols) show normal sensitivity of the M cone pathway and a marked loss in sensitivity of the S cone pathway. Foveal increment threshold curves were obtained for all diabetic eyes, and the sensitivity loss of the M cone (pi 4) pathway and the S cone (pi 1) pathway compared to the normals was calculated. A comparison of these sensitivity losses is shown in Figure 4. Each data point represents an individual diabetic's loss in sensitivity compared to the normal. The (0,0) point represents the mean normal value, and the dashed horizontal and vertical lines indicate the upper bound of the range of normal values. Twelve of 24 patients (50%) show an S cone pathway sensitivity loss; of these, only 1 showed a significant M cone sensitivity loss. The patient with losses in both pathways had slight juxtafoveal macular edema (grade 3) as well as decreased visual acuity (20/30—). All 12 who showed S cone pathway sensitivity losses had diabetic retinopathy. An additional 5 patients had diabetic retinopathy but did not show an S cone sensitivity loss.

The loss in sensitivity of the S cone pathway was compared to the level of retinopathy and the degree of macular edema (Figs. 5A, B, respectively). The correlations between S cone pathway sensitivity loss and the level of retinopathy and the degree of edema were determined using Spearman Rank correlation coefficients. Both the level of retinopathy ($r = 0.598$, $P < 0.01$) and the degree of macular edema ($r = 0.574$, $P < 0.01$) correlated significantly with S cone sensitivity loss. There was no significant correlation between the level of retinopathy and the degree of macular edema.

Fig. 3. Mean foveal increment threshold data for 14 normal observers (open triangles) and increment threshold data for a diabetic eye (filled triangles). The branched solid curve is the best fit of the equation $\log T = \log To + \log ((Ao + A)/Ao)$, where $T$ is the threshold of the test, $To$ is the dark-adapted test threshold, $Ao$ is a constant, and $A$ is the adapting intensity of the 600-nm background. $To$ and $Ao$ were estimated to minimize the value of chi-squared. The curve has been moved vertically to fit the patient's data (dashed curve).

Fig. 4. A comparison of the log sensitivity loss of the S cone pathway to that of the M cone pathway for 24 diabetic patients (triangles). The dashed vertical and horizontal lines represent the limits of the normal range.
Fig. 5. Log S cone pathway sensitivity loss for 24 diabetic patients as a function of (A) the level of retinopathy; (B) the degree of macular edema; and (C) the duration of disease.

Since the duration of diabetes is reported to be strongly associated with the frequency and severity of retinopathy, we compared the loss in sensitivity of the S cone pathway to the duration of the disease (Fig. 5C). Duration of disease was determined by history from time of diagnosis of diabetes to time of participation in the study. No significant correlation between the S cone pathway losses and duration of disease was found using Spearman Rank correlation coefficients.

Discussion

A selective loss in S cone pathway sensitivity was found in 70.6% of patients with early background retinopathy. In all but one of these patients, M cone pathway sensitivity was within the normal range. Because of the use of a 480-nm test light to measure both M and S cone pathways, the selective S cone sensitivity loss cannot be attributed to increased lens density in diabetics. Our findings are in agreement with the results of previous studies; however, unlike other investigators, we did not find evidence of decreased S cone pathway sensitivity in patients with no signs of retinopathy.

For the majority of our patients, hue discrimination as tested with the FM 100-hue test was normal. Although 33% of the diabetic eyes studied showed error scores greater than the 95th percentile score for age-matched normals, Spearman rank correlation co-
efficient analysis revealed no significant correlation between error scores and the level of retinopathy. This result is in contrast to Bresnick et al., who showed a significant correlation between FM 100-hue error scores and the level of retinopathy. One possible explanation for the discrepancy between our results and those of Bresnick et al. is that most of their diabetic subjects had retinopathy levels greater than level 3, with the majority of the patients classified in the range level 4–7. Our study included only those diabetics with early retinopathy (levels 1–4). Close examination of their data reveals that the early diabetics (level 1–3) had FM 100-hue error scores in the normal range. Quadrant analysis of our data revealed that 24% of patients with retinopathy exhibited a significant tritan axis; however, no significant correlation with either level of retinopathy or S cone pathway loss was found. Therefore, in agreement with an earlier study by Zwas et al. on seven diabetics with early diabetic retinopathy, it seems that the FM 100-hue test may not be sufficiently sensitive to detect color vision deficits in the early stages of retinopathy. A more sensitive method appears to be two-color increment threshold testing. Since the sensitivity losses of the cone pathway being tested are not necessarily independent of the level of adaptation used, there is a need to compare thresholds obtained at two or more levels of adaptation. For some retinal diseases, greater sensitivity losses have been measured in the unadapted than in the adapted state.

As a first step in determining whether a selective loss of S cone pathway sensitivity can be used as a possible predictor of progression of retinopathy or macular edema, we compared the loss in sensitivity to the level of retinopathy and to the degree of macular edema. Spearman correlation coefficient analysis revealed a significant correlation between S cone sensitivity loss and both level of retinopathy and degree of macular edema. The latter finding is in agreement with Adams et al., who reported increased amounts of sensitivity loss in the presence of macular edema. In contrast to other investigators, who have reported S cone sensitivity loss in patients prior to the development of retinopathy, we did not find S cone sensitivity loss in patients who had no signs of diabetic retinopathy (Fig. 5A). One possible explanation is that in the current study, the level of retinopathy was determined not only on the basis of color photographs and contact lens examination but also on the results of fluorescein angiography. Without the use of fluorescein angiography, microvascular abnormalities may be overlooked and patients inappropriately classified.

Despite many reports of color vision deficits in diabetics, the underlying mechanisms and anatomic sites are still not fully understood. Diabetes is a disease that affects the midretinal, inner retinal, and probably also the outer retinal layers. A single anatomic site cannot be ruled out, but given the nature of the disease, the results suggest that we are not dealing with either a pure receptor or postreceptoral locus.

With regard to the underlying mechanisms in this study, the foveal S cone sensitivity loss we found appears to correlate with peripheral and central vascular changes. Both levels of retinopathy and degree of macular edema are measures of vascular abnormalities within the retina. It is well known that diabetes exerts much of its damage through its effect on vasculature. Diabetic vessels become less competent and leaky, and therefore, inefficient in the transport of nutrients and oxygen to the tissues. Thus, there exists a state of relative hypoxia within the diabetic retina. There are reports of acute hypoxia resulting in tritanlike defects in normals. Since losses of S cone sensitivity were associated with both level of retinopathy and edema, it would seem that hypoxia is playing some role in the mechanism of S cone vulnerability. One way hypoxia could preferentially affect the S cone pathways was suggested by Hood et al., the S cone pathways may have a more limited response range than the other cone pathways. Because of the difference in response ranges, an equivalent change in maximal response of each pathway may affect the S cone pathway more adversely. The loss of maximal response in an already limited response range of the S cone pathway may raise the threshold above the level that is detected psychophysically. This would make the S cones appear more vulnerable to hypoxia.

In conclusion, we found no evidence of color deficits in patients with normal fluorescein angiograms. Our results indicate that increment threshold testing at two or more levels of adaptation is a very sensitive method for detecting color vision deficits in early diabetic retinopathy. Reliance on the FM 100-hue test alone may result in a failure to detect large losses of sensitivity in these early diabetics. Whether these losses of sensitivity are an early indicator of progression of retinopathy can be determined only by a prospective study. We are currently following these patients to test this hypothesis.

Key words: diabetic retinopathy, S cone pathway, hue discrimination, increment threshold

References
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