Cone–Photoreceptor Density in Adolescents With Type 1 Diabetes

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WT and TW contributed equally to the work presented here and should therefore be regarded as equivalent authors.

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PURPOSE. Changes to retinal structure and function occur in individuals with diabetes before the onset of diabetic retinopathy. It is still unclear if these changes initially affect vascular or neural retina, or if particular retinal areas are more susceptible than others. This paper examines the distribution of cone photoreceptor density in the retina of adolescents with type 1 diabetes.

METHODS. This cross-sectional prospective study includes 29 adolescents and young adults with type 1 diabetes and no diabetic retinopathy and 44 control participants recruited at the Hospital for Sick Children. Adaptive-optics enhanced retinal imaging of the cone photoreceptor mosaic was performed in four quadrants at an eccentricity of ~7° from the fovea. After image registration and averaging, cone photoreceptors were counted and photoreceptor density was calculated. Analysis of variance with repeated measures was used to assess the differences in photoreceptor density between groups.

RESULTS. Cone density was similar in both control participants and participants with diabetes. There was a small effect of retinal hemisphere; participants with diabetes did not show the expected radial asymmetry observed in control participants.

CONCLUSIONS. Cone density in the parafoveal retina is not reduced in adolescents with type 1 diabetes.

Keywords: type 1 diabetes, retina, adaptive optics, adolescent

The neural retina of people with diabetes has been shown to have atypical function and structure. A number of electrophysiological studies have reported deficits of retinal functions in multiple layers of the retina.1–4 Structural damage to the neural retina has been reported in animals and people with diabetes. A recent study demonstrated a reduction in photoreceptor density close to the fovea of adults with type 1 diabetes (T1D).5 Animal models have shown cell death and thinning of specific retinal layers shortly after experimental induction of diabetes. Most notable, photoreceptor apoptosis occurred as early as 4 weeks after the onset of diabetes in streptozotocin-induced diabetic rats; by 24 weeks, there was a dramatic reduction of the outer nuclear layer.5 Neural apoptotic cell death was also observed in postmortem humans with diabetes and no clinically apparent diabetic retinopathy (DR).5

Diabetic retinopathy is a progressive disease. Classification of disease severity is primarily based on examination of the fundus either directly or with fundus photography. In addition, disruption of the retinal vasculature is studied using fluorescein angiography. Adaptive optics (AO) retinal imaging is a noninvasive tool that enables high resolution imaging of the retina, in vivo. Adaptive optics-enhanced imaging has demonstrated subclinical changes to the retinal vasculature in people with diabetes, including increased capillary tortuosity, potential drop-out of arterial-venule capillaries,6 increases in the wall to lumen ratio of retinal arterioles, and increased parafoveal capillary diameter in individuals with diabetes.7

Previous work in our lab with the multifocal electroretinogram has shown global retinal dysfunction but no regional susceptibility.8,9 The purpose of the current study is to examine the effect of T1D on the structural integrity of the outer retina and to determine if there are regional susceptibilities for photoreceptor deficit. Using AO-enhanced scanning laser ophthalmoscopy (AOSLO) we compare cone photoreceptor density between adolescents and young adults with T1D and control participants.

METHODS

Subjects
Adolescents and young adults with T1D and no DR were recruited from The Hospital for Sick Children. Inclusion criteria were duration of T1D of ≥5 years and age 10 to 25 years. Participants with any signs of DR were excluded based on 7-field, 30° stereoscopic fundus photographs graded by a retinal specialist according to the modified Airlie House classification system.10 Typically-developing, age-similar participants without diabetes, recruited from the community, acted as control participants. All participants with other eye diseases, hemoglobinopathy, high refractive error (worse than ±5 diopters [D]),
poor visual acuity (worse than 0.3 logMAR), neurologic disorders, and those on medications affecting retinal function were excluded. Informed consent was obtained from all participants. All procedures were conducted in compliance with the tenets of the Declaration of Helsinki and were approved by the Research Ethics Board at The Hospital for Sick Children.

**Data Acquisition**

All participants were tested at The Hospital for Sick Children. One eye was selected randomly for testing from each participant and the untested eye was occluded. All participants were assessed for visual acuity (Early Treatment of Diabetic Retinopathy Study vision chart, logMAR), contrast sensitivity (Pelli-Robson), and color vision (Hardy-Rand-Rittler pseudoisochromatic plates and the Mollon-Reffin Minimalist Test). The tested eye was anesthetized with a topical corneal anesthetic (0.5% proparacaine) and dilated pharmacologically (1% tropicamide and 2.5% phenylephrine). Refractive error was measured post dilation for the majority of participants. In participants where refraction was not performed, uncorrected visual acuity was tested to rule out large refractive errors. Axial length from the front corneal vertex to the anterior retina was measured using an I3 system—ABD ultrasound (Innovative Imaging, Inc., Sacramento, CA, USA). Glycated hemoglobin (HbA1c) values were obtained from a review of hospital charts, only the value closest in time; within 6 months of the AO imaging session was considered. Valid HbA1c values (within 6 months of testing) were not available for 15 participants with T1D.

**AO Imaging**

Retinal imaging was performed using a multimodal AO retinal imaging system (Physical Sciences, Inc., Andover, MA, USA). Retinal structure was assessed using the scanning laser ophthalmoscope (SLO) channel of the system. The SLO operated at a wavelength centered at 760 nm. Scanning in a raster pattern, the SLO operated at a frame rate of 24 Hz, creating 1024 × 1000 pixel images. The images were taken while focusing on the photoreceptor layer. The AOSLO is capable of resolving retinal structures ~3 μm apart. The imaging protocol is outlined in the fundus photograph in Figure 1. The SLO videos, 1.8° × 1.8° in size, were taken at each retinal quadrant, these areas were centered approximately 5° horizontal × 5° vertical from the fovea.

Optimal participant alignment was achieved by centering the pupil on the wavefront sensor and keeping the AO image bright while locating the field in the line scanning ophthalmoscope (LSO) channel of the AO system. The wide-field LSO beam operated at a wavelength of 915 nm, covering a field diameter of 33° on the retina.

**Participant Alignment**

Participants were seated in front of the imaging system with their head stabilized between temple and chin rests. The three-axis stage permitted the participant to be moved in the lateral (x-y axes) and axial (z-axis) directions. Continuous alignment adjustments were made by the operator throughout testing. Participants were asked to fixate on a target and to ignore the scanning laser.
Image Analysis

Each SLO video consisted of 30 to 100 frames. Videos were postprocessed to generate the final cone density value, using custom software, as follows: abnormally dim frames, normally caused by participant blinks during recording, were automatically removed. A frame from each video, with no eye movement artifacts, was manually selected. A strip registration algorithm was used to register the remaining frames to this key-frame. After frame registration, three to eight frames with the greatest correlation to the key frame were averaged together to form a final image for cone counting. Cone counting was performed only on images where the cone photoreceptor mosaic was visible in at least part of the image (Fig. 2). Cone counting was performed using custom software based on the Mahotas image processing library. A region, no smaller than 0.2° x 0.2° in size, of each registered image with high quality visualization of the cone photoreceptor mosaic was sampled. Care was taken to avoid large blood vessels when selecting the sampled areas. The selected sample was processed with a two-dimensional Gaussian filter and an intensity threshold calculated using the Otsu method.17 The number of regions exceeding the threshold was counted and divided by the size of the sampled region (measured in degrees of visual field). This value is reported as the angular cone density. The cone counting algorithm was validated against counts obtained using the graph theory and dynamic programming algorithm proposed by Chiu et al. with data from Garrioch et al. (mean difference = −5%, 95% limits of agreement = −12% to 3%). Cone density calculated as cones per millimeters squared were also calculated based on individual participant axial length measurements. In cases where axial length was not measured, axial length was estimated using a linear model relating refractive error to axial length based on participant data where both measures were available.

Data Analysis

Average cone densities for each of the four retinal quadrants were compared between participants with and without diabetes using mixed model analysis. This method allows for unequal sample sizes of each retinal quadrant to be accounted for. To account for the nonindependence of retinal quadrants imaged within the same participant, an “id” variable was included as a random-effect term. The response variable, angular cone photoreceptor density, was analyzed. The fixed effects of the model included group (diabetes versus control), quadrant (superior temporal [ST], superior nasal [SN], inferior temporal [IT], inferior nasal [IN]), distance from the fovea, and group*quadrant interaction.

RESULTS

The demographic data for all participants are shown in the Table. There were no significant group differences in age, visual acuity, refractive error, or axial length. Most participants with T1D (18/29) were diagnosed before the age of 10. It is notable that HbA1c values in participants with T1D were significantly higher (P < 0.01) than recommended guidelines (<7%).

Sample Sizes

A total of 37 participants with diabetes (11 males, 26 females) and 61 control participants (19 males, 42 females) undertook AO retinal imaging. Usable images were extracted from 29 participants with diabetes (78%) and 44 control participants (72%). The most common causes for failure to extract usable images were excessive eye movements or failure of the AO system to focus correctly on the retina. Due to technical challenges, all four retinal quadrants were not imaged for every participant, and some data were discarded due to poor recording quality. The sample sizes for each quadrant imaged are included in the Table.

<table>
<thead>
<tr>
<th>Table. Demographic Data for All Participants</th>
<th>T1D Participants, n = 29</th>
<th>Control Participants, n = 44</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, male/female</td>
<td>18/11</td>
<td>12/32</td>
</tr>
<tr>
<td>Age at testing, y</td>
<td>19.06 ± 3.0</td>
<td>18.51 ± 3.36</td>
</tr>
<tr>
<td>Age at diagnosis, y</td>
<td>8.44 ± 4.7</td>
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<tr>
<td>Duration of T1D, y</td>
<td>10.69 ± 3.9</td>
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</tr>
<tr>
<td>HbA1c, % (n)</td>
<td>8.5 ± 1.3 (14)</td>
<td>N/A</td>
</tr>
<tr>
<td>Visual acuity, logMAR (n)</td>
<td>−0.04 ± 0.14 (26)</td>
<td>−0.05 ± 0.11 (42)</td>
</tr>
<tr>
<td>Refractive error, D (n)</td>
<td>−1.52 ± 1.2 (15)</td>
<td>−0.81 ± 1.22 (30)</td>
</tr>
<tr>
<td>Axial length, mm (n)</td>
<td>23.5 ± 0.7 (23)</td>
<td>23.7 ± 0.8 (43)</td>
</tr>
<tr>
<td>Quadrants, n</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST</td>
<td>24</td>
<td>26</td>
</tr>
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<td>SN</td>
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<td>IT</td>
<td>16</td>
<td>35</td>
</tr>
<tr>
<td>IN</td>
<td>8</td>
<td>26</td>
</tr>
</tbody>
</table>

Data presented as mean ± standard deviation. Refractive error presented as spherical equivalent. N/A, not applicable.
Axial Length
Axial length was measured in 23 participants with diabetes and 43 control participants. The remaining axial length measurements were missing because of unavailability of A-scan equipment. There was no significant difference in measured axial length between control participants and those with T1D.

Cone Density
Figure 3 compares angular cone densities between groups, in each retinal quadrant. There is no difference in cone photoreceptor density between groups (T1D mean = 821 ± 116 cones/deg²; control mean = 849 ± 129 cones/deg²; P = 0.46), or as cones/mm² (T1D mean = 10,298 ± 1635 cones/deg; control mean = 10,224 ± 1631 cones/deg; P = 0.60). Post hoc analysis reveals evidence for a radial asymmetry in control participants with reduced cone densities in the temporal retina compared with nasal retina (P = 0.01); this asymmetry was not observed in participants with T1D. There was no measurable influence of blood glucose control (HbA1c), duration of diabetes, or age on photoreceptor density.

DISCUSSION
Cone photoreceptor density, in either angular or linear coordinates, was no different in the parfoveal retina of adolescents with T1D compared with control participants.

A recent study by Lombardo et al.⁵ reported cone densities that were around 10% less in a population with T1D compared with controls. In the current study, we imaged more peripheral retinal regions (~2 mm peripheral versus a maximum of 460 μm). Our study population is younger (mean age in current study = 19 years versus 41 years) and is limited to participants with no DR. A factor perhaps of particular importance is the age at which participants were diagnosed with diabetes. In the current study, the age of diagnosis was 8.5 ± 4.1 years; the age of diagnosis in the study by Lombardo et al.⁵ was 27 ± 8 years. This gives rise to the possibility that participants in the Lombardo et al.⁵ study have undergone a prolonged period of undiagnosed diabetes with potentially poor control of insulin and glucose levels. Latent autoimmune diabetes in adults, an adult onset diabetes characterized by autoimmune attack on β-cells and insulin resistance, should also be considered as an alternate diagnosis for these older patients.

The cone densities observed in this study (25th–75th interquartile range [IQR], 8493–11,683 cones/mm²) are in agreement with previously published data. The observed nasal/temporal asymmetry in participants without T1D is consistent with that observed by Osterberg⁶ and Curcio et al.⁷ Interestingly, this asymmetry was not significant in the subjects with T1D (P = 0.26 for angular densities). The intersubject variability in cone densities are similar to those published by other groups working with both AO imaging and histologic data.⁸,⁹

The ability to observe cone photoreceptor structure reveals nothing about their function. Measures of retinal function such as the multifocal electroretinogram have revealed functional deficits in those with diabetes.⁵,¹¹,²⁵ It is possible that deficits in photoreceptor function precede changes to photoreceptor structure and that the observed decrease in nasal/temporal asymmetry in our population with T1D may become more significant with increased disease duration.

A potential weakness of this study lies in the relatively small retinal regions selected for cone density analysis within each imaged area. While the region chosen for cone density analysis was placed without knowledge of the diabetic status of the participant, variations of cone density across the 1.8° imaged field may lead to a bias. An analysis of the position of the analyzed areas within each AO-SLO image did not reveal any systematic differences between participants with diabetes and controls.

Difficulties obtaining images of suitable quality in all retinal areas of all subjects may introduce another source of variation. Retinal regions where it was not possible to image the cone mosaic were of necessity excluded from this study. A chi-squared test, comparing the imaging success rates of participants with T1D and control participants for each retinal quadrant, indicated no difference in success rates between the two groups. The reasons why imaging is not always possible are not clear but may be associated with reduced tear film breakup time associated with duration of T1D.²⁶

In summary, this study demonstrates that cone photoreceptor density in the parfoveal retina of adolescents and young adults, with T1D, before the onset of retinopathy is similar to that of age similar control participants.

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References