Corneal Function during Normal and High Serum Glucose Levels in Diabetes

Nancy A. McNamara, Richard J. Brand, Kenneth A. Polse, and William M. Bourne

PURPOSE. To assess corneal structure and the effects of acute hyperglycemia on corneal function in subjects with type 1 diabetes.

METHODS. Twenty-one diabetic and 21 nondiabetic volunteers of similar age were recruited. Baseline measurements of intraocular pressure (IOP), corneal thickness (CT), corneal autofluorescence (CAF), corneal sensitivity (CST), central and temporal endothelial cell density (DenC and DenT), and coefficient of variation in cell area (CVC and CVT) were taken. Corneal edema was induced, and the percent recovery per hour (PRPH) from hypoxic edema and endothelial permeability to fluorescein were determined. These procedures were done twice in the diabetic subjects under controlled euglycemic (EG) and hyperglycemic (HG) conditions, and once in control subjects while they were fasting.

RESULTS. Substantial differences in baseline measurements were found for IOP, CT, CAF, CST, DenC, and CVT. The mean ± SE corneal swelling in the HG diabetic subjects (51.6 ± 2.3 μm) was less when compared to the swelling in the EG diabetic subjects (56.2 ± 1.87 μm, P = 0.05) and the control subjects (58.9 ± 1.56 μm, P = 0.011). During euglycemia, the mean ± SE PRPH was less in diabetic subjects than in control subjects (65.0 ± 3.20 versus 73.8 ± 1.81%/hour, P = 0.02) but did not differ in diabetic subjects under EG and HG conditions (65.0 ± 3.20 versus 67.7 ± 3.1%/hour, P = 0.56). No significant differences were noted between groups in endothelial permeability.

CONCLUSIONS. In addition to differences in baseline corneal structure, diabetic subjects showed less corneal swelling and reduced corneal recovery from hypoxia than did control subjects. During acute hyperglycemia, corneal swelling was less than during euglycemia in diabetic subjects, which suggests that hyperglycemia affected corneal hydration control.

Diabetes mellitus is associated with structural changes in corneal epithelial and endothelial cells. Although it is not known whether these changes directly affect corneal function, clinical evidence shows that patients with diabetes have functional abnormalities such as recurrent corneal erosion, persistent epithelial defects, and increased endothelial permeability to fluorescein after intraocular surgery. Diabetes has also been shown to increase the risk for corneal decompensation after argon laser iridectomy. Unfortunately, the mechanisms responsible for these changes in corneal structure and function are not well understood.

Hyperglycemia is thought to be a major factor in the pathogenesis of diabetes, and several hyperglycemia-induced biochemical processes have been implicated. Of particular interest is recent evidence linking elevated glucose to reduced Na⁺,K⁺-ATPase activity in corneal endothelial cells. In vitro studies show that polyhydroxyl compounds (glucose, galactose, galactitol, sorbitol, or xylitol) inhibit Na⁺,K⁺-ATPase activity in cultured bovine corneal endothelial cells, whereas in vivo studies show reduced Na⁺,K⁺-ATPase activity in the corneal endothelium of diabetic rabbits after only 10 weeks of alloxan-induced hyperglycemia. Because this enzyme is a major component of the endothelial fluid pump, it is not surprising that these same diabetic rabbits also showed greater baseline corneal thickness, decreased corneal swelling response, and slower recovery from hypoxic edema than did nondiabetic rabbits.

Although animal studies suggest that exposure to high glucose in vitro and hyperglycemia in vivo can influence endothelial function, human studies have not provided clear evidence to support these animal data. For example, Weston and colleagues did not find lower corneal function, as measured by recovery from hypoxic edema, in patients with dia-
betes; however, others have demonstrated significant reductions in corneal deswelling when comparing patients with diabetes with control subjects.19-21 It is possible these disparate findings could be caused by the differences that exist among the methods used to measure corneal recovery or by the degree of the advance of the disease: the diabetic patients selected for the latter studies may have had more advanced diabetes or more hyperglycemia at the time of testing.

In the present study we sought to provide a more complete understanding of the mechanisms that lead to alterations in the diabetic cornea by examining how corneal structure and function differ in human diabetic and nondiabetic subjects and by assessing whether acute hyperglycemia affects endothelial function.

To investigate differences in corneal structure between diabetic subjects and control subjects, we compared measurements of corneal sensitivity, corneal autofluorescence, corneal thickness, and endothelial morphology obtained from 21 subjects with insulin-dependent (type 1) diabetes mellitus and 21 nondiabetic control subjects. To compare corneal function in these diabetic and nondiabetic subjects, we measured the corneal swelling response to hypoxia, the exponential recovery from hypoxic edema, and endothelial permeability to fluorescein. These comparisons of corneal structure and function provided information about the chronic effects of diabetes.

Because previous studies of corneal function in human diabetic subjects were not performed under controlled glucose conditions, the effects of acute hyperglycemia on corneal function have not been directly assessed. To provide clues about the metabolic effects of glucose on endothelial cell function and the role of the glycemic state in diabetic subjects, we also compared the corneal swelling response to hypoxia, the exponential recovery from hypoxic edema, and the endothelial permeability to fluorescein in the same diabetic corneas under randomly ordered normal and high serum glucose conditions.

METHODS

Subjects

We used a computer search to identify Mayo Clinic employees and patients between the ages of 21 and 60 years with insulin-dependent diabetes and signs of diabetic retinopathy. Careful review of more than 350 patient records yielded 91 eligible diabetic subjects who were invited by mail to participate. Respondents were scheduled for a screening visit, which was completed by 27 volunteers with diabetes. Of these, 21 subjects (7 female, 14 male) between 23 and 54 years of age (mean ± SD = 39.6 ± 8.8 years), who had background or proliferative diabetic retinopathy and met all eligibility requirements, participated in the study. We also recruited 21 volunteers without diabetes, with normal retinas, by posting study announcements on the Mayo Campus. To ensure that diabetic and nondiabetic subjects were of similar age, we stratified diabetic participants into three age-range categories (18-27, 28-38, and 39-60 years) and entered an equal number of nondiabetic subjects into each category. Thus, our control group was between 21 and 53 years of age (mean ± SD = 38.5 ± 9.7 years: 12 female, 9 male). Volunteers, with or without diabetes, with a history of contact lens wear, ocular surgery (including laser), glaucoma, trauma, ptosis, corneal opacities, fluorescein allergy, severe keratoconjunctivitis sicca, or systemic disease other than diabetes affecting the eyes were excluded, as were current users of topical ophthalmic medications or systemic medications that might affect corneal thickness (such as birth control, diuretics, or beta-blockers). This study observed the tenets of the Declaration of Helsinki and was approved by the Mayo Clinic Institutional Review Board.

Patients with diabetes were classified as type 1 (insulin-dependent) if at least two of the following criteria were present: significant ketonuria, insulin treatment started within 1 year after diagnosis, age of diagnosis more than 40 years, and weight below 110% of ideal weight.22 All the diabetic subjects in our study met at least two of these clinical classification criteria. In addition, we estimated pancreatic islet beta-cell function by measuring the stimulated C-peptide level of each diabetic patient during induced hyperglycemia. In the absence of impaired insulin production, plasma C-peptide levels increase in accordance with pancreatic insulin production after stimulation with glucagon or after a meal. An assay level of less than 0.32 nmol/l after stimulation is representative of the minimal or absent beta-cell function found in type 1 diabetes.22 Twenty of 21 subjects had C-peptide levels below 0.02 nmol/l; the remaining subject's C-peptide level was 1.6 nmol/l.

Instrumentation

Pachometry. Corneal thickness was measured using a modified Haag-Streit optical pachometer equipped with fixation lights for improved measurement accuracy. This instrument, which has been more fully described elsewhere, has a potentiometer linked to an IBM-compatible microcomputer through an analog-to-digital converter to allow direct entry of the readings into computer memory for accurate time monitoring. Each corneal thickness measurement was the mean of 10 successive pachometry readings. Two measurements were completed on each eye at each session, and these were averaged to provide a single value for the eye at that session.

Fluorophotometry (Endothelial Permeability Measurement). Central corneal autofluorescence and endothelial permeability to fluorescein were estimated using a two-dimensional scanning ocular fluorophotometer. This device uses a 488-nm argon laser for excitation, and the emission window accepts fluorescent wavelengths between 515 and 600 nm, as previously described. To estimate endothelial permeability, fluorescein dye was instilled topically, and fluorescein concentrations in the cornea and aqueous humor were monitored over a period of several hours. Each fluorophotometric scan produced a profile of corneal and aqueous humor fluorescence. At each measurement session we scanned both eyes twice and calculated the average corneal and aqueous humor fluorescence for each eye. Using these data, we estimated two different aspects of endothelial permeability per study visit; fluorescence measurements obtained in the morning were used to estimate permeability during contact lens-induced hypoxia (AM $P_{\text{endo}}$), and those obtained in the afternoon were used to estimate the permeability during normal corneal oxygen conditions (PM $P_{\text{endo}}$). A more detailed description of the experimental design and the method used to estimate AM and PM $P_{\text{endo}}$ is provided under Experimental Procedures.

Endothelial Specular Microscopy. A wide-field, contact specular microscope was used to obtain endothelial photographs of the central and temporal cornea. The negatives were projected at 500X magnification, and the apices of 100 endothelial cells in each eye were digitized. We calculated the mean and standard deviation of the endothelial cell size, the endo-
Corneal Aesthesiometry. To estimate paracentral corneal sensitivity, we used the Cochet–Bonnet aesthesiometer. This instrument consists of a 0.12-mm diameter nylon monofilament encased in a cylinder that can be extended to lengths between 5 mm and 60 mm, which correspond, to pressures ranging from 200 to 11 mg/0.0113 mm² (the cross-sectional area of the filament). To begin the measurement procedure, the examiner advanced the fully extended aesthesiometer filament perpendicularly to a corneal location about 2 mm superior to the 6 o’clock limbal position, until a slight flexure of the filament was first perceived. This corneal position was chosen to avoid apprehension and/or a reflex blink often provoked when the central cornea is stimulated. The patient was instructed to tap a pen once against the examination table to indicate a positive corneal sensation, while the examiner repeatedly stimulated the cornea at a variable rate with decreasing fiber lengths (increasing pressures). Four to six contacts were made with the cornea at each fiber length, and blank runs were introduced (the filament was advanced but the cornea was untouched) to assess the reliability of a patient’s responses. Testing continued until the subject responded to at least 50% of the trials at a given length; additional testing with both longer and shorter fiber lengths was conducted to ensure that the correct response had been elicited.

Experimental Procedures

Screening Exam

We obtained informed consent from all subjects and verified the fulfillment of inclusion and exclusion criteria by completing an ophthalmic examination, including tests for visual acuity, aesthesiometry, applanation tonometry, slit-lamp biomicroscopy, and dilated ophthalmoscopy. At the conclusion of the screening examination, we measured each volunteer’s anterior chamber volume using a photogrammetric technique and obtained specular micrographs of the central and temporal corneal endothelium. All subjects were then scheduled for admission to the General Clinical Research Center at St. Mary’s Hospital for a 24-hour study of corneal function.

24-Hour Visit: Overview. The corneal swelling response (CSR), the percentage of recovery per hour (PRPH) in corneal deswelling, and the endothelial permeability to fluorescein under hypoxic (AM Pendo) and normal oxygen (PM Pendo) levels were assessed during pharmacologically induced euglycemic (80–120 mg/dl) and hyperglycemic (200–250 mg/dl) conditions in each diabetic subject, and during fasting glucose conditions in each nondiabetic subject (80–120 mg/dl). Each volunteer with diabetes was admitted to the research center for 24 hours on two separate occasions. During each visit, the diabetic subject’s blood glucose level was controlled by infusion of intermediate-acting insulin in the morning (duration of action, from 10 to 48 hours), and their last dose of regular insulin at lunch time (duration of action, from 3 to 8 hours) on the day of admission. We measured weight and height on admission, and drew blood samples to test for pregnancy, hemoglobin level, and percentage of glycosylated hemoglobin.

On that evening, we placed an 18-gauge catheter in the subject’s lower forearm vein to obtain blood samples for plasma glucose measurements. These were made every 30 to 60 minutes for the remainder of the study visit. An identical catheter was also placed in the mid-forearm of the other arm of each diabetic subject so we could regulate their plasma glucose level by infusing intravenous insulin (Humulin-R) and 20% dextrose. We measured the open-eye, steady-state corneal thickness (OESS) by making two replicate corneal thickness measurements every 20 minutes for approximately 2 hours. Two measurements of central corneal autofluorescence were also taken on each eye and averaged to give a single value for the eye. After these measurements, at approximately 6:00 PM, all subjects ate a standard mixed meal (636 calories; 48% carbohydrate, 52% fat, and 19% protein).

Euglycemic Visit: After the evening meal during the euglycemic (EG) visit, we lowered the glucose concentration to our goal range of 80 to 120 mg/dl using a variable intravenous infusion of insulin. To maintain a glucose concentration between 80 mg/dl and 120 mg/dl, the insulin infusion rate was adjusted throughout the euglycemic study visit using a reduced version of a previously published algorithm. Supplemental dextrose was given only if plasma glucose concentrations fell below 80 mg/dl.

Hyperglycemic Visit: On the hyperglycemic (HG) visit, we began a basal insulin infusion immediately after the meal at a rate of 0.15 mU/kg per minute. Approximately 3 hours after the meal, small amounts of 20% dextrose were infused to raise and then maintain serum glucose at the target level between 200 mg/dl and 250 mg/dl. The basal insulin infusion was continued throughout the 24-hour study period, and no additional insulin was infused on the hyperglycemic day unless plasma glucose concentrations exceeded 300 mg/dl.

Using this strategy, we obtained plasma glucose concentrations of between 80 mg/dl and 120 mg/dl on the euglycemic day and between 200 mg/dl and 300 mg/dl on the hyperglycemic day. Although it is recognized that these two approaches do not result in a precise clamp of glucose, they resulted in substantial differences in blood glucose levels during the hyperglycemic and euglycemic study visits. In addition, we anticipated that free insulin concentrations differed minimally on the two study days, because the average basal insulin infusion required to maintain euglycemia was equivalent to the basal concentration.
insulin infusion rate used in our protocol during the hyperglycemic visit (0.15 mU/kg per minute). Thus, any differences in corneal function observed between the hyperglycemic and euglycemic visits were likely a result of elevated blood glucose not insulin deficiency.

To ensure equilibration of glucose across the blood-aqueous barrier, we admitted the patients to the hospital overnight and began to adjust serum glucose levels approximately 13 hours before starting ocular testing. We typically achieved the euglycemic or hyperglycemic glucose goal between midnight and 2 AM, which gave approximately 5 to 7 hours for glucose to reach a steady-state concentration in the aqueous humor. In vivo studies with normal rats suggest that, after bolus injection, t-glucose and 3-O-methyl-D-glucose enter the cornea with equal rapidity and reach a concentration greater than in the plasma and aqueous humor (from which they are presumed to have originated through the endothelium) in about 20 minutes and 8 minutes, respectively.30 Moreover, the concentration ratio of glucose between aqueous humor and plasma reached steady-state approximately 100 to 120 minutes after injection, whereas the cornea-to-plasma and cornea-to-aqueous humor concentration ratios reached steady-state in just over 120 minutes.30-31 Although similar data are not available for human subjects, fluorophotometric studies of the human eye report that fluorescein concentrations in the aqueous humor peak approximately 1 to 2 hours after intravenous injection.31 Because the glucose molecule is nonpolar and approximately one half of the molecular weight of fluorescein, we suggest that it appears in the aqueous humor at a rate comparable to or faster than fluorescein. Hence, we assumed a delay of no greater than 2 hours for equilibration of aqueous humor and plasma glucose levels.

24-Hour Visit: Corneal Function Testing. At 1:00 AM the next morning, we woke all subjects and instilled 2% fluorescein drops in both eyes. The drops were instilled at 5-minute intervals, and the total dose of fluorescein was based on age, with subjects younger than 26 years receiving 5 drops, subjects between 26 and 35 years old receiving 4 drops, and subjects older than 35 years receiving 3 drops in each eye. Using this standard dosing schedule, we found that corneal uptake of fluorescein was considerably greater in diabetic subjects than in those without diabetes, which agrees with a previous study.32 Thus, we reduced the total dose of fluorescein for volunteers with diabetes to obtain similar corneal and aqueous humor dye levels to those observed in control subjects and to minimize fluorophotometer self-absorption problems that can occur in the following morning when corneal fluorescein levels are too high. After instillation of the dye, subjects returned to sleep and were reawakened at 6:00 AM. We removed residual fluorescein from the ocular adnexa with a moist swab and instructed the participants to remain awake with both eyes open for the next hour. During this hour, we obtained a 4-ml blood sample from each diabetic subject to assay for the presence of C-peptide enzyme (hyperglycemic visit only).

At 7:00 AM we began monitoring corneal and aqueous humor fluorescence and central corneal thickness. Then, between 7:30 AM and 9:30 AM, we induced corneal edema by inserting thick, low-water hydrogel lenses (Durasoft 2; Westley-Jessen, Chicago, IL) with +20D power, 8.3-mm base curve, and 38% water content, in both eyes and instructed the subjects to close their eyes. Based on the manufacturer’s calculated oxygen transmissibility, the estimated oxygen tension at the tear–cornea interface during closed-eye conditions was 0 mm Hg.33 After 2 hours, we removed the hypoxic contact lenses, measured the CSR, and quantified the fluorescence of the cornea and aqueous humor. We repeated fluorophotometry and pachometry measurements in 30 minutes and continued to monitor the fluorescence (hourly) and the thickness (every 30 minutes) for the next 6.5 hours (approximately 10:00 AM to 4:30 PM).

Calculation of Percentage of Recovery per Hour

To estimate PRPH based on a composite analysis of the corneal thickness data obtained from the OESS and corneal deswelling measurements, we used an augmented three-parameter exponential model as previously described.34 To minimize any residual effects from the hypoxic contact lens used to induce corneal edema,35,36 we used only corneal thickness measurements that were made more than 55 minutes after removal of the hypoxic lens.

Calculation of Endothelial Permeability

We used the corneal and anterior chamber fluorescence measurements in conjunction with the corneal thickness values to calculate endothelial permeability to fluorescein. The basic method and assumptions of Jones and Maurice37 were used to calculate the mean permeability between each fluorescence measurement and the succeeding one. We made two additional adjustments for changes in corneal thickness. The methods and references that we used to obtain estimates of endothelial permeability have been previously described.19 Because they are distributed over several publications, however, we have included a comprehensive description of the permeability calculation in the appendix.37-39

Previous studies have shown that a reduction in corneal pH after closed-eye contact lens wear can lead to a decrease in the fluorescence efficiency of fluorescein.40,41 Cohen et al.36 reported that it takes approximately 50 minutes for the stromal pH to recover to its steady-state level after 2 hours of hypoxic contact lens wear. In the present study, endothelial permeability to fluorescein (Penendo) was estimated during hypoxic and normal oxygen levels. To avoid an artifact from a potential pH-induced reduction in fluorescence efficiency, we calculated permeability during hypoxia (AM Penendo), using the fluorescence measurements obtained just before contact lens insertion and approximately 90 minutes after contact lens removal at 7:00 AM and 11:00 AM, respectively. Pachometry readings taken at 7:00 AM and 9:30 AM were used to correct for changes in corneal thickness. To calculate permeability during normal oxygen conditions (PM Penendo), we used fluorescence and pachometry measurements obtained hourly between noon and 4:30 PM.

Statistical Methods

To make comparisons between diabetic subjects under hyperglycemic and euglycemic conditions and control subjects, we used linear normal mixed-model analysis for repeated measures data. The analysis was implemented with the program PROC MIXED (SAS Institute; Cary, NC).42 This made it possible to perform one-way ANOVA, regression analysis, and analysis of covariance while allowing for statistical dependence between repeated measurements caused by multiple visits, two eyes, and replicate determinations (see the following three paragraphs for details). Additionally, PROC MIXED corrects some
TABLE 1. Comparison of Baseline Ocular Characteristics for 21 Subjects with Diabetes and 21 Subjects without Diabetes

<table>
<thead>
<tr>
<th>Baseline Variable</th>
<th>21 Subjects without Diabetes</th>
<th>21 Subjects with Type 1</th>
<th>P value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corneal thickness (μm)§</td>
<td>507</td>
<td>524</td>
<td>0.0857</td>
</tr>
<tr>
<td>Corneal autofluorescence (ng/ml fluorescein equivalents)§</td>
<td>3.00</td>
<td>4.63</td>
<td>0.0001</td>
</tr>
<tr>
<td>IOP (mm Hg)§</td>
<td>13.9</td>
<td>17.3</td>
<td>0.0011</td>
</tr>
<tr>
<td>Corneal sensitivity (mm of monofilament)</td>
<td>54.4</td>
<td>43.8</td>
<td>0.0005</td>
</tr>
<tr>
<td>CV central (mean/SD)</td>
<td>0.274</td>
<td>0.284</td>
<td>0.4587</td>
</tr>
<tr>
<td>CV temporal (mean/SD)</td>
<td>0.285</td>
<td>0.316</td>
<td>0.0529</td>
</tr>
<tr>
<td>Den central (cells/mm²)</td>
<td>2842</td>
<td>2634</td>
<td>0.0412</td>
</tr>
<tr>
<td>Den temporal (cells/mm²)</td>
<td>2534</td>
<td>2525</td>
<td>0.9594</td>
</tr>
<tr>
<td>Hex central (%)</td>
<td>63.8</td>
<td>61.9</td>
<td>0.4454</td>
</tr>
<tr>
<td>Hex temporal (%)</td>
<td>67.1</td>
<td>62.9</td>
<td>0.1259</td>
</tr>
</tbody>
</table>

* = 95% confidence interval.
† = Two-sided P value for comparison of means.
§ = Measured twice on each diabetic eye.
CV = Coefficient of variation in corneal endothelial cell area.
Den = Density of corneal endothelial cells per square mm.
Hex = Percentage of hexagonal corneal endothelial cells.

To compare corneal function in euglycemic diabetic subjects, hyperglycemic diabetic subjects, and fasting nondiabetic subjects, we chose a group-specific compound symmetry covariance structure that allowed for the amount of between- and within-subject variations to be different under the three comparison conditions. This same covariance structure was used for studying OESS corneal thickness and AM Pcono as predictors of the CSR. This more elaborate covariance model was needed for the analysis of corneal function data, because the amounts of variability were found to differ between groups according to the Akaike Information Criterion, which was used for covariance model selection.

RESULTS

Overview

The results are presented in four parts: (1) a description of the clinical characteristics of the 21 diabetic subjects, (2) a comparison of baseline ocular characteristics of the 21 diabetic and 21 nondiabetic subjects, (3) a comparison of corneal function measured under pharmacologically controlled EG and HG conditions in diabetic subjects, and during a fasting glucose state in control subjects (ND), and (4) an analysis of the relationship between the CSR to hypoxia as an outcome variable predicted by two corneal properties: endothelial permeability during hypoxia and baseline (OESS) corneal thickness.

Clinical Characteristics of Diabetic Subjects

The mean ± standard deviation (SD) duration of diabetes for the 21 study participants was 22.1 ± 10.1 years (range, 9–42 years), and the daily insulin dose was 46.8 ± 16.9 U/day (range, 23–84). Glycosylated hemoglobin was used to estimate diabetic control over the previous 2 to 3 months, and the mean ± SD HbA1c was 10.2% ± 1.52% (range, 8.1–12.6%). By the interpretation of Mayo’s clinical laboratory, four of our subjects had good diabetic control (HbA1c < 9.0%), 14 had fair control (HbA1c = 9–12%), and three had poor control (HbA1c >
Measurement

Table 2. Parameters of Corneal Function in Hyperglycemic and Euglycemic Subjects with and without Diabetes

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Hyperglycemic Subjects with Diabetes (mean ± SE)</th>
<th>Euglycemic Subjects with Diabetes (mean ± SE)</th>
<th>Fasting Subjects without Diabetes (mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cornal swelling (µm)</td>
<td>51.6 ± 2.30</td>
<td>56.2 ± 1.87</td>
<td>58.9 ± 1.56</td>
</tr>
<tr>
<td>PRPH-55 minutes excluded (%/hr)</td>
<td>67.7 ± 3.09</td>
<td>65.0 ± 3.20</td>
<td>73.8 ± 1.82</td>
</tr>
<tr>
<td>AM endothelial permeability (× 10^{-4} cm/min)</td>
<td>2.99 ± 0.13</td>
<td>3.17 ± 0.14</td>
<td>3.19 ± 0.14</td>
</tr>
<tr>
<td>PM endothelial permeability (× 10^{-4} cm/min)</td>
<td>3.90 ± 0.09</td>
<td>3.92 ± 0.07</td>
<td>3.85 ± 0.10</td>
</tr>
<tr>
<td>OESS corneal thickness (µm)</td>
<td>524 ± 6.8</td>
<td>507 ± 2.5</td>
<td>518 ± 3.5</td>
</tr>
</tbody>
</table>

* Abbreviations used: EG, euglycemic subjects with diabetes; HG, hyperglycemic subjects with diabetes.
† = 95% CI confidence interval for the mean difference.

Table 3. Differences in Corneal Function between Subjects without Diabetes, Euglycemic Subjects with Diabetes, and Hyperglycemic Subjects with Diabetes

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Non-diabetic-EG ( \Delta )</th>
<th>Non-diabetic-HG ( \Delta )</th>
<th>EG Diabetic-HG ( \Delta )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cornal swelling (µm)</td>
<td>2.73 (-2.13, 7.59) (95% CI)</td>
<td>7.27 (1.72, 12.8) (95% CI)</td>
<td>4.54 (0.9, 9.08) (95% CI)</td>
</tr>
<tr>
<td>PRPH-55 minutes excluded (%/hr)</td>
<td>8.8 (1.42, 16.1) (95% CI)</td>
<td>6.1 (-1.10, 13.2) (95% CI)</td>
<td>-2.7 (-11.9, 6.5) (95% CI)</td>
</tr>
<tr>
<td>AM endothelial permeability (× 10^{-4} cm/min)</td>
<td>0.027 (-0.37, 0.42) (95% CI)</td>
<td>0.202 (-0.18, 0.58) (95% CI)</td>
<td>0.175 (-0.12, 0.46) (95% CI)</td>
</tr>
<tr>
<td>PM endothelial permeability (× 10^{-4} cm/min)</td>
<td>-0.070 (-0.31, 0.17) (95% CI)</td>
<td>-0.047 (-0.31, 0.22) (95% CI)</td>
<td>0.023 (-0.12, 0.17) (95% CI)</td>
</tr>
</tbody>
</table>

For each baseline ocular characteristic, we checked for possible systematic differences in the mean response between right and left eyes. In the case of OESS corneal thickness, left eye pachometry measurements were, on average, 3.8 ± 0.72 µm (mean ± SE) thicker than right eye measurements of the same patient \( (P = 0.0001) \), suggesting some asymmetry existed in the measurement procedure. The difference, on average, between right and left eyes was not found to be substantially or significantly different for any of the other baseline characteristics shown in Table 1.

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Corneal Function Assessment during the Regulation of Blood Glucose

We assessed corneal function by measuring the CSR to hypoxia, the PRPH from hypoxic edema, and \( P_{endo} \) under both hypoxic (the AM measurement) and normal oxygen conditions (the PM measurement). Measurements were obtained under controlled EG and HG conditions in each diabetic subject, and during a fasting glucose state in control subjects. This gave three different sets of corneal function data leading to three possible comparisons: ND versus EG, ND versus HG, and EG versus HG (paired comparison). Table 2 provides the mean ± SE estimates for each characteristic of corneal function, and Table 3 summarizes the mean differences in corneal function for the three possible comparisons between the EG, HG, and ND data groups along with related 95% confidence intervals. A plot of the right-left eye average values for each subject under each glucose test condition is provided for three different corneal function characteristics (see Figs. 1-3). The results for each subject are plotted with an open circle, and the paired EG

Baseline Ocular Characteristics of Diabetic Subjects and Control Subjects

Table 1 provides the mean ± SE, and 95% confidence interval for each baseline characteristic measured for diabetic and non-diabetic eyes. Results were obtained by a method that accounted for the two repeated measurements available for each eye for baseline corneal thickness and autofluorescence on the diabetic eyes, and for each subject's right and left eye paired data, which was available except when measurement problems occasionally led to some missing values.

Compared with the non-diabetic group, the diabetic group, on average, had higher intraocular pressure (17.3 ± 0.49 versus 13.9 ± 0.49 mmHg; \( P = 0.0001 \)), lower corneal sensitivity (43.8 ± 1.97 versus 54.4 ± 1.97 mm fiber length; \( P = 0.0005 \)), and higher corneal autofluorescence (4.63 ± 0.24 versus 3.00 ± 0.25 ng/ml fluorescein equivalents; \( P < 0.0001 \)). We also found substantially lower density of central endothelial cells (2634 ± 70.0 versus 2842 ± 70.0 cells/mm²; \( P = 0.0529 \)), greater OESS corneal thickness (524 ± 6.8 versus 507 ± 6.8 µm; \( P = 0.0857 \)) in the corneas of diabetic subjects.

12%). Nonproliferative background diabetic retinopathy was present in all volunteers with diabetes and was graded using a previously reported classification system. Based on the findings of the more severe eye, nine of the diabetic subjects had minimal background retinopathy (grade 2), 11 had moderate background retinopathy (grade 3), and 1 had signs of preproliferative retinopathy (grade 4).
and HG values for the 21 diabetic subjects are connected by line segments. The method we used to analyze these data provides a quantitative assessment of two contributors to residual variation in the diabetic subjects under EG and HG conditions, between-subject and within-subject variability. The magnitude of each source of variability is revealed in Figures 1–3, in which the vertical separation between the lines represents systematic differences between eyes of different subjects (leading to between-subject variation in measurements) and the fluctuations in measurements of the same eye under different levels of blood glucose (shown by a lack of parallelism between connected points) represent within-subject variability.

Percent Recovery Per Hour. For each available glucose condition, the averages of right and left eye PRPH estimates are plotted in Figure 1. The primary data for making these estimates were obtained by monitoring the exponential change in corneal thickness that occurred after 2 hours of closed-eye contact lens wear. We estimated the mean ± SE PRPH to be 67.7 ± 3.09, 65.0 ± 3.20, and 73.8 ± 1.82%/hour for the HG, EG, and ND groups, respectively (see Table 2). As shown in Table 3, the mean (95% confidence interval [CI]) PRPH was 8.8 (1.42, 16.1)%/hour less in the diabetic group during euglycemic conditions compared with the control subjects, and this difference was significantly different from zero \(P = 0.02\). The mean (95% CI) difference of 6.1 (−1.10–13.2) was less pronounced between the control subjects and the diabetic subjects under hyperglycemic conditions, and it was not statistically significant \(P = 0.096\). There was little indication of real difference in the mean level under the different glucose conditions in the diabetic subjects. As can be seen in Figure 1, some extreme swings in measured PRPH were noted in the diabetic subjects from one glucose condition to another. Additional investigation confirmed that these were not data errors but instead appear to stem from a combination of inherent measurement variability in the assessment of PRPH and/or lability in PRPH itself.

Endothelial Permeability. Figure 2 displays estimates of corneal endothelial permeability to fluorescein during contact lens-induced hypoxia \(\left(P_{\text{endo}}\right)\). The mean ± SE \(P_{\text{endo}}\) was 2.99 ± 0.13, 3.17 ± 0.14, and 3.19 ± 0.14 \(\times 10^{-4}\) cm/minute for the HG, EG, and ND groups, respectively, as shown in Table 2. On average (95% CI), the \(P_{\text{endo}}\) estimated for HG was 0.175 (−0.12, 0.46) \(\times 10^{-4}\) cm/minute \(P = 0.23\) and 0.202 (−0.18, 0.58) \(\times 10^{-4}\) cm/minute \(P = 0.30\) less than that measured for EG and ND, respectively, as shown in Table 3. The estimates of mean ± SE \(P_{\text{endo}}\) were equivalent for the three groups; however, in Table 2 we show \(P_{\text{endo}}\) values were 0.929 ± 0.090, 0.769 ± 0.136, and 0.651 ± 0.147 \(\times 10^{-4}\) cm/minute greater than \(P_{\text{endo}}\) for HG, EG, and ND, respectively, and these differences were all significant \((P < 0.0001)\). Against the backdrop of observed variability in \(P_{\text{endo}}\) between diabetic subjects and control subjects, and between diabetic subjects under the EG and HG states, there were neither substantial nor statistically significant.

Corneal Swelling Response. The measured changes in corneal thickness induced by 2 hours of closed-eye contact lens wear (that is, the difference between the 7:00 AM and 9:30 AM corneal thickness readings) are plotted in Figure 3. The mean ± SE changes were 51.6 ± 2.30, 56.2 ± 1.87, and 58.9 ± 1.56 \(\mu\)m for the HG, EG, and ND groups, respectively, as shown in Table 2. On average (95% CI), the CSR measured in HG was 4.54 (0.0, 9.08) \(\mu\)m \((P = 0.05)\) and 7.27 (1.72, 12.8) \(\mu\)m \((P = 0.01)\) less than that measured in EG and ND, respectively (see Table 3). It appears that the diabetic subjects have less corneal swelling as a result of the hypoxic stimulus and that the reduction is more pronounced when the diabetic subjects are in the HG state.

Of interest in Figure 3 is the degree of parallelism between connected points, suggesting that some diabetic subjects demonstrated higher amounts of corneal swelling under both the EG and HG conditions, whereas others demonstrated lower amounts of swelling. In light of the fact that an acute increase...
in blood glucose appeared to substantially decrease the CSR compared with that measured in the same subject under eu-glycemia, it is possible that some of the variability between diabetic subjects in the CSR can be explained by differences in their recent history of blood glucose control. We, therefore, conducted a supplemental investigation to see whether decreased corneal swelling may also be related to the HbA1C level. We found no evidence of a relationship between HbA1C and the CSR, and this relationship was not pursued further.

**Corneal Swelling Response in Relation to AM \( P_{\text{endo}} \) and OESS Corneal Thickness**

Investigators have hypothesized that diabetic subjects may have reduced corneal swelling during hypoxia because they tend to have lower endothelial permeability and/or thicker corneas at baseline than do control subjects. This explanation requires that one or both of these characteristics must also be related to the hypoxic CSR. Consequently, we studied absolute CSR as the dependent variable with AM \( P_{\text{endo}} \) and/or OESS corneal thickness as predictor variables in a repeated-measures analysis of covariance model that also included terms to represent differences in the mean CSR for nondiabetic subjects, EG diabetic subjects, and HG diabetic subjects. These latter terms in the models provide estimates of differences between these data groups after adjustment for AM \( P_{\text{endo}} \) OESS corneal thickness, or both, depending on whether the first (model 1), second (model 2), or both (model 3) of these covariables is included in the model. Table 4 summarizes the results of the analyses with these three models. The coefficients reported for the continuous predictors give the difference in mean corneal thickness per unit difference in the value of the predictor, but they require careful interpretation, because AM \( P_{\text{endo}} \) and OESS corneal thickness have quite different numerical ranges.

The results for the fitted model with either AM \( P_{\text{endo}} \) and OESS corneal thickness as the predictor are plotted in Figures 4 and 5, respectively. The plotted points fall on a straight line, which gives the predicted mean CSR for each comparison group, depending on the value of the predictor variable. The horizontal coordinates of the plotted points on a line are the observed values for the mean of the right and left eye values observed for a nondiabetic subject or a diabetic subject under one of the regulated glucose conditions. The range of values used for the vertical axes in Figures 4 and 5 is the same as the range used for plotting the CSR data in Figure 3. Consequently, the trend in mean CSR, as it depends on the value of the predictor, is shown in the context of the overall background variability in the CSR data.

The vertical differences between the three lines of data plotted in Figures 4 and 5 represent the adjusted difference between the data groups after accounting for AM \( P_{\text{endo}} \) or OESS corneal thickness differences between groups using a parallel line model. The corresponding estimates of CSR differences between groups after adjusting for both predictors is provided by the results from the third model in Table 4. Comparison of

**Table 4. Estimated Regression Coefficients and Their Corresponding P Values before and after Adjusting for the Effects of Endothelial Permeability during Hypoxia, (AM \( P_{\text{endo}} \)) OESS Corneal Thickness, and the Combined Effect of AM \( P_{\text{endo}} \) and OESS on Hypoxic Corneal Swelling**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>No Adjustments</th>
<th>Model 1: Adjustment for AM ( P_{\text{endo}} ) Only</th>
<th>Model 2: Adjustment for OESS Only</th>
<th>Model 3: Adjustment for OESS &amp; AM ( P_{\text{endo}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>48.9 ± 3.85 0.0001</td>
<td>58.91 ± 1.56 0.0001</td>
<td>58.05 ± 1.59 0.0001</td>
<td>58.13 ± 1.62 0.0001</td>
</tr>
<tr>
<td>EG*</td>
<td>2.73 ± 2.43 0.205</td>
<td>-2.98 ± 2.84 0.484</td>
<td>-1.45 ± 2.51 0.567</td>
<td>-2.06 ± 2.57 0.427</td>
</tr>
<tr>
<td>HG*</td>
<td>7.27 ± 2.78 0.011</td>
<td>-6.82 ± 2.86 0.02</td>
<td>-6.00 ± 2.80 0.036</td>
<td>-5.93 ± 2.92 0.047</td>
</tr>
<tr>
<td>AM ( P_{\text{endo}} ) (( \times 10^{-4} ) cm/min)†</td>
<td>1.26 ± 0.98 0.204</td>
<td>1.10 ± 0.976 0.264</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OESS corneal thickness (( \mu m ))†</td>
<td>0.075 ± 0.036 0.045</td>
<td>-0.066 ± 0.039 0.101</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For each analysis, we first centered the observed AM \( P_{\text{endo}} \) and OESS values by subtracting the mean values of all AM \( P_{\text{endo}} \) and OESS outcomes, 3.064 \( \times 10^{-4} \) cm/minute and 518.604 \( \mu m \), respectively.

* Indicator variables: HG = 0 and EG = 0 when predicting mean corneal swelling for a fasting nondiabetic volunteer (reference level); HG = 1 and EG = 0 for a diabetic subject during hyperglycemic; and, HG = 0 and EG = 1 for a euglycemic diabetic.

† Change in mean corneal swelling response per 1.0 \( \times 10^{-4} \) cm/min change in AM \( P_{\text{endo}} \) or per 1.0 \( \mu m \) change in OESS corneal thickness.
predicted for a cornea with an OESS thickness of 496 μm with thinner corneas. To avoid statistical bias in this relation-
greater OESS corneal thickness had a lower CSR than subjects
8.87) jam after we added AM Pendo to the model.
parallel best-fit lines for the three groups shown in Figure 5 was
mood; decreasing from 4.544 (0.0, 9.08) jam to 3.84 (-1.19,
used in the calculation of the CSR connected with the patient's
exposure to the stress lens. The estimated slope (±SE) of the
diabetic subjects showed more than a 10% change in magni-
dicitors and CSR, which is not sufficient to explain a very large
part of the observed unadjusted group differences. In Table 5, we
provide the estimated mean (95% CI) differences in corneal
swelling between ND versus EG, ND versus HG, and EG versus
HG, predicted using the three models described above.
When AM Pendo is the only adjustment variable, the rela-
tionship between AM Pendo, and CSR is described by the parallel
lines in Figure 4 with estimated slopes (±SE) of 1.26 ± 0.984
μm (P = 0.20). Thus, from the best estimate of slope, the
change in mean corneal swelling predicted for a 0.62 X 10^-4
cm/minute increase in AM Pendo (that is, the difference be-
tween the 25th and 75th percentile of the AM Pendo outcomes)
is 0.78 (−0.44, 2.0) μm.
In Table 5, we see that estimates and 95% confidence intervals
for the mean between-group differences in corneal swelling estimated with model 1 are similar to those estimated
without the predictor AM Pendo in the model. Only the mean
(95% CI) difference in corneal swelling between HG and EG
diabetic subjects showed more than a 10% change in magnitude; decreasing from 4.544 (0.0, 9.08) μm to 3.84 (−1.19,
8.87) μm after we added AM Pendo to the model.
In the second analysis, we assessed whether subjects with
greater OESS corneal thickness had a lower CSR than subjects
with thinner corneas. To avoid statistical bias in this relation-
ship related to the phenomenon known as “regression to the
mean,” the baseline corneal thickness was determined inde-
pendently from the pre-insertion measurement, which was
used in the calculation of the CSR connected with the patient’s
exposure to the stress lens. The estimated slope (±SE) of the
parallel best-fit lines for the three groups shown in Figure 5 was
−0.0751 ± 0.036 (P = 0.043); thus, the mean (95% CI) CSR
predicted for a cornea with an OESS thickness of 496 μm
would be 3.16 (0.11, 6.20) μm greater than that predicted for
a thickness of 538 μm, where 496 μm and 538 μm are the 25th
and 75th percentile of OESS outcomes. Compared with the
between-group differences in corneal swelling calculated with-
out OESS as a predictor, the mean (±SE) difference between
ND versus EG decreased nearly 50% (that is, from 2.73 ± 2.43
μm to 1.45 ± 2.51 μm) when we added OESS to the model. By
contrast, the mean (±SE) difference in corneal swelling be-
tween HG versus ND predicted using model 2 changed from
7.27 ± 2.78 μm to 6.00 ± 2.80 μm, and the difference be-
tween HG and EG diabetic subjects was essentially unchanged
from that predicted using the unadjusted model. Although
adjusting for OESS corneal thickness substantially reduced the
mean difference in corneal swelling between diabetic and
nondiabetic subjects during euglycemia, differences between
hyperglycemic diabetic subjects and both euglycemic diabetic
subjects and control subjects could not be substantially ex-
plained by differences in baseline corneal thickness.
As a final step in the study of this set of variables, we
examined the relationship between AM Pendo and OESS thick-
ness and found that subjects in our study with thicker corneas
had lower endothelial permeability during hypoxia than did
subjects with thinner corneas. When we examined OESS as a
predictor of AM Pendo, the estimated slope (±SE) was 0.0064 ± 0.0029 (P = 0.03); thus, the mean (95% CI) AM Pendo
predicted for a cornea with an OESS thickness of 496 μm would be 0.27 (0.021, 0.52) X 10^-4 cm/minute greater than that predicted

![FIGURE 4. Relationship between endothelial permeability during hypoxia (AM Pendo) and corneal swelling. Each point represents the corneal swelling response predicted for an observed value of AM Pendo using the calculated regression equation: Mean CSR = 58.913 - 6.823 (HG) - 2.98 (EG) + 1.263 (AM Pendo) - 3.064. EG, euglycemic; HG, hyperglycemic.](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933426/)

![FIGURE 5. Relationship between open-eye steady-state corneal thickness (OESS) and corneal swelling. Each point represents the corneal swelling response predicted for an observed value of OESS using the calculated regression equation: Mean CSR = 58.05 - 6.002 (HG) - 1.447 (EG) +0.0751 (OESS - 518.604). EG, euglycemic; HG, hyperglycemic.](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933426/)
for a thickness of 538 μm (that is, the 25th and 75th percentiles of OESS outcomes). The mechanism responsible for this relationship between AM $P_{\text{endo}}$ and OESS is unclear, but may suggest that less fluid moves across the endothelium of a thicker cornea during hypoxia because of an average reduction in stromal swelling pressure.

**DISCUSSION**

This study compares several aspects of corneal hydration dynamics in subjects with type 1 diabetes and nondiabetic subjects, and shows that some of the between-group differences in function are dependent on the diabetic subject's blood glucose level at the time of measurement. The comparisons between diabetic subjects in the EG and HG states are experimental in nature with paired comparisons and random order of the times when the two glucose conditions were applied. Consequently, comparisons between these two groups are balanced with respect to both the personal and temporal factors and should give a fairly direct assessment of the acute effects of the glucose state per se. By contrast, the various comparisons give a fairly direct assessment of the acute effects of the method used to calculate PRPH. For example, in our PRPH calculation we corrected for pH effects by eliminating the first 55 minutes of corneal recovery data, whereas Weston eliminated only 30 minutes. However, on re-analysis of Weston's PRPH data with the initial 55 minutes of corneal thickness data eliminated, there was still no significant difference between the diabetic and nondiabetic groups and, thus, the reported outcome did not change. An alternative explanation for the difference in our findings and Weston's may be that the diabetic cohort in the latter study consisted of volunteers with both type 1 and type 2 diabetes. By including older subjects with type 2 diabetes, the mean ± SD age of Weston's diabetic cohort was 51.2 ± 14.2 years compared with 39.6 ± 8.8 years in the present investigation. Because PRPH decreases with age, it may be more difficult to detect differences in PRPH between an older group of diabetic subjects and age-matched control subjects. Corneal function may also be less affected by type 2 diabetes than by type 1.

The mechanism responsible for decreased PRPH in diabetic subjects requires further exploration. Aldose reductase has been demonstrated immunohistochemically in the corneal endothelium, and the osmotic stress that occurs secondary to sorbitol accumulation could recur periodically over the life of the diabetic patient and eventually lead to altered endothelial morphology, cell loss, and reduced pump function. In addition, corneal epithelial hypoxia in response to contact lens wear results in the time-dependent formation of the NADPH-cytochrome P450-dependent arachidonate metabolite, 12(R)-hydroxycicosatetraenoic acid (12(R)-HETE), a Na⁺,K⁺-ATPase inhibitor. Williams et al. have demonstrated that 12(R)-HETE can diffuse through the stroma to the endothelium and may adversely affect endothelial metabolism. Whether or not the production and/or diffusion of 12(R)-HETE in response to contact lens wear differs in subjects with and without diabetes requires additional investigation. If 12(R)-HETE production in patients with diabetes was increased during the corneal stress test compared with control subjects, it may further explain the finding of reduced corneal recovery from edema in our diabetic group. We have no explanation for why the PRPH mea-

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### Table 5. Mean (95% Confidence Interval) Differences in Corneal Swelling (in Microns) between Euglycemic (EG) Diabetic, Hyperglycemic (HG) Diabetic, and Nondiabetic Subjects before and after Adjustments for Endothelial Permeability during Hypoxia (AM $P_{\text{endo}}$) and OESS Corneal Thickness

<table>
<thead>
<tr>
<th>Group/Comparison</th>
<th>Model 1: Adjustment for AM $P_{\text{endo}}$ Only</th>
<th>Model 2: Adjustment for OESS Only</th>
<th>Model 3: Adjustment for OESS and AM $P_{\text{endo}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean Difference (95% CI)</td>
<td>P value</td>
<td>Mean Difference (95% CI)</td>
</tr>
<tr>
<td>Nondiabetic</td>
<td>2.73 (−2.13, 7.59)</td>
<td>0.265</td>
<td>2.73 (−2.13, 7.59)</td>
</tr>
<tr>
<td>EG Diabetic</td>
<td>7.27 (1.72, 12.8)</td>
<td>0.011</td>
<td>7.27 (1.72, 12.8)</td>
</tr>
<tr>
<td>HG Diabetic</td>
<td>4.54 (1.00, 11.61)</td>
<td>0.059</td>
<td>4.54 (1.00, 11.61)</td>
</tr>
<tr>
<td>Diabetic</td>
<td>0.00 (−1.19, 8.87)</td>
<td>0.567</td>
<td>0.00 (−1.19, 8.87)</td>
</tr>
</tbody>
</table>

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ased in diabetic subjects during controlled hyperglycemia was not significantly different from that measured during euglycemia; however, the PRPH measurement is inherently noisy, and this may have obscured any subtle differences in PRPH between the two glucose conditions given our small group of diabetic subjects.

In this study we explored possible mechanisms for the lower CSR observed in our diabetic subjects. Previous investigators have suggested that decreased endothelial permeability during hypoxia and/or increased baseline corneal thickness in diabetic subjects may contribute to the difference in corneal swelling compared with control subjects. We examined the influence of these two corneal characteristics on swelling by analyzing the relationship between corneal swelling as an outcome variable predicted by endothelial permeability during hypoxia (AM Pendo) and open-eye steady-state (OESS) corneal thickness.

Our first analysis demonstrated that AM Pendo was not a significant predictor of the CSR, although the estimated difference in swelling between hyperglycemic and euglycemic diabetic subjects was approximately one fifth less than that observed without adjusting for the effects on AM Pendo on corneal swelling. This result is interesting considering that, on average, AM Pendo in diabetic subjects during hyperglycemia was lower than that measured in both the same diabetic cornea during euglycemia and in control subjects. This suggests that glucose levels may affect endothelial permeability during hypoxia and thereby influence corneal swelling. Reduced endothelial permeability during hypoxia, however, will not fully explain the mechanism by which glucose affects corneal swelling.

In our second analysis, we found that baseline corneal thickness was a significant predictor of absolute corneal swelling (for example, subjects with the thinnest corneas experienced substantially greater swelling after hypoxia than those with the thickest corneas). After adjusting for this thickness-swelling relationship, the difference in swelling between diabetic subjects and control subjects during controlled euglycemia decreased by nearly 50%. However, this same adjustment, explained only about one sixth of the difference in corneal swelling between hyperglycemic diabetic subjects and control subjects, and essentially none of the difference between diabetic subjects measured under the high and low glucose states. These analyses suggest that, during euglycemia, the corneas of diabetic subjects swell less than the corneas of nondiabetic control subjects because of increased baseline corneal thickness. In contrast, when the diabetic patient’s blood glucose is elevated, a substantial amount of the difference in corneal swelling between the diabetic and nondiabetic groups cannot be accounted for by differences in baseline corneal thickness, suggesting that there is an additional glucose effect on corneal swelling.

Other possible mechanisms that may account for the swelling differences between diabetic and nondiabetic subjects include reduced corneal lactate production and/or increased endothelial pump function during corneal hypoxia. Less osmotic swelling of the cornea would be expected during hypoxia if the corneal epithelium of the diabetic patient produced less lactate during anaerobic glycolysis. A decrease in corneal oxygen consumption has been demonstrated in both diabetic humans and rats, which suggests that reduced epithelial metabolic activity occurs in the diabetic cornea and occurs to an even greater extent in diabetic subjects during hyperglycemia. It is less likely that increased endothelial pump function in the diabetic cornea during hypoxia is responsible for lower corneal swelling, because our euglycemic diabetic subjects recovered more slowly from corneal edema than the nondiabetic subjects.

We were unable to measure a significant difference in endothelial permeability during either hypoxic (AM Pendo) or normal corneal oxygen levels (PM Pendo) in the diabetic and nondiabetic subjects. Other investigators have also failed to demonstrate any functional abnormality in the corneal endothelial barrier using both in vitro and in vivo human diabetic models. Weston and colleagues, however, recently demonstrated decreased endothelial permeability during hypoxia in a diabetic group compared with age-matched control subjects. In Figure 2, we illustrate that AM Pendo was lower in the diabetic group during hyperglycemia than in both euglycemic diabetic subjects and fasting control subjects. Although these differences were not statistically significant, this trend, together with Weston’s findings, suggests the need for further study to assess whether diabetic hyperglycemia influences endothelial permeability during hypoxia.

When we compared AM Pendo and PM Pendo, within groups, we revealed significantly lower permeability estimates during hypoxia than with normal corneal oxygen in all three groups, and the magnitude of this difference was similar across groups. Possible explanations for lower endothelial permeability during hypoxia are either a decrease in the simple diffusion of fluorescein across the endothelial cell layer caused by interference from water moving into the cornea as it swells (that is, solvent drag) or a reduction of paracellular fluid flow resulting from an alteration in endothelial morphology. If solvent drag were occurring, it would cause a falsely low estimate of permeability during corneal hypoxia, particularly in corneas that swell the most. This explanation is unlikely, however, because our study and others demonstrated higher permeability values in corneas that swelled the most. The second explanation is more likely because altered endothelial morphology has been hypothesized to occur during hypoxia as a result of corneal acidosis, and this could influence endothelial hydraulic conductivity.

Although acute hyperglycemia was associated with a decrease in the CSR in our diabetic group, we found no relationship between the recent history of blood glucose control (that is, the HbA1C) and the absolute level of corneal swelling measured in our diabetic subjects. There was also no relationship between the HbA1C and the percentage of recovery from contact lens-induced edema or the permeability of the endothelium to fluorescein during hypoxia. Only endothelial permeability during normal corneal oxygen levels (PM Pendo) appeared to be significantly related to the diabetic subject’s HbA1C level, at which the mean ± SE increase in PM Pendo for a 4.5% increase in HbA1C (the range of HbA1C levels measured in our diabetic subjects) was 0.514 ± 0.194 × 10⁻⁴ cm/minute (P = 0.01). Although we were unable to demonstrate a significant difference in endothelial permeability under EG and HG glucose conditions in our diabetic subjects, endothelial permeability has been demonstrated to be increased approximately 23.5% in patients with severe diabetic retinopathy as compared with those with more mild retinopathy. Metabolic stress and poor glycemic control contribute to the development of diabetic retinopathy, and the barrier function of the endothelium may be associated with both the recent history of blood glucose (HbA1C) and the long-term history of glucose control (retinopathy) in diabetic subjects. The exact mechanism by
which glucose affects endothelial function could not be elicited in the present study.

Finally, our structural assessment of the cornea during steady-state revealed several differences between the diabetic and nondiabetic groups. On average, our diabetic subjects had higher corneal autofluorescence, lower corneal sensitivity, greater baseline corneal thickness, fewer endothelial cells centrally, and increased endothelial coefficient of variation of cell area temporally than did the control subjects. Although changes in central endothelial morphology, such as decreased density, increased coefficient of variation, and decreased percentage of hexagonal cells, have been previously documented in patients with diabetes,5,7 information on temporal endothelial morphology in diabetes has not been reported. Studies of central versus peripheral endothelial morphology in the human cornea using specular microscopy are also limited in number, and there are inconsistencies in the findings reported.54-59 Information about central and peripheral morphology in the normal cornea are important to establish the effect of various interventions, such as contact lens wear, refractive surgery, and disease on corneal endothelial health. Recent studies suggest that peripheral endothelial morphology is affected by long-term contact lens wear and excimer laser photorefractive keratectomy.60,61 Our results suggest that temporal endothelial morphology also differs in diabetic subjects when compared with control subjects of similar age; however, additional studies will be necessary to determine whether consistent alterations in peripheral morphology are present in the corneas of diabetic subjects and whether these changes differ from those found centrally.

The finding of greater corneal autofluorescence has been consistently demonstrated in patients with type 1 diabetes, but not in control subjects,18,49,52,62-64 and may result from the accumulation of advanced glycation end products over time.65 Others, however, suggest that increased corneal autofluorescence in diabetes results from an increased fluoroscent signal of reduced pyridine nucleotides (NADH and NDPH) located in the cytoplasm and mitochondria of corneal cells.66 The ratio of NADH/NAD+ (the redox state) can be estimated by measuring the ratio of the fluorescence signal of reduced pyridine nucleotides to oxidized flavoproteins with a fluorophotometer, and an investigator recently demonstrated that both the ratio of pyridine nucleotides to flavoproteins (measured by fluorophotometry) and NADH/NAD+ (measured histochemically) were increased in the corneal endothelium of type 1 diabetic mice.67 Studies using in vitro organ-cultured rabbit corneas also showed that exposure to high-glucose media significantly increased the fluorescence signal of pyridine nucleotides and increased the pyridine nucleotide:flavoprotein ratio.66 Thus, both diabetes-induced hyperglycemia in vivo and exposure to high glucose in vitro appear to increase the fluorescence signal of pyridine nucleotides, and this may indicate a reduced metabolic state of the tissue.68 In sum, this study provides unique information about the acute effects of hyperglycemia on corneal function. We originally hypothesized that diabetic hyperglycemia would lead to increased aqueous humor glucose levels and directly inhibit corneal endothelial function. Although corneal recovery from hypoxic edema did not differ substantially between hyperglycemic and euglycemic diabetic subjects, the recovery rate was significantly slower in diabetic subjects than in control subjects during euglycemia. The CSR was also reduced in our diabetic subjects, and the same diabetic cornea swelled significantly less during hyperglycemia than during euglycemia. The present investigation does not explain the cause of reduced corneal swelling in diabetic subjects during hyperglycemia, but it does provide initial evidence that hyperglycemia can directly influence the mechanisms that control corneal hydration.

Acknowledgments

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References


APPENDIX

The basic method and assumptions of Jones and Maurice were used to calculate the permeability of the endothelial
cell layer by measuring how the concentration of fluorescent tracers in the cornea and aqueous humor change relative to each other with time. This model assumes that epithelial losses and limbal losses of fluorescein are minimal when compared with the loss of corneal fluorescein across the endothelium to the aqueous humor. It also assumes that the rate of loss of fluorescein from the cornea is dependent on the total mass of dye in the cornea and anterior chamber, and that exchanges between the cornea and aqueous humor obey simple first-order dynamics.

Using these assumptions, for the exchange between the cornea and aqueous humor we may write:

\[
\frac{dC_{\text{c(a)}}}{dt} = k_{\text{c,a}}(C_a - C_{\text{c(a)}}) \tag{1}
\]

where \(k_{\text{c,a}}\) is the transfer coefficient from the cornea to the aqueous humor, referring to the volume of the cornea, and \(C_a\) and \(C_{\text{c(a)}}\) are the effective or unbound fluorescein concentrations in the aqueous humor and cornea. The corresponding equation for the aqueous humor is:

\[
\frac{dC_a}{dt} = -k_a C_a + k_{\text{c,a}} (C_{\text{c(a)}} - C_a) \tag{2}
\]

where \(k_a\) is the loss coefficient from the anterior chamber and \(k_{\text{c,a}}\) is the corneal transfer coefficient referring to the volume of the anterior chamber. The solution of these two equations is biexponential and is more fully discussed in Jones and Maurice.\(^\text{37}\)

The rate of exchange of fluorescein across the corneal endothelium is dependent on the unbound concentration of fluorescein. Protein binds fluorescein in the corneal stroma, and the measurements of stromal fluorescein include both bound and unbound fractions. Ota et al.\(^\text{38}\) estimated the steady-state distribution ratio for fluorescein between the cornea and the anterior chamber, \(r_{\text{ca}}\), to be 1.6 for human corneas of normal thickness. Thus, when the concentration of fluorescein in the cornea and aqueous humor reach equilibrium, the total (bound + unbound) concentration of corneal fluorescein, \(C_c\), is equal to the product of the unbound fluorescein concentration in the cornea and aqueous humor, \(C_{\text{c(a)}}\), and the measurements obtained between noon and 4:00 PM.

The average of a concentration that decreases linearly is simply the arithmetic mean of the start and end concentrations. When concentrations decrease exponentially, the arithmetic mean accurately represents the average on short intervals, because the change between the endpoints is approximately linear. However, on a long interval, when the change is not linear, the mean of the endpoints does not represent the true average concentration. A better estimate of average concentration on long intervals, therefore, is the area under the curve divided by the change in time:

\[
\bar{C} = \frac{C_1 - C_2}{\ln \left( \frac{C_1}{C_2} \right)} \tag{5}
\]

Thus, the average concentration used to calculate \(k_{\text{c,a}}\) for each of the five intervals was calculated using equation 5.

Endothelial permeability in cm/minute is defined as\(^\text{38}\):

\[
\text{Permeability} = k_{\text{c,a}} q r_{\text{ca}} \tag{6}
\]

where \(k_{\text{c,a}}\) is calculated for each time interval, \(\Delta t\), between successive fluorescence measurements as described above, \(q\) is the mean of the corneal thickness at the beginning and end of each interval, and \(r_{\text{ca}}\) is assumed to be 1.6.\(^\text{38}\)

We made two additional adjustments to correct for errors introduced as corneal thickness changed throughout the time of the study. We assumed that thickness was constant over each interval and was equal to the mean of the beginning and end thickness as previously described.\(^\text{18,39}\) First, we adjusted the distribution ratio to account for the decrease in stromal protein concentration during an increase in corneal hydration:

\[
r_{\text{ca}} = q_1 (r'_{\text{ca}} - 1) + 1 \tag{7}
\]

The term \(r'_{\text{ca}}\) is the unadjusted steady-state distribution ratio for fluorescein (assumed to be 1.6),\(^\text{38}\) \(q_0\) is the thickness at 7:00 AM, and \(q_1\) is the mean of the thicknesses at the beginning and end of the measurement interval. This relationship is derived from the law of mass action.

Then, we adjusted the estimate of corneal fluorescein to account for a change in the efficiency of the fluorophotometer that occurs with changes in corneal thickness. The focal diamond, or measurement window, of the fluorophotometer is formed by the intersection of the excitation beam and emission path, and its anterior–posterior depth is slightly greater than the thickness of the cornea. Thus, the volume of the focal diamond includes the cornea plus air, tears, and aqueous humor, and, therefore, it is not filled uniformly. However, fluorescein was calibrated on the assumption that the focal diamond was entirely filled with a solution of uniform fluorescence. Because the depth of the cornea only partially fills the focal diamond, corneal fluorescence will be underestimated, and as long as the thickness of the cornea is less than...
the anterior-posterior depth of the focal diamond, the measured fluorescence will depend on thickness. When the cornea swells, a greater proportion of the cornea is included in the diamond. The efficiency of the focal diamond, $ef.d$ (the ratio of the fluorescence measured from a solution in a thin structure to the fluorescence measured from the same solution in a thick chamber) is approximately linear with thicknesses ($q$) between 400 $\mu$m and 800 $\mu$m:

$$ef.d = mq + b$$

(8)

The values calculated for the scanning ocular fluorophotometer used in our study are $m = 0.70$ mm$^{-1}$ and $b = 0.35$, whereas $q$ is the corneal thickness at the time of the scan. We used this linear relationship to correct corneal fluorescence for a given corneal thickness:

$$C_c = \frac{C'_c}{ef.d}$$

or

$$C_c = \frac{C'_c}{mq + b}$$

(9)

where $C'_c$ is the uncorrected corneal fluorescence and $C_c$ is the corrected corneal fluorescence.