

## CLINICAL SCIENCE

## Extent of foveal tritanopia in diabetes mellitus

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**Aim:** To use a colour matching technique to test the hypothesis that the foveal tritanopic zone is increased in size in diabetes mellitus.

**Method:** A Wright tristimulus colorimeter was adapted for small field colour matching and colour matches were performed on bipartite fields in the range 12' to 60' of arc. The reference stimulus was 490 nm desaturated with 650 nm and the matching stimulus consisted of either two wavelengths (530 nm and 650 nm) or three (460 nm, 530 nm, and 650 nm). The size of the zone of foveal tritanopia was measured using two alternative forced choice presentations of dichromatic and trichromatic matches made by the observer for different field sizes. 21 diabetic and 12 controls performed the experiment.

**Results:** The results for the controls show a normal distribution, with a median foveal tritanopic zone of 18' of arc. The median for the diabetic patients was also 18' of arc, but the distribution showed a significant skew to the right. A non-parametric test shows a significant difference in comparison with the controls ( $p = 0.01$ ), with several subjects having extensive zones of foveal tritanopia, reaching up to 1 degree.

**Conclusions:** In the majority of diabetic subjects the extent of foveal tritanopia is normal; however, there is good evidence that in a small number of subjects the size of the zone is significantly increased. This indicates S-cone pathway damage that is sufficiently severe to lead to dichromatic colour vision in the fovea.

Short wavelength sensitive (S) cones are fewer in number than medium wavelength (M) and long wavelength sensitive (L) cones, representing approximately 10% of the total cone population.<sup>1</sup> An early study showed that the fovea was relatively insensitive to short wavelength light and that colour vision was impaired, colour matching being possible with two primaries instead of the usual three.<sup>2</sup> This finding was reproduced in a later study.<sup>3</sup> It was thought initially that an absence of rods was responsible for the changes seen in visual function. Willmer and Wright, however, demonstrated the tritanopic nature of the colour vision defect.<sup>4</sup> They showed that dichromatic colour matches consistent with congenital tritanopia could be made for a 20' bipartite field. A similar tritanopic colour vision deficit was reported for small fields generally,<sup>5,6</sup> suggesting that the fovea may not be unique in this respect. Other theories suggested that the apparent tritanopic deficit in the fovea might be due to Troxler fading<sup>7</sup> or to preretinal screening of short wavelength light by the increasing density of the macular pigment.<sup>8</sup> Williams *et al*<sup>9</sup> performed a series of elegant psychophysical experiments demonstrating that the tritanopic response is most likely to be due to absence of S-cone function in the fovea. Their findings could not be explained by Troxler fading nor by screening by the macular pigment. Small field extrafoveal tritanopic effects for steady fields did, however, appear to be explainable by Troxler fading, as normal S-cone responses were seen for transient stimuli. Anatomical study has also demonstrated that the centre of the fovea appears to be devoid of S-cones in primates.<sup>10,11</sup> The extent of the foveal tritanopic region is around 20–25 minutes of arc.

Studies of the Farnsworth Munsell 100 Hue test in diabetics have shown an increased error score.<sup>12–25</sup> Some studies have shown generalised loss of hue discrimination with no specific axis,<sup>13,14</sup> while others have demonstrated a red-green axis loss.<sup>20,24</sup> However, three studies have shown a loss of colour discrimination on a tritanopic axis in patients with diabetes.<sup>12,21,24</sup> Using a probe-flash technique, Greenstein<sup>13</sup> has shown an early selective S-cone pathway deficit in diabetics

and further study has suggested that this is post-receptoral in origin.<sup>26,27</sup>

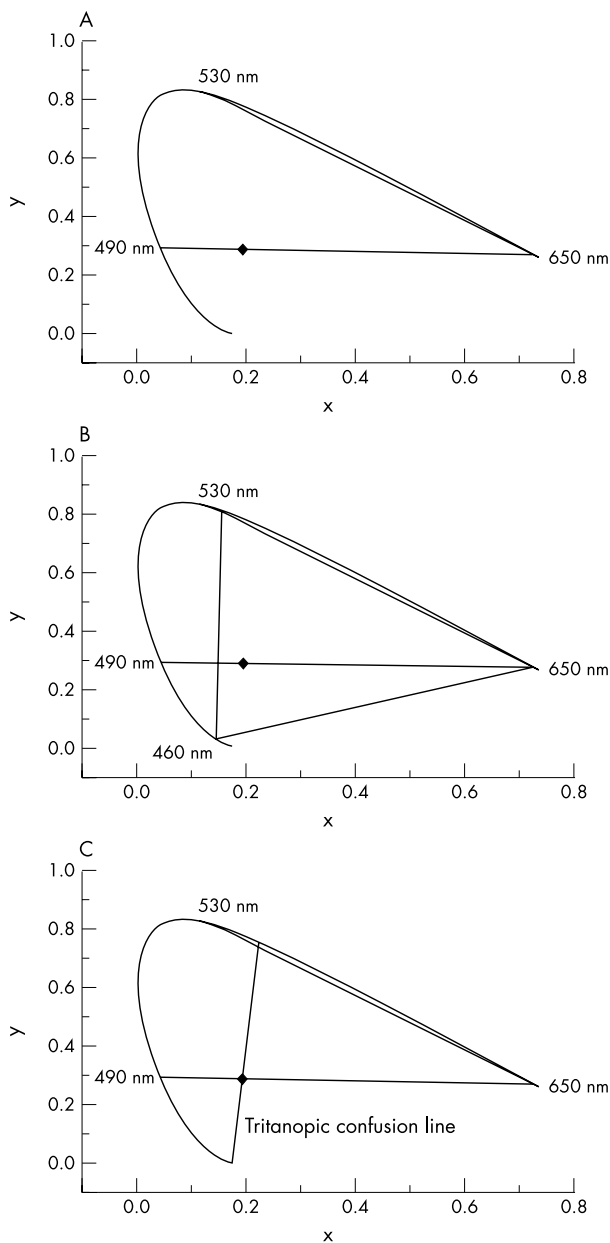
The hypothesis tested in this study was that S-cone pathway dysfunction in diabetes may result in an increased extent of foveal tritanopia.

## METHODS

A two alternative forced choice method using the results of two colour matches made by the subject while observing bipartite small fields in the range 12' to 60' of arc in 6' steps was used to establish the presence of tritanopia. The Wright tristimulus colorimeter<sup>28</sup> was adapted for small field colour matching using apertures of decreasing size to produce a series of circular, bipartite fields of view of low photopic luminance. The lower semicircle contained a reference spectral primary of 490 nm, desaturated with 650 nm. The upper semicircle contained the matching stimulus, which consisted of a mixture of either two spectral primaries (530 nm and 650 nm) or three (460 nm, 530 nm, and 650 nm). The unmodified bipartite field of the colorimeter is a 1 degree 20' square field and this was used as the largest assessment of tritanopia if necessary. The colour matches were performed using the right eye only (a constraint resulting from the design of the colorimeter).

The colour matching procedure was as follows. The subject dark adapted for 10 minutes. A dental bite bar was used to ensure that the subject's pupil was aligned with the exit pupil of the colorimeter. The subject was asked to maintain fixation on the centre of the stimulus (which was of very small angular diameter) at all times. The subject's task was to adjust the radiance of a mixture of the matching primaries (upper semicircle) to the test stimulus (lower semicircle) such that the test and matching fields were equivalent in colour and brightness. This was achieved by altering the position of gradient neutral density filters mounted in front of each matching beam. Once the initial match was made, the positions of the neutral density wedges were noted. One of the neutral density wedges

was then moved to a different, random position and the observer was required to use the appropriate control to re-attain the match. This procedure was repeated three times for each matching wavelength. Considering the chromaticity diagrams shown in Figure 1, for a trichromatic observer, a colour match to the reference is not possible using only 530 nm and 650 nm as these two chromaticity lines are well separated on the diagram, the reference appearing blue and the matching stimulus colour only variable from red to yellow to green (Fig 1A). If a third primary of 460 nm is added to the stimulus field, a colour match becomes possible as the chromaticity of the reference is within the triangle formed by the three spec-



**Figure 1** Chromaticity diagrams illustrating the principle of colour matching to establish the presence of S-cones. (A) The possible colours obtainable using mixtures of 530 nm and 650 nm light and a separate mixture of 490 nm and 650 nm light. The circle on the 490–650 nm line indicates the position of the test colour. For this combination of primaries, no colour match is possible for a trichromatic subject. (B) This shows that the addition of 460 nm light to 530 nm and 650 nm allows a trichromatic subject to achieve a colour match. (C) Shows that in tritanopia, a colour match is possible as the ratio of 530 nm to 650 nm light can be adjusted so that both colours lie on a tritanopic confusion line.

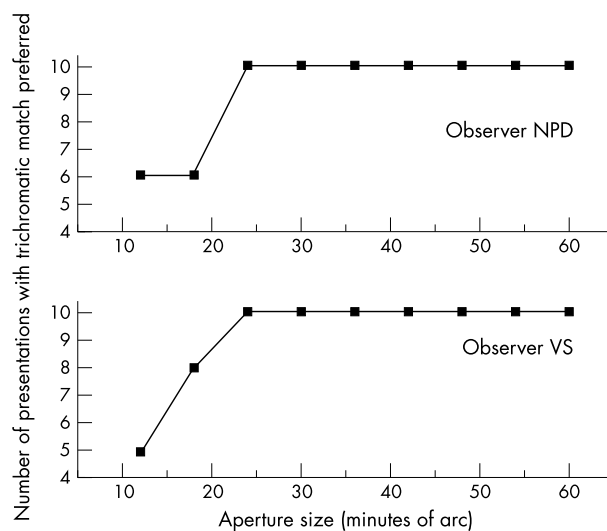
tral primaries in the stimulus (Fig 1B). If the subject is tritanopic in the retinal area illuminated, a colour match to the reference is possible with two primaries of 530 nm and 650 nm as the chromaticity points lie along a tritanopic confusion line (Fig 1C).

Consequently the dichromatic match is possible only in areas of tritanopia, whereas the trichromatic match is possible in both tritanopic and trichromatic retinal areas.

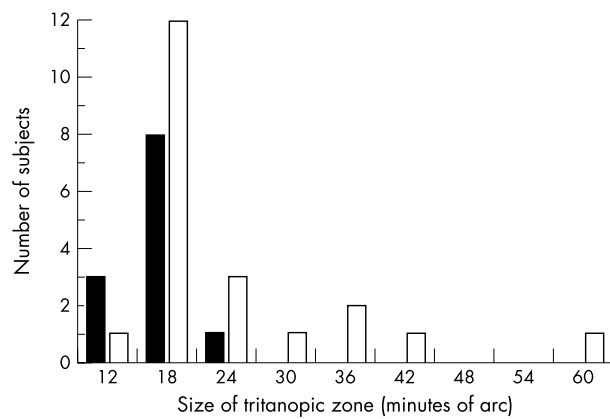
The bipartite field was flickered at 5 Hz using a beam chopper, allowing maximal cone summation time but inhibiting fading from Troxler's phenomenon. Validation of the method was demonstrated using two observers (NPD and VS). Dichromatic and trichromatic matches were made for each aperture in order of decreasing size. For the larger aperture sizes it was found that no match was possible for the dichromatic mixture, the colour appearance of the matching field always being too green in comparison with the reference field. As aperture size decreased, a point was reached at which the colour match was possible for the dichromatic mixture. The colorimeter settings were noted and the match repeated three times for each wavelength as described above. The third spectral primary was then added to the stimulus and a further three matches were made.

A two alternative forced choice technique was then used to ascertain which of the two colour matches the observer consistently preferred.

Either the dichromatic or trichromatic match was presented to the observer and called "trial 1." After a delay of a few seconds, the other match was presented and called "trial 2." The observer was asked to state which trial contained the better match. This procedure was repeated 10 times, the order of presentation of dichromatic and trichromatic matches was varied randomly and the observer was unaware which match was presented as "trial 1" and "trial 2" at all times. The result of the forced choice presentations was recorded as the number of trichromatic presentations preferred. If the aperture illuminates a trichromatic retinal zone, the trichromatic match will be preferred and the result of the forced choice experiment should be close to 10 (100%). If, however, the retinal area illuminated is tritanopic, assuming the colour matches are appropriate, the observer will be unable to distinguish between the two and the result of the forced choice experiment will be near five (50%). The results for NPD and VS are shown in Figure 2.



**Figure 2** Results of forced choice presentations of dichromatic and trichromatic colour matches as a function of aperture size for observers NPD and VS. Note that with aperture sizes below 20' of arc the forced choice response approaches five, which would be expected by chance (that is, the observers have reached a tritanopic zone)



**Figure 3** Histograms of the measured size of the zone of foveal tritanopia in the control (solid) and diabetic (open) groups.

For both subjects the tritanopic nature of the fovea was revealed at aperture sizes of 18' and 12' of arc, in agreement with other studies.<sup>4,9,11</sup>

This method of examining the two and three wavelength matches for all apertures was found to be lengthy and fatiguing for the observer and a shorter paradigm was developed using a single staircase with respect to aperture size to determine the size of the tritanopic zone for a larger number of naive subjects.

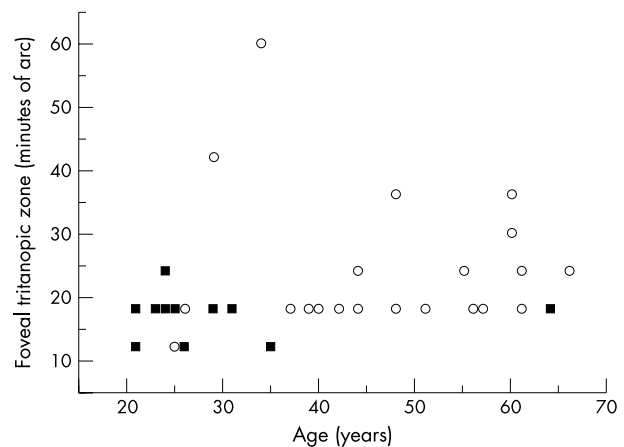
A dichromatic match was attempted with a 60' aperture. If the subject was unable to achieve a colour match (which should be the case for such a large aperture), the aperture size was reduced to 24' of arc and the dichromatic match repeated. The aperture size was decreased further if a match was still not possible. Once a match was found it was repeated three times and the settings noted. Following this the 460 nm primary was added to the matching wavelengths and the match repeated. The matches were then presented to the observer 10 times in random order and the forced choice response recorded. If the results were consistent with a trichromatic response, the aperture was reduced in size and the test repeated. If the results were consistent with tritanopic response, the aperture size was increased and the test repeated. The end result was recorded as the largest aperture size that produced a tritanopic response.

All subjects also performed the Rayleigh match to assess their colour vision, a colour match to assess the optical density of the lens, and two colour matches (one foveal and the other extrafoveal) to measure the macular pigment density. These matches were performed on a bipartite field of 1 degree 20'. The full analysis of the results of these matches are published elsewhere.<sup>29</sup> Of importance here the findings of the Rayleigh match allowed subjects with inherited red/green colour vision deficiencies to be excluded (in actuality, none were found) and the results of the tritanopia measurement could be compared with the findings of the ocular media measurements.

The study had the approval of the research and ethics committee of St Mary's, Imperial College of Science, Technology and Medicine. Twenty one diabetic patients and 12 controls were involved in the study. The level of diabetic retinopathy was graded using the modified Airlie House classification system.<sup>30</sup> The mean level of retinopathy was 3.1 (SD 1.9) and the mean grade of maculopathy was 0.5 (SD 0.9). The mean visual acuity of the patients was 0.04 (SD 0.08) logMAR.

## RESULTS

The mean size of the largest aperture at which tritanopia was detected was 17' of arc for the control group (SD 3.4' of arc) and 24' of arc for the diabetics (SD 11' of arc). The median size of tritanopia in both groups was 18' of arc. Statistical analysis



**Figure 4** Foveal tritanopic zone versus age in control (solid) and diabetic (open) groups.

of the data revealed that the control group data were normally distributed but that the diabetic group showed a significant skew to the right. In view of this a non-parametric test (Mann-Whitney U test) was used to compare the results of the two groups. This showed a statistically significant increase in the size of the foveal tritanopic zone in the diabetic group ( $p=0.01$ ). A number histogram is shown in Figure 3. The control subjects were significantly younger than the diabetic patients (control group mean 29 years (SD 11) and diabetic group mean 46 years (SD 12)), but no age dependence was seen in the data for control subjects ( $r = 0.01$ ,  $p = 0.97$ ) or diabetic patients ( $r = -0.7$ ,  $p = 0.77$ ). The tritanopic zone size as a function of age is shown in Figure 4.

The mean Rayleigh match ratio was  $-0.26$  (SD 0.07) for the controls and  $-0.23$  (SD 0.08) for the diabetics (non-absolute log units), with no significant difference between the groups ( $p=0.24$ ). The mean macular pigment optical density for the controls was 0.30 log units (SD 0.25) and for the diabetic subjects 0.16 log units (SD 0.21), with no significant difference ( $p=0.14$ ). The optical density of the lens was significantly increased in the diabetic patients ( $p=0.001$ ), with a mean of 0.70 log units (SD 0.32) in the diabetic group and 0.30 log units (SD 0.25) in the control group.

A multivariate analysis within the diabetic group was also performed, but showed no significant association of increased size of foveal tritanopia with subject age ( $p = 0.48$ ), duration of diabetes ( $p = 0.47$ ), level of retinopathy ( $p = 0.67$ ), grade of maculopathy ( $p = 0.94$ ), previous laser treatment ( $p = 0.79$ ), Rayleigh match result ( $p = 0.75$ ), macular pigment density ( $p = 0.55$ ), or lens density ( $p = 0.88$ ).

## DISCUSSION

To date there are few published studies that have measured the extent of foveal tritanopia in a large number of subjects. The results of the colour matching experiment performed by the controls above give results that are similar to those made using other methods,<sup>9,31,32</sup> suggesting a lack of functioning S-cones in a region of approximately 20' of arc (diameter) centred on the fovea. This finding agrees with the findings of histological studies.<sup>10,11</sup>

The accurate measurement of the size of the zone is difficult. It requires good fixation and, in our experiment, multiple colour matches and a forced choice procedure to generate a psychometric function. If fixation were poor, the retinal image of the bipartite field would be incident on trichromatic as well as dichromatic (tritanopic) areas. The effect of this would be to artificially decrease the apparent size of the zone of tritanopia, as dichromatic matches would not be possible in the trichromatic areas. The finding of increased

foveal tritanopia in some of the diabetic subjects cannot therefore be explained by poor fixation. No age dependence was seen in either the control or diabetic groups and no change in the extent of foveal tritanopia has been reported in other studies.<sup>9,32</sup> As the ocular media age the formation of a small retinal image would become more difficult; this would also lead to an underestimation of the size of the foveal tritanopic zone in older subjects. Our findings for the older diabetic subjects with increased foveal tritanopia can not therefore be explained by changes in the ocular media.

Previous studies of colour response in diabetes have shown a tendency towards deficits of the S-cone pathway. The FM 100 Hue test has been used either solely or as part of a battery of tests to assess visual dysfunction in diabetes.<sup>12-25</sup> Patients with diabetes have increased error scores compared to age matched normals. The error score, however, did not correlate with the degree of retinopathy present in some studies,<sup>13,23</sup> but did in others.<sup>12,22,24</sup> The error score is raised in aretinopathic patients,<sup>14,16</sup> although less so than in retinopathic patients.<sup>24</sup> The error score does not correlate with duration of disease, blood glucose, or glycosylated haemoglobin levels.<sup>14,21</sup> The axis of the error score appears to be variable. The most consistent finding was a blue/yellow axis discrimination loss, which was present in 26%,<sup>24</sup> 66.5%,<sup>12</sup> and 70%<sup>21</sup> of subjects. Other axes (red/green) were found in some studies,<sup>20,24</sup> whereas others found a general increase in error score with no specific axis.<sup>13,14</sup>

Hardy *et al*<sup>15</sup> estimated the contribution of the optical density of the lens<sup>33</sup> to the FM 100 Hue test and used this to compare error scores of lens matched rather than age matched normals with aretinopathic IDDM patients. They found that some, but not all of the increased error score could be accounted for by changes in the ocular media. This points to a direct effect of the disease on neurosensory retinal function, rather than retinal dysfunction arising as a consequence of an embarrassed vasculature.

Dean *et al*<sup>14</sup> used a CRT based chromatic contrast sensitivity test to investigate change in protan and tritan axes in a group of diabetic subjects before and after breathing supplementary oxygen. They found that the contrast thresholds fell significantly in the diabetics but did not improve to the level of the controls after breathing oxygen, concluding that retinal hypoxia contributes to the colour vision loss (assuming that an acute rise in blood oxygen levels has no acute effect on the ocular media absorption spectra).

To avoid potential confounding effects from the crystalline lens, Kessel<sup>15</sup> studied FM 100 Hue error scores in a group of diabetic and normal pseudophakes. The diabetic patients in that study had higher error scores than the normal group, but the amplitude of the difference was smaller than that measured previously in phakic subjects, and all error scores were within the normal limits for subject age. They concluded that a proportion of the error score in phakic subjects is due to lens yellowing. However, a chromatic contrast sensitivity test has shown that red-green and blue-yellow discrimination is reduced in both diabetic and normal pseudophakes in comparison with phakic subjects,<sup>36</sup> suggesting that cataract surgery per se has an effect on colour vision (possibly due to preoperative exposure to the operating microscope light or to postoperative subclinical cystoid macula oedema). Using the same CRT based chromatic contrast sensitivity system,<sup>37</sup> Tregear found a predominantly tritanopic sensitivity loss, in retinopathic and aretinopathic patients. The tritan contrast sensitivity loss correlated well with duration of disease and was removed by estimating the contribution of the crystalline lens.<sup>33</sup> Given the strong correlation of lens yellowing with duration of disease<sup>38</sup> an ocular media induced tritan sensitivity loss is understandable. They did, however, find that in the retinopathic patients there was a loss of tritan and red/green contrast sensitivity after correction for the changes in the lens. This correlated weakly with the grade of maculopathy and the

level of retinopathy, indicating a direct effect of retinopathy on colour processing.

Greenstein *et al* assessed<sup>39</sup> Stiles two colour increment thresholds in patients with retinitis pigmentosa, diabetes, and glaucoma. They assessed S and M-cone pathway function and used the different pathological states as illustrations of diseases affecting photoreceptors, the middle and inner retina, and the inner retina alone respectively. They found preferential S-cone pathway dysfunction in all subjects, although the diabetic patients showed a more selective loss. Further work showed that the two colour increment threshold was more sensitive at detecting early visual dysfunction than the FM 100 Hue test.<sup>13</sup>

S-cone pathway dysfunction has been also been shown to occur in diabetes using a probe flash technique<sup>26,27</sup> which is free from ocular media effects. Greenstein *et al*<sup>26</sup> selected eight patients in whom the Stiles  $\pi_r$  and  $\pi_s$  mechanisms were normal from their earlier study. They measured thresholds for detecting S-cone or (L+M) cone increments and decrements and showed that in these patients the loss of sensitivity in the S-cone pathway was most likely to occur at the opponent (postreceptoral) site.

Terasaki<sup>27</sup> measured thresholds for a 50 ms 1 degree blue flash on top of a 500 ms 2 degree blue flash. A yellow background isolated the S-cone system, although neither spectral composition nor chromaticity used were reported. The mean flash on flash threshold curve shifted up as the level of retinopathy increased in patients with IDDM and was raised in those without retinopathy. In 25 patients with NIDDM, those with preproliferative retinopathy showed S-cone sensitivity loss, but those with background or no retinopathy showed no statistically significant change in sensitivity. They concluded that early functional changes in the aretinopathic IDDM patients illustrate a direct effect of diabetes on neurosensory retinal function.

The "lens corrected" visual field has been assessed<sup>40</sup> to examine the peripheral S-cone system and achromatic sensitivity in patients with diabetes compared to normal controls. Perimetric measurements were obtained to study peripheral S-cone system and achromatic sensitivity. Measures of individual lens absorption of short wavelength light were used to correct visual field sensitivity values for attenuation of the test light as a result of lens absorption, using extrafoveal absolute thresholds.<sup>38</sup> Both before and after correction for lens absorption of test spot light, peripheral field averaged S-cone system and achromatic sensitivities were not significantly reduced among patients with diabetes compared to normal controls of the same age. However, localised sensitivity losses in the visual field were found in most patients with diabetes both before and after lens absorption correction. The amount of localised loss (number of field locations with reduced sensitivity) was significantly correlated with the level of retinopathy. Statistical analysis showed that after the effects of age and duration were removed, field averaged S-cone system sensitivity in patients with diabetes was also significantly reduced as a function of increasing severity of retinopathy. The authors concluded that patients with diabetes may have areas of reduced S-cone system sensitivity before and after the onset of diabetic retinopathy.

This study has shown a normal distribution of foveal tritanopia size in control subjects, but a distribution skewed to the right in the diabetic patients, which gives statistical significance on a non-parametric test. The study, however, did not reveal any association of foveal tritanopic zone size with other colour measures or with disease attributes in diabetes. The results show an increased foveal tritanopic zone in some patients with diabetes, indicating severe local S-cone pathway dysfunction in the fovea sufficient to reduce colour vision to a dichromatic state, although in the majority of subjects tested the foveal tritanopic zone was normal in size.

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