The Relationship Between Corneal Nerve Density and Hemoglobin A1c in Patients With Prediabetes and Type 2 Diabetes

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Purpose. Decreased corneal nerve fiber density and higher corneal epithelial dendritic cells have been reported in established patients with type 2 diabetes; however, alterations in the subbasal nerve plexus in prediabetes with healthy subjects or subjects with diabetes is limited. The study aimed to determine corneal nerve fiber density and morphology and dendritic cell density between healthy subjects and those with prediabetes or type 2 diabetes.

Methods. Fifty-two subjects (aged 30–70 years) were recruited. Blood samples and body metrics were taken. Subjects were grouped as: healthy controls (hemoglobin A1c [HbA1c] < 5.7%), prediabetes (5.7–6.4%), and type 2 diabetes (> 6.4% or physician diagnosis). Central corneal subbasal nerve plexus was imaged using in vivo confocal microscopy. Corneal nerve fiber density and morphology, including interconnections and tortuosity, and dendritic cell density were assessed. Kruskal-Wallis tests were carried out to compare differences in the examined variables between groups. Spearman correlations were carried out to examine the associations between body metrics with HbA1c and corneal findings.

Results. Seventeen healthy controls, 20 subjects with prediabetes, and 15 subjects with type 2 diabetes completed this study. Central corneal nerve fiber density was significantly lower in type 2 diabetes compared to prediabetes ($P = 0.045$) and healthy controls ($P = 0.001$). No differences were found in central corneal nerve fiber interconnections, tortuosity, or dendritic cell density between groups. There was a significant association between HbA1c and corneal nerve fiber density (rho = −0.45, $P = 0.001$) and body mass index (BMI; rho = −0.30, $P = 0.04$).

Conclusions. Increased HbA1c values are associated with decreased corneal nerve fiber density across the spectrum of type 2 diabetes.

Keywords: corneal nerve, hemoglobin A1c, type 2 diabetes, prediabetes

According to the 2020 Centers for Diseases Control National Diabetes Statistics Report, 34.2 million (13%) adults have type 2 diabetes.[1] Of these, 7.3 million adults had laboratory defined diabetes, but were unaware of their condition.[1] Diabetes can result in significant health issues, including cardiovascular disease, blindness, and kidney disease.[2] It also carries a major economical toll (US $327 billion per year).[3] Being older and/or overweight are the most significant contributing factors to type 2 diabetes.[4] Prediabetes is considered a risk factor for type 2 diabetes because blood glucose is elevated and there is an increase in insulin resistance.[2,5] This condition is preventable and reversible by changes in diet and exercise. Hemoglobin A1c (HbA1c) is the American Diabetes Association (ADA) recommended diagnostic test for diagnosis of prediabetes (5.7%–6.4%) and diabetes (> 6.5%).[2,6] The ADA also recommends the use of a seven-question Diabetes Risk Test to screen adults for type 2 diabetes.[6,7] However, the survey has a relatively poor specificity (47%) for adults over age 45 years.[7] Ideally, the survey could be combined with a clinical test to improve its accuracy in early detection of individuals with diabetes.

It is well known that small peripheral nerve fibers begin to be affected even in the early diabetic state and can lead to diabetic neuropathy, including distal peripheral neuropathy.[8,9] In addition to peripheral neuropathy, up to 70% of patients with diabetes have some form of neurotrophic keratitis and poor wound healing.[10–14] Chronic hyperglycemia affects both corneal nerve function and epithelial cell repair,[15] and, consequently, asymptomatic ocular surface complications, such as keratitis, can be observed.[16] Hypoesthesia[17–20] and lower nerve fiber density[17,21–24] have been demonstrated in patients with established diabetes as well as prediabetes based on a glucose tolerance test.[25,26] Researchers have also reported an increase in central corneal dendritic cell density in patients with diabetes compared

to healthy controls. The dendritic cell density was negatively associated with corneal nerve fiber density.[21,27] This association suggests an immune mediated component to diabetic neuropathy. In addition, according to Gao et al. 2016,[28] corneal sensory nerves and dendritic cells, which is considered the central of neuro-immune interactions,[29,30] are structurally and functionally independent of each other. For example, the induction of type 1 diabetes[31] in animal models demonstrate the role of dendritic cells in modulating corneal nerve reinnervation during wound healing. However, the association between diabetic disease processes based on HbA1c concentration and other contributing factors, such as greater body mass index (BMI)[32] and other body biometrics, in corneal dendritic cells has not been investigated.

Therefore, this study aimed to (1) determine the differences in corneal subbasal nerve parameters between healthy patients and those with prediabetes and type 2 diabetes based on HbA1c and (2) examine the association between known risk factors for type 2 diabetes, such as body metrics, and corneal nerve and dendritic cell variables.

Methods

This was a cross-sectional study conducted at the University of Houston, College of Optometry, Houston, Texas, between June 2018 and August 2019. The study followed the tenets of the Declaration of Helsinki, and the University of Houston Institutional Review Board approved the research prior to study recruitment. Written informed consent was obtained after explanation of the nature and possible consequences of the study from all subjects prior to their participation.

A sample size was calculated based on corneal nerve fiber density between healthy controls and patients with type 2 diabetes (21.5 ± 7.0 mm/mm^2 vs. 16.1 ± 5.7 mm/mm^2),[33] indicating that at least 15 subjects in each group in this study was sufficient to show a significant difference mentioned above in central corneal nerve fiber density with 95% confidence and 80% power. Potential subjects were recruited from the University of Houston, College of Optometry, the University Eye Institute, Texas Obesity Research Center and the surrounding community in Houston, Texas, by way of email, flyers, and word of mouth.

Subjects were between 30 and 70 years of age. Exclusion criteria included type 1 diabetes, pregnancy, autoimmune diseases, eye diseases, such as glaucoma, cataract, or macular degeneration, as well as previous retinal and anterior segment surgeries, which may affect the corneal structure. A slit lamp examination with white light was conducted to confirm the health of the anterior segment. In addition, the presence or absence of diabetic retinopathy was recorded via retinal fundus photography. Contact lens use was recorded.

In order to calculate the BMI, height and weight were measured. Body fat percentage (Tanita Body Composition Analyzer, Arlington Heights, IN, USA), hip, waist, and neck circumference were measured. The waist to hip ratio was calculated. Subjects were asked to complete the ADA Diabetes Risk Test[6,34] and Ocular Surface Disease Index (OSDI)[35] in order to assess diabetes risk and dry eye symptoms. The ADA Diabetes Risk Test contains seven questions based on age, sex, family history of diabetes, physical activity level, and BMI. A total score of 5 or higher (out of 11) is considered at-risk for having type 2 diabetes.[6,34] The OSDI questionnaire contains 12 questions in 3 areas: symptoms, visual function, and the environmental contributing factors affecting the eye comfort over the past week.[35] A total score of > 21 of 100 is considered having at least mild-moderate of dry eye symptoms.[35]

To determine HbA1c levels, the subject's finger was pricked using a standard commercial lancing device to collect < 0.5 mL of peripheral blood. This blood sample was used to quantify HbA1c concentration and was determined by using the Siemens HbA1c analyzer (Siemens, Munich, Germany). The final subject grouping was based on the A1c testing conducted during the study: healthy controls: < 5.7%, prediabetes (5.7%–6.4%), and type 2 diabetes (> 6.4% or physician diagnosis).

In vivo laser scanning confocal microscopy (Heidelberg Retinal Tomograph II with Rostock Corneal Module; Heidelberg Engineering GmbH, Heidelberg, Germany) was conducted on the left eye to image the central corneal subbasal nerve plexus. In brief, a drop of gel (Genteal; Alcon, Fort Worth, TX, USA) was applied between the sterile TomoCap (Heidelberg Engineering GmbH) and the tip of the objective lens. Prior to the confocal microscopy, the left eye was anesthetized with a drop of 0.5% proparacaine hydrochloride (Alcon) and an artificial tear was placed in both eyes to help with comfort.[36,37] Subjects were asked to look at a fixation light in front of their right eye and the examiner manually located the central cornea on the left eye based on the external alignment (reported by subjects) and the vertical appearance of the nerves. Up to 100 images were taken using the volume mode for each subject; the two clearest and representative 400 × 400 μm^2 images with < 20% overlapped with each other[38] were used for analysis. Corneal subbasal nerve fiber density (NFD; mm/mm^2) using NeuronJ[39] and nerve fiber interconnections (NFI), as the number of bifurcations of the corneal nerve per nerve mm/mm^2, were quantified as described previously.[36,37] Corneal nerve fiber tortuosity (coefficient) was also examined automatically by uploading the jpg file into a custom MatLab (MathWorks, Natick, MA, USA) program developed by BioImLab, Spain.[40] In addition, the numbers of corneal dendritic cells were counted manually and the average from the two images was recorded and presented as cells/mm^2.[41] The percentage of corneal epithelial dendritic cells contacting the nerve was also counted and calculated. An investigator masked to subject status (author C.C.) performed all subjective nerve and dendritic cell measurements.

Statistical Analysis

The data were analyzed using SPSS version 25 (SPSS for Microsoft, Chicago, IL, USA). Kruskal-Wallis tests were carried out to compare differences in continuous variables between the groups. Mann-Whitney *U* tests were used for post hoc analysis with Bonferroni adjustments. Fisher's Exact tests were used to examine differences in categorical variables, such as sex, race, and use of contact lenses. Spearman correlation tests were carried out to examine the associations between body biometrics, HbA1c concentration, and in vivo confocal microscopy findings. Potential cofounding factors, such as age, were included in the linear mixed models as a covariant to examine if it affects the findings in both body biometrics, HbA1c and concentration, and corneal nerve variables. In vivo confocal microscopy findings were then used as the dependent variables, and demographics, body biometrics, and blood tests ($P < 0.10$) were used as independent variables for multiple backward linear regres-

Table 1. Subject Demographic Data

	Healthy Controls ($n = 17$)	Prediabetes ($n = 20$)	Type 2 Diabetes ($n = 15$)	*P* Value
Hemoglobin A1c (%)	**5.3 (5.1–5.4)**	**5.8 (5.7–6.0)**	**7.1 (6.7–7.5)**	**<0.001**
Age	**43.0 (37.0–53.5)**	**54.0 (41.4–63.3)**	**58.0 (53.0–61.0)**	**0.02**
Sex	5M: 12F	4M: 16F	4M: 11F	0.79*
Race	2A: 4B: 10W	6A: 2B: 12W	4A: 3B: 7W: 1O	0.44*
Soft contact lens wear (Yes:No)	6: 11	3: 17	3: 12	0.29*
OSDI score (0–100)	4.2 (1.0–20.8)	9.6 (6.3–18.2)	12.5 (8.3–18.8)	0.46

Data presented in median and interquartile range (IQR); *P* values were generated from Kruskal-Wallis tests or Fisher's Exact tests (*). Bold = $P < 0.05$. A, Asians; B, Black/African Americans; W, White/Caucasian; O, Other race; OSDI, The Ocular Surface Disease Index.

Table 2. Body Biometrics, ADA DM Risk Test and Glucose Concentration Between Healthy Controls, Prediabetes and Type 2 Diabetes

	Healthy Controls ($n = 17$)	Prediabetes ($n = 20$)	Type 2 Diabetes ($n = 15$)	*P* Value
Body mass index	**25.3 (22.4–29.4)**	**29.3 (25.6–34.2)**	**29.6 (29.0–33.1)**	**0.02**
Body fat (%)	**25.3 (23.0–36.5)**	**39.1 (20.6–45.5)**	**39.7 (33.8–41.3)**	**0.03**
Neck circumference (cm)	36.0 (23.5–39.0)	36.0 (31.4–38.8)	38.0 (35.0–42.5)	0.19
Waist circumference (cm)	92.4 (81.5–98.1)	99.7 (99.8–110.8)	102.0 (91.5–111.0)	0.056
Hip circumference (cm)	**104.0 (98.3–110.5)**	**114.0 (103.5–118.4)**	**114.0 (107.0–117.8)**	**0.048**
Waist: hip ratio	0.83 (0.79–0.91)	0.87 (0.83–0.90)	0.89 (0.85–0.95)	0.31
ADA DM Risk Test (0–11)	**3.0 (1.0–4.5)**	**5.0 (4.0–5.8)**	**6.0 (5.0–7.0)**	**<0.001**

Data presented in median and interquartile range (IQR); *P* values were generated from Kruskal-Wallis tests; Bold = $P < 0.05$. ADA DM Risk Test, American Diabetes Association Diabetes Mellitus Risk Test.

sion models. The final models were determined based on the maximum number of significant factors and the higher R^2 value. Significance was determined at $P < 0.05$.

Results

Subject Demographics

Seventeen healthy subjects, 20 subjects with prediabetes, and 15 subjects with type 2 diabetes, based on HbA1c concentration, completed the study. In this study, 75% (15 of 20) of the prediabetic subjects who were stratified by HbA1c concentration were not aware of having prediabetes. Of the 5 prediabetic subjects, the average self-reported disease duration was 5.2 ± 3.4 years. In terms of the subjects with type 2 diabetes who were stratified by HbA1c concentration, 2 subjects reported they had prediabetes and another 2 subjects were not aware of having type 2 diabetes. Of the 11 subjects with type 2 diabetes who knew they had diabetes, the average disease duration was 9.2 ± 7.5 years. Only two subjects with diabetes mellitus (DM) had moderate retinopathy with retinal edema and none of the subjects reported having kidney and neuropathy. Age was not confounded the HbA1c concentration ($P = 0.37$).

The use of exogenous insulin was reported by two of the subjects with diabetes. Ten diabetes and two prediabetes subjects used metformin. Subjects in the diabetic group were about 15 years older than healthy controls (post hoc: $P = 0.03$), but there was no significant difference in age between healthy controls and prediabetes or prediabetes and diabetes (all $P > 0.05$). There were no differences in sex, race, and self-reported current soft contact lens wear ($P > 0.18$; Table 1). Subjects reported trace dry eye symptoms based on the total score of the OSDI, and there was no difference between groups ($P = 0.46$).

Risk Factors Associated with Diabetes

Subjects with type 2 diabetes had a higher BMI and hip circumference (both $P = 0.04$) compared to healthy controls. There were no significant differences between the healthy and prediabetics subjects or between the prediabetic and subjects with type 2 diabetes (Table 2). Subjects with prediabetes and diabetes had a higher body fat percentage when compared to the healthy controls ($P = 0.045$ and $P = 0.046$, respectively), whereas the differences between subjects with prediabetes and diabetes did not reach a significant level ($P > 0.99$). There was no significant difference in neck and waist circumference or waist to hip ratio among the three groups (Table 2).

The ADA DM Risk Test score increased with diabetes progression (Table 2). There was a trend of a higher score in the diabetes group compared to the prediabetes group ($P = 0.054$), with both diabetic and prediabetic groups having a significantly higher score than healthy controls ($P < 0.001$ and $P = 0.006$, respectively; Table 2). In addition, age was associated with ADA DM Risk Test score (rho = 0.55, $P < 0.001$) but not with body biometrics, such as BMI ($P = 0.22$).

In Vivo Confocal Microscopy Findings

Four subjects did not have 2 clear images available for nerve analysis, thus a total of 16 healthy controls, 18 subjects with prediabetes and 14 subjects with diabetes were included in this analysis. Central corneal nerve fiber density was significantly lower in subjects with diabetes compared to those with prediabetes ($P = 0.04$) or healthy controls ($P = 0.003$; Table 3). There was no difference between healthy controls and subjects with prediabetes ($P = 0.18$; Table 3). There was no difference in central corneal nerve fiber interconnection, tortuosity, dendritic cell density, or the percentage of dendritic cells contacting nerves among the groups ($P > 0.20$; Table 3).

Central corneal nerve fiber density was negatively associated with HbA1c concentration (rho = −0.43, $P = 0.002$; Fig. A) and was still significant after the removal of the outlier (rho = −0.42, $P = 0.003$). Corneal nerve fiber density was also associated with BMI (rho = −0.30, $P = 0.04$; Fig. B) and

Table 3. In Vivo Confocal Microscopy Findings at the Central Cornea of Healthy Controls, Subjects with Prediabetes, and Subjects with Type 2 Diabetes

	Healthy Controls ($n = 16$)	Prediabetes ($n = 18$)	Type 2 Diabetes ($n = 14$)	*P* Value
Nerve fiber density (mm/mm^2)	**14.1 (12.5–18.4)**	**12.0 (8.6–15.5)***	**8.1 (4.2–10.9)****	**0.004**
Nerve fiber interconnection (numbers/mm of nerve)	5.2 (4.2–7.8)	5.8 (3.2–8.0)	4.0 (0.4–6.5)	0.20
Nerve fiber tortuosity (score)	0.18 (0.11–0.48)	0.21 (0.10–0.39)	0.25 (0.0–0.52)	0.94
Dendritic cell density (cells/mm^2)	28 (9–59)	23 (9–39)	16 (9–53)	0.66
% **dendritic cell** contacting nerve (%)	27.8 (14.3–37.0)	33.3 (26.9–42.9)	36.7 (14.4–66.7)	0.57

Data presented in median and interquartile range (IQR); *P* values were generated from Kruskal-Wallis tests; Bold = $P < 0.05$. *Post hoc analysis: $P < 0.05$ compared to healthy controls; **Post hoc analysis: $P < 0.01$ compared to healthy controls.

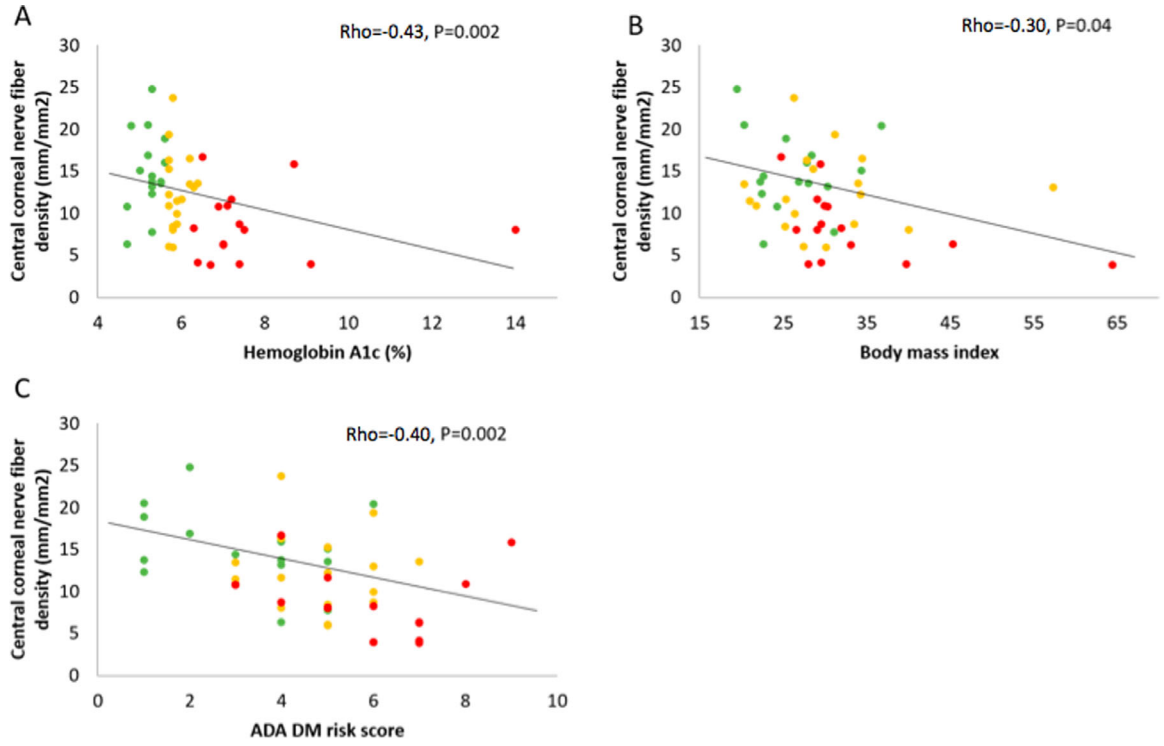

Figure. Associations between corneal nerve fiber density and hemoglobin A1c (**A**), body mass index (**B**) and American Diabetes Association Diabetes Risk test score (**C**). *Green* = healthy controls; *yellow* = subjects with prediabetes, and *red* = subjects with type 2 diabetes.

ADA DM Risk Test score (rho = −0.40, $P = 0.005$; Fig. C). There was no effect of age ($P = 0.59$) on the associations with corneal nerve density mentioned above as assessed by linear mixed models as a covariant.

Because corneal nerve fiber density was significantly associated with several risk factors and/or biomarkers of type 2 diabetes, but not age, a multiple linear regression model was conducted. Central corneal nerve fiber density was associated with BMI ($\beta = -0.28$, $P = 0.047$) and HbA1c concentration ($\beta = -0.29$, $P = 0.04$) with an R^2 of 0.19, indicating that BMI and HbA1c explained 19% of the variance in the corneal nerve fiber density. The equation generated for the central corneal nerve fiber density = 23.46 −0.28* BMI-0.29* HbA1c. The ADA DM Risk Test was not a significant predictor of corneal nerve fiber density.

Discussion

Reduced central corneal nerve fiber density was found in subjects with type 2 diabetes compared to subjects with prediabetes and healthy controls, as seen in the literature.[25,26,33,42,43] However, this study examined the corneal nerve fiber density in subjects with prediabetes based on HbA1c, which was found to be higher than in subjects with diabetes, but with no significant difference compared to healthy controls. This is consistent with the De Clerck et al. findings that categorized diabetic disease based on the oral glucose tolerance test.[44] This could be due to the current sample size that was calculated based on the differences in corneal nerve fiber density between the healthy and diabetes groups. According to backward post hoc analysis, a sample size of 26 in each group is required to demonstrate a difference in central corneal nerve fiber density between healthy

controls and subjects with prediabetes with $P < 0.05$ and 80% power. However, based on the Asghar et al. findings, a total sample size of four in each group should be sufficient to detect the difference in corneal nerve fiber density between healthy controls and subjects with prediabetes.[25]

There was a significant association between HbA1c and central corneal nerve fiber density, which further suggests that reduced corneal nerve fiber density begins to occur in the early stages of development toward type 2 diabetes. Although it was noted that subjects with type 2 diabetes were 15 years older than the healthy controls, studies examining the effect of aging on corneal nerve fiber density are contradictory. Based on the analysis, age was not a covariant affecting the corneal nerve fiber findings between groups. Some studies show that decreased nerve fiber density examined using in vivo confocal microscopy was associated with aging,[45,46] whereas other studies found that corneal nerve fiber density (mm/mm^2) was independent to aging in a healthy population.[47–51] However, Niederer et al. did not exclude subjects who may have systemic health issues.[45] Tavakoli et al. showed that nerve fiber density significantly decreased with aging in women (−0.06 mm/mm^2 per year) but not men (−0.045 mm/mm^2 per year) in a population age ranged between 8 and 82 years.[46] In addition, Dehgani et al. found a linear decrease in corneal nerve fiber density with age (−0.05 mm/mm^2 per year) in a senior population (52 ± 15 years old).[52] Based on the Tavakoli et al. and Dehgani et al. findings,[46,52] the differences in corneal nerve fiber density in this study between healthy and prediabetes (age difference: 11 years) or type 2 diabetes (15 years) were 0.50 to 0.66 mm/mm^2 and 0.68 to 0.90 mm/mm^2, which is less than the standard error of the differences of our results (1.55–1.62 mm/mm^2) and is unlikely contributing the differences between groups. In the current study, based on the inclusion of age as a covariant, age did not affect the association between corneal nerve fiber association and HbA1c concentration. This indicates that reduced corneal nerve fiber density may be an indicator of increased HbA1c concentration, independent of aging, and may serve as an early anterior segment alteration biomarker, which may appear prior to neuropathic changes in other areas of the body and end stage complications in type 2 diabetes, such as macular edema and diabetic retinopathy. However, it requires confirmation in future studies.

A higher score of the ADA DM Risk Test has been suggested in screening type 2 diabetes.[34] However, the test has sensitivity that ranged between 76% and 86% and specificity ranged from 38% to 59% depending on the three largest US based databases.[7] The ADA also suggested the addition of HbA1c concentration because it is a more accurate biomarker of detecting diabetes. However, HbA1c was not consistently used in the database to determine the sensitivity and specificity of the survey.[7] This indicates that this survey along with another biomarker may further improve its accuracy in early detection of individuals with prediabetes and type 2 diabetes based on HbA1c concentration.[53] It was previously shown that corneal nerve fiber density of 12 mm/mm^2 provided area under the curve (AUC) of 0.68 with the 69% sensitivity and 63% specificity in predicting established type 2 diabetes.[54] This suggests that corneal nerve fiber density along with the use of ADA DM Risk Test may further improve the sensitivity and specificity of detection in type 2 diabetes. Therefore, the diagnostic ability of the combination of corneal nerve fiber density and the ADA DM Risk Test should be assessed in future studies with a larger sample size.

Increased BMI was also associated with lower nerve fiber density. Higher BMI, body fat percentage, and hip circumference are known risk factors of diabetic status,[55,56] and are consistent with our findings. BMI is the standard reference for obesity[32] and higher amounts of visceral fat by measuring waist circumference or waist to hip ratio[57] have been shown to be associated with type 2 diabetes,[4,32,58–60] even though it is not consistent across all races. This is also confirmed with the backward linear regression model that increased the body metric coefficients along with HbA1c concentration to predict corneal nerve fiber density. Previously, our laboratory reported a correlation between HbA1c concentration and both waist and hip circumference but not BMI in the same population.[61] It indicated that increased body biometrics may be associated with poorer adipose function and increased inflammation in metabolic syndrome.[62,63] Even though HbA1c concentration and body biometrics, including BMI, did not affect the fifth percentile normative values for corneal nerve findings in a healthy population,[46] obesity and metabolic syndrome may have an effect on corneal nerve fiber density.

Dendritic cell density has been examined as a biomarker of corneal immune tone[64] during diabetes. In order to examine the neuro-immune cross-talk, the percentage of dendritic cells contacting nerves were examined.[65] There was no difference in dendritic cell density, contrasting with other studies,[21,27] nor in the percent of cells contacting the nerves between groups in this study. Qu et al.[21] only recruited subjects with type 2 diabetes who had significant corneal punctate epitheliopathy from a hospital setting, whereas Tavakoli et al.[27] recruited subjects with diabetes with neuropathy. Therefore, the association between dendritic cell density and diabetic status in these studies could be due to the advanced corneal and neuropathic conditions observed in their subject groups as compared to our relatively healthy, early stage of subjects with type 2 diabetes, as recruited in the current study.

There were two subjects with diabetes that reported use of exogenous insulin, indicating that their diabetes cannot be controlled by changing their diets and/or use of metformin. There was no difference in corneal findings after the exclusion of the two subjects who used insulin. This is consistent with a previous study[66] that indicated no difference in corneal structure between subjects regardless of diabetes duration or treatments. However, the effect of using insulin in type 2 diabetes on corneal nerves should be further investigated.

Although measures were taken to ensure minimal confounding influences, there are several limitations of this study. First, sex and race are well-established to be associated with body biometrics, including BMI, body fat percentage, and body circumference[67,68] and are the leading risk factors of type 2 diabetes.[2,69] Although there was no difference in the race and sex distributions between groups in this study, further analysis of the effects of sex and race on ocular findings in each group should be addressed in future studies with bigger sample sizes and balanced race and sex distributions in each group. In addition, due to the small sample size of HbA1c matched to self-known subjects with diabetes ($n = 11$), there is insufficient power to detect the effects of disease duration on the ocular surface findings. Other limitations include body metrics measurement, which can be taken with more accurate methods, such as hydrostatic

weighing and dual-energy X-ray absorptiometry.[70] However, BMI and body circumference methods were chosen for this study due to patient comfort and capability to implement in the research laboratory. In regard to acquisition of corneal confocal imaging in this study, we used two images, whereas research has suggested that 4 to 6 nonoverlapping or 8 < 20% nonoverlapping images should be used for corneal nerve analysis,[26,38,71] because it can improve accuracy and repeatability (1 image vs. 4.3 images with < 5% error).[71] Although the volume scan is not recommended to determine corneal nerve parameters,[71] the methods used in this study allowed us to maximize patient comfort, decrease contact time, and be user-friendly to those, particularly clinicians, who are not experienced in using in vivo confocal microscopy. In addition, because all the subjects were examined using the same methodology, the results are comparable between groups in this study but may not be able to compare to the other studies that reported corneal nerve fiber density. Other corneal nerve analysis software, such as CCMetrics or ACCmetrics, may provide more information regarding the changes in corneal nerves in different diabetic stages and is considered for use in future studies.

In summary, this is the first study that has examined the effect of prediabetes and type 2 diabetes based on HbA1c concentration on in vivo confocal microscopy findings. Corneal nerve fiber density was negatively associated with HbA1c concentration, indicating that corneal nerves may be a useful screening tool to detect prediabetes and early type 2 diabetes. The impacts of sex, race, duration of the disease, and use of insulin on the in vivo confocal microscopy findings are required to be investigated in future studies. Decreased corneal nerve fiber density may be a useful screening tool to detect prediabetes and early type 2 diabetes.

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