Rat Model of Type 2 Diabetes Mellitus Recapitulates Human Disease in the Anterior Segment of the Eye

Cheryl L. Wang,* Jessica M. Skeie,*† Chantal Allamargot,*‡ Andrew S. Goldstein,*§ Darryl Y. Nishimura,*† James M. Huffman,* Benjamin T. Aldrich,*† Gregory A. Schmidt,* Leandro B.C. Teixeira,§ Markus H. Kuehn,*§ Mark Yorek,* and Mark A. Greiner*†‡

From the Department of Ophthalmology and Visual Sciences,* Carver College of Medicine, and the Office of the Vice President for Research,† Central Microscopy Research Facility, University of Iowa, Iowa City, Iowa; Iowa Lions Eye Bank,‡ Coralville, Iowa; the Department of Pathobiological Sciences,§ University of Wisconsin—Madison School of Veterinary Medicine, Madison, Wisconsin; and the Center for the Prevention and Treatment of Visual Loss, Iowa City Veterans Affairs Health Care System, Iowa City, Iowa

Accepted for publication February 9, 2024.

Address correspondence to Mark A. Greiner, M.D., Department of Ophthalmology and Visual Sciences, Coralville, Iowa 52242.
E-mail: mark-greiner@uiowa.edu.

Changes in the anterior segment of the eye due to type 2 diabetes mellitus (T2DM) are not well-characterized, in part due to the lack of a reliable animal model. This study evaluates changes in the anterior segment, including crystalline lens health, corneal endothelial cell density, aqueous humor metabolites, and ciliary body vasculature, in a rat model of T2DM compared with human eyes. Male Sprague-Dawley rats were fed a high-fat diet (45% fat) or normal diet, and rats fed the high-fat diet were injected with streptozotocin i.p. to generate a model of T2DM. Cataract formation and corneal endothelial cell density were assessed using microscopic analysis. Diabetes-related rat aqueous humor alterations were assessed using metabolomics screening. Transmission electron microscopy was used to assess qualitative ultrastructural changes ciliary process microvessels at the site of aqueous formation in the eyes of diabetic rats and humans. Eyes from the diabetic rats demonstrated cataracts, lower corneal endothelial cell densities, altered aqueous metabolites, and ciliary body ultrastructural changes, including vascular endothelial cell activation, pericyte degeneration, perivascular edema, and basement membrane reduplication. These findings recapitulated diabetic changes in human eyes. These results support the use of this model for studying ocular manifestations of T2DM and support a hypothesis postulating blood–aqueous barrier breakdown and vascular leakage at the ciliary body as a mechanism for diabetic anterior segment pathology. (Am J Pathol 2024, 1–16; https://doi.org/10.1016/j.ajp.2024.02.004)

Diabetes mellitus (DM) is a highly prevalent disease with a rapidly increasing incidence; there are 34.2 million people with the disease in the United States and 1.5 million new cases each year. The characteristic pathophysiology of chronic hyperglycemia and tissue glycation is well-known to cause macrovascular and microvascular damage resulting in neuropathy, retinopathy, nephropathy, and atherosclerotic changes. While diabetic changes to the retina have been a huge focus of research and clinical attention, the cornea has not received similar consideration. Due to its avascular nature, the cornea was previously thought to be unaffected clinically by DM, and eye banks still do not require the tracking of diabetes as a specific category in their corneal donors. It is only in recent years that a growing body of literature has begun to describe the detrimental effects of diabetes throughout corneal tissue. There are now well-described morphologic, functional, and biochemical changes in the corneal endothelium. Alterations at the level of specific corneal layers have now been associated with deleterious effects in both diabetic
Wang et al

donors, and recipients in the context of endothelial keratoplasty, the most commonly performed corneal transplantation technique. Although these pathologic changes are increasingly well-documented, there is little research to explain their causal mechanisms. One of the key barriers to exploring these mechanisms has been the lack of a reliable model that recapitulates the effects of type 2 DM (T2DM) in the human cornea. The difficulty of carrying out randomized, prospective studies with adequate controls among humans makes the prospect of finding an animal model that replicates the pathophysiology of T2DM a compelling project. As T2DM accounts for up to 95% of diabetes cases in the United States, a model that could explain the observed pathologies in corneas with T2DM would have important and clinically relevant significance.

Multiple animal models of type 1 DM (T1DM) are available, including chemical-induction models (by high-dose streptozotocin [STZ] and alloxan injection), autoimmune models (nonobese diabetic mice, BioBreeding rats, and LEW.1AR1/1-iddm rats), genetically induced models (Akita mice), and other models such as viral or surgical induction. However, none of these animal models are adequate for reproducing the natural history of T2DM, with its progression from insulin resistance to compensatory hyperinsulinemia and resultant pancreatic β-cell exhaustion. A model that has shown promise for replicating this natural history is the Sprague-Dawley rat that is fed a high-fat diet (HFD) to induce hyperinsulinemia and insulin resistance, followed by STZ injection to promote hyperglycemia. The HFD-STZ rat also has the benefit of being well-established as a reliable model for the investigation of therapeutics in T2DM, making it a useful model to study the anterior segment of the eye.

In addition to an appropriate model, a biologically credible pathophysiological mechanism must be developed that permits an understanding of how diabetes as a vascular disease can impact the cornea as an avascular tissue. Diabetic neuropathy has been described in animal models as anurysm formation, capillary occlusion, and ischemia leads to the up-regulation of vascular endothelial growth factor (VEGF), which in turn further increases vascular permeability by compromising tight-junction proteins. The study hypothesis was that diabetes-related pathologic findings in the anterior segment also involve blood–aqueous barrier breakdown as a mechanism.

This study investigated the HFD-STZ Sprague-Dawley rat model of T2DM to determine its utility as a model of human disease with respect to diabetes-associated complications of the anterior segment, with particular attention to the posterior cornea as the most commonly transplanted ocular tissue. Multimodal evaluations were performed in this model and in nonaffected controls of cataract formation, corneal endothelial cell density, aqueous humor, and ultrastructural evaluation of ciliary body vascular endothelial cells and mitochondria—areas of well-established pathology in humans with T2DM—with comparative analysis against human donor tissues to ensure a thorough assessment of this model.

**Materials and Methods**

The study protocol was approved by the University of Iowa (Iowa City, IA) Institutional Review Board and adhered to the tenets of the Declaration of Helsinki. All human tissue and animal use followed institutional, NIH, and Eye Bank Association of America guidelines. The outlined studies also adhered to the Association for Research in Vision and Ophthalmology’s statement on the use of animals in research. None of the human eye donors from whom samples were procured for this investigation were from a vulnerable population, and all donors or next of kin provided appropriate consent for tissue donation and research.

**Human Donor Corneas and Diabetes Status**

Human donor eyes were procured via whole-globe enucleation performed by eye bank technicians from the Iowa Lions Eye Bank (Coralville, IA). Aqueous humor (100 µL) was collected by research personnel within 5 hours post-mortem using a 23-gauge needle inserted through the central clear cornea into the anterior chamber. Samples were stored immediately in a −80°C freezer. Donor eyes dedicated for transmission electron microscopy (TEM) of the anterior segment were prepared from whole globes by research personnel within 24 hours postmortem. Anterior segments were removed from the whole globes and placed in half-strength Karnovsky solution in preparation for TEM and stored at 4°C. Human ocular tissue samples were collected from a total of 29 donors.

Human aqueous and tissue samples from donors with DM who had a history of home insulin use and end-organ damage (diabetic neuropathy, diabetic retinopathy, renal failure due to diabetes, and/or amputations due to diabetes) specifically noted in the medical history as occurring as a result of diabetes were classified as the advanced diabetes (AD) sample group. Samples from diabetic donors with no notation of medical complications secondary to diabetes (with or without a history of home insulin use) were classified as the...
Table 1  Demographic Characteristics and Medical History of Donors with Type 2 Diabetes Mellitus and Controls Analyzed for Aqueous Humor Proteomic Changes

<table>
<thead>
<tr>
<th>No.</th>
<th>Diagnosis</th>
<th>Age, years</th>
<th>Sex</th>
<th>Medical history</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AD, CHF, arthritis/OA, weakness, falls, SOB, morbid obesity, IDDM, hypothyroidism, A-fib, HTN, depression, gout, ulcers, nausea, constipation</td>
<td>76</td>
<td>F</td>
<td>COPD, CHF, arthritis/OA, weakness, falls, SOB, morbid obesity, IDDM, hypothyroidism, A-fib, HTN, depression, gout, ulcers, nausea, constipation</td>
</tr>
<tr>
<td>2</td>
<td>AD, HTN, dermatoses, hyperlipidemia, tinnitus, chronic sinusitis, UTI, pain, localized superficial swelling, bicipital tenosynovitis, osteomyelitis of left foot, cellulitis of foot excluding toe, hemorrhoids, osteopenia, fibro-osteoma, kidney surgery, toe amputation</td>
<td>70</td>
<td>F</td>
<td>IDDM, retinopathy, nephropathy, neuropathy, diabetic foot ulcers and amputation, chronic kidney disease, HTN, prostate adenocarcinoma, melanoma, gout, hyperlipidemia, superficial femoral artery stent, anxiety, hallucinations, hypercalcemia, mental disorder, PVD, hearing loss, A-fib</td>
</tr>
<tr>
<td>3</td>
<td>AD, 89 M</td>
<td></td>
<td></td>
<td>Below-knee amputation, PVD, end-stage renal disease, IDDM, HTN, A-fib, hemodialysis, stage V chronic kidney disease, nephrotic range proteinuria, anemia, disorder of mineral metabolism, primary open-angle glaucoma, hypercholesteremia, OA, lumbago, edema, kidney calculus, hearing loss, mental disorder, nervous system disorder, dermatoses, osteomyelitis, acquired musculoskeletal deformity, malnutrition, toe infection, diabetic neuropathy, hypoalbuminemia, hyponatremia, sacral ulcer, encephalopathy, AV fistula stenosis, cellulitis, abscess, atherosclerosis, depression, left ventricular hypertrophy, hypercholesterolemia, left foot amputation, cataract surgery, vascular surgery, joint replacements, femoral artery—to—aortic bypass</td>
</tr>
<tr>
<td>4</td>
<td>AD, 84 F</td>
<td></td>
<td></td>
<td>Traumatic brain injury, CAD, IDDM, hyperthyroidism, hyperlipidemia, AD, advanced diabetes; A-fib, atrial fibrillation; AV, atrioventricular; CABG, coronary artery bypass grafting; CAD, coronary artery disease; CHF, congestive heart failure; COPD, chronic obstructive pulmonary disease; F, female; GERD, gastroesophageal reflex disease; HTN, hypertension; IDDM, insulin dependent type 2 diabetes mellitus; M, male; NAD, nonadvanced diabetes; ND, noninsulin dependent type 2 diabetes mellitus; OA, osteoarthritis; PVD, peripheral vascular disease; SOB, shortness of breath; UTI, urinary tract infection.</td>
</tr>
<tr>
<td>5</td>
<td>AD, 84 M</td>
<td></td>
<td></td>
<td>IDDM, HTN, CHF, fibromyalgia syndrome, dystonic syndrome, degenerative arthritis, atherosclerotic CAD with stenosis of the left circumflex with stenting, pancytopenia, nephrotic syndrome, anxiety, ischemic cardiomyopathy, bilateral retinal surgery, cholecystectomy</td>
</tr>
<tr>
<td>6</td>
<td>AD, 45 F</td>
<td></td>
<td></td>
<td>IDDM, CAD, abdominal pain, constipation, abdominal wall hernia, CHF, A-fib, hyperlipidemia, HTN, ischemic cardiomyopathy, PVD, chest pain, SOB, dizziness, UTI, permanent pacemaker, hysterectomy, tonsillectomy, cystocele repair, iliac artery stent, coronary angioplasty, CABG, implantable cardioverter defibrillator placement</td>
</tr>
<tr>
<td>7</td>
<td>NAD, 78 F</td>
<td></td>
<td></td>
<td>IDDM, CAD, abdominal pain, constipation, abdominal wall hernia, CHF, A-fib, hyperlipidemia, HTN, ischemic cardiomyopathy, PVD, chest pain, SOB, dizziness, UTI, permanent pacemaker, hysterectomy, tonsillectomy, cystocele repair, iliac artery stent, coronary angioplasty, CABG, implantable cardioverter defibrillator placement</td>
</tr>
<tr>
<td>8</td>
<td>NAD, 77 M</td>
<td></td>
<td></td>
<td>IDDM, emedatous colon polyp, hypercholesteremia, HTN, A-fib with ablation, CAD, back pain, stroke, kidney failure, acid reflux, arthritis, atrial flutter, GERD, cataracts, heart artery stent replacement, cholecystectomy, CABG, coronary angioplasty, cataract surgery</td>
</tr>
<tr>
<td>9</td>
<td>NAD, 82 M</td>
<td></td>
<td></td>
<td>A-fib, CHF, COPD, NIDDM, hypothyroidism, ASHD, bladder tumor, cardiac catheter, cholecystectomy, right femoral tibial bypass, transurethral resection</td>
</tr>
<tr>
<td>10</td>
<td>NAD, 87 F</td>
<td></td>
<td></td>
<td>NIDDM, HTN, laminectomy, neuropathy, COPD, SOB, chest tightness</td>
</tr>
<tr>
<td>11</td>
<td>NAD, 72 F</td>
<td></td>
<td></td>
<td>Liver cirrhosis secondary to nonalcoholic steatohepatitis, portal HTN, NIDDM, obesity, chronic kidney disease, cellulitis in left lower extremity, hypokalemia, GERD, OA, finger deformity, abdominal swelling, ascites, gastric bypass, knee joint replacement</td>
</tr>
<tr>
<td>12</td>
<td>ND, 81 M</td>
<td></td>
<td></td>
<td>Lung cancer, COPD, cerebral metastasis, HTN, depression</td>
</tr>
<tr>
<td>13</td>
<td>ND, 88 F</td>
<td></td>
<td></td>
<td>Abdominal aortic aneurysm, tachydyssrhythmia, HTN, GERD, arthritis, primary AV block, tonsillectomy, cholecystectomy, pacemaker</td>
</tr>
<tr>
<td>14</td>
<td>ND, 87 F</td>
<td></td>
<td></td>
<td>Hyperlipidemia, urge incontinence, cholecystectomy</td>
</tr>
<tr>
<td>15</td>
<td>ND, 93 M</td>
<td></td>
<td></td>
<td>Hyponatremia, benign prostatic hyperplasia, A-fib, hypothyroidism, transurethral resection of the prostate, hypothyroidism, hyperlipidemia, insomnia, depression, hip replacement, skin cancer, hernia repair, hearing loss, HTN, CAD</td>
</tr>
<tr>
<td>16</td>
<td>ND, 67 M</td>
<td></td>
<td></td>
<td>Lymphocytic leukemia, depression, basal cell carcinoma nose and neck, actinic keratosis, seborrheic dermatitis, CAD, hyperlipidemia, diverting enterocoloectomy, appendectomy, tonsillectomy, adenoidecotomy, cervical spondylitis, diverticulitis</td>
</tr>
</tbody>
</table>
nonadvanced diabetes (NAD) sample group. Finally, samples from donors lacking a diagnosis of DM were defined as the nondiabetic (ND; control) sample group. For TEM analysis, ND samples were further stratified into those with a history of cardiovascular disease (ND-CH) and those without (ND). This stratification was based on the modified Diabetes Complications Severity Index.25,30,40,61 Donor details can be found in Tables 1 and 2.
Rat Corneas and Diabetes Induction

For this study, a highly characterized rat model of late-stage T2DM, developed in 2000 by Reed et al., was used. These rats are hyperglycemic (blood glucose, 20.9 ± 1.2 versus 6.0 ± 0.2 mmol/L in controls; P < 0.05) and insulin resistant without marked elevation in serum insulin concentrations (2.2 ± 0.5 versus 1.6 ± 0.3 ng/mL in controls; P > 0.05), and are directly analogous to the development of frank hyperglycemia and decline in compensatory hyperinsulinemia that occur in human T2DM.69,52 Male Sprague-Dawley rats (Harlan Sprague Dawley, Indianapolis, IN) were approved under the Animal Component of Research Protocol of the US Department of Veterans Affairs, obtained at 10 to 11 weeks of age, and housed in a certified animal care facility with food and water provided ad libitum. Beginning at 12 weeks of age, Sprague-Dawley rats were fed either a HFD (45% fat) or normal diet (4.25% fat) for 8 weeks. At age 20 weeks, HFD rats were injected with STZ i.p. (30 mg/kg in 0.1 mol/L citric acid buffer pH 4.5; EMD Chemicals, Gibbstown, NJ).64 Rats fed the normal diet were injected with buffer alone. Beginning at week 20, rats were weighed and their blood glucose was measured once weekly. Blood was collected from the tail vein and measured using a OneTouch glucometer (LifeScan Inc, Malvern, PA). Rats were considered diabetic if they had a resting blood glucose concentration of ≥250 mg/dL after STZ injection, indicative of insulin resistance (ie, reduced blood glucose clearance). Measurements were continued on each rat until euthanasia to ensure proper classifications during the study. At age 48 weeks, a time point when corneal nerves are damaged,54 rats were euthanized and tissues collected. Rat eyes used for this study were obtained from an investigator (M.A.Y.) as control eyes from another study using this T2DM model; as such, they were healthy eyes with respect to all but the presence or absence of experimentally induced T2DM. Prior to ocular enucleation, aqueous humor was aspirated with a 30-gauge needle. The aqueous humor was then placed into a 1.5-mL Eppendorf tube on wet ice for transportation to the laboratory, frozen in liquid nitrogen, and then stored at −80°C. Four samples of aqueous humor from two unique animals were pooled to obtain enough sample for metabolomics screening. Ocular globes were fixed in either half-strength Karnovsky solution for TEM preparation or zinc-formalin fixatives for light-microscopy preparation. Eyes for TEM were stored at 4°C and eyes for light microscopy were stored at room temperature.

Light-Microscopy Analysis

Complete central cross sections of rat eyes were collected and observed using light microscopy. Diabetic rats (n = 10) and controls rats (n = 10) were analyzed for the presence of lens opacity indicative of cataract. Cataract grading was completed at the University of Iowa ocular pathology laboratory.

Corneal Endothelial Cell Density Analysis

Rat whole corneas were mounted on a cover-glass bottom dish after nuclear staining with To-Pro3 iodide (catalog number T-3605; InvitroGen/Thermo Fisher Scientific, Waltham, MA). Confocal imaging was performed using the Leica SP8 microscope (Leica Microsystems Inc., Buffalo Grove, IL) using a 10× lens to collect the whole cornea with Z-stacks and tiling. Upon completion, the images were analyzed on a MicroBrightField stereology platform (MBF Bioscience, Williston, VT) using MicroBrightField Stereo Investigator software and optical fractionator to count nuclei. Counting frame width × height was set at 50 × 50 μm and the sampling grid was 250 × 250 μm. Total counts of corneal endothelial cell nuclei were made for the entire area of each cornea and compared between diabetic (n = 8) and control (n = 10) groups using the t-test. Corneas with an area of <6 mm² were not analyzed.

Aqueous Humor Metabolomic Analysis

Aqueous humor samples from diabetic rat eyes (n = 8, with each n consisting of three pooled aqueous samples to obtain necessary volume) and control rat eyes (n = 8, with each n consisting of three pooled aqueous samples to obtain necessary volume) were pooled according to disease status and prepared for metabolomic analysis as described previously.66 Small aqueous humor quantity limited the present analysis to metabolomics screening, which was prioritized because T2DM is a metabolic disease. Metabolomics analysis was performed at the Fraternal Order of Eagles Diabetes Research Center Metabolomics Core (University of Iowa, Carver College of Medicine) via gas chromatography/mass spectrometry. For gas chromatography/mass spectrometry metabolite profiling, samples were extracted with a methanol-acetonitrile-water mix followed by conversion to a trimethylsilyl.65 Metabolites were separated by gas chromatography, detected using either a single-quadrupole mass spectrometer or a Q-Exact mass spectrometer (Thermo Fisher Scientific), and data were collected in either full mass range (50 to 700 Da) or by single-ion monitoring. A set of nine isotopically labeled internal standards were used to provide correction of extraction, derivatization, and/or loading effects. Pooled quality-control samples were injected in duplicate at the beginning and end of each run, as well as after every 10 runs. These quality-control samples were used to correct for instrument drift over time using local regression analysis by NOREVA software.66 Metabolite identification was based on comparison to an in-house mass spectrum library of authenticated standards and their retention times using Tracefinder software version 4.1 (Thermo Fisher Scientific).57 Metabolite intensities were...
Table 3  Morphologic Grading Criteria for Human Ciliary Body TEM Images

<table>
<thead>
<tr>
<th>Grading scale</th>
<th>Mitochondria</th>
<th>Pericyte Coverage</th>
<th>EC Activation</th>
<th>BM</th>
<th>Autophagy</th>
<th>Edema</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Oval shape, neatly layered cristae organization</td>
<td>Circumference of vessel almost entirely covered by pericytes</td>
<td>ECs demonstrate filamentous activation</td>
<td>Thin, even layer of BM all of the way around the vessel</td>
<td>Few vacuoles and autophagocytic inclusions</td>
<td>Collagen fibers tightly packed around vessel perimeter</td>
</tr>
<tr>
<td>2</td>
<td>Swollen, distending appearance, loss of some cristae structure</td>
<td>80% covered</td>
<td>ECs demonstrate filamentous activation</td>
<td>BM appears thickened in areas around the vessels</td>
<td>Moderate vacuoles and autophagocytic inclusions</td>
<td>Perimeter of edema surrounding the vessel</td>
</tr>
<tr>
<td>3</td>
<td>Near-complete obliteration of cristae structure, evidence of increased autophagy</td>
<td>60% covered</td>
<td>ECs demonstrate filamentous activation</td>
<td>Multiple layers of thick, reduplicated BM around the vessel</td>
<td>Many vacuoles and autophagocytic inclusions</td>
<td>Edema extends beyond the perivascular space</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>40% covered</td>
<td>ECs demonstrate filamentous activation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>20% covered</td>
<td>ECs demonstrate filamentous activation</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

BM, basement membrane; EC, endothelial cell.

Aqueous Humor Proteomic Analysis

Aqueous humor samples from human donor eyes were prepared for mass spectrometry and proteomic analysis as described previously. Aqueous humor samples were collected from 16 donor eyes, including 6 from the AD group and 5 from the NAD group (diabetic eyes, n = 11; mean age, 76.73 years), and 5 from the ND group (control eyes, n = 5; mean age, 83.20 years).

Peptides were analyzed by ultra-high-performance liquid chromatography—tandem mass spectrometry (UHPLC-MS/MS). Liquid chromatography was performed on the Easy-nLC 1000 UHPLC system (Thermo Fisher Scientific), which was interfaced to a quadrupole-Orbitrap MS (Q-Exactive) via nano-electrospray ionization (Thermo Easy Spray source; Thermo Fisher Scientific). The MS acquired tandem mass spectra from the top 20 ions in the full scan from 400 to 1200 m/z. Dynamic exclusion was set to 15 seconds, singly charged ions were excluded, and isolation width was set to 1.6 Da. Full MS resolution was set to 70,000 and MS/MS resolution, to 17,500; normalized collision energy was set to 25; automatic gain control, to 2 × 10^5 eV; max fill MS, to 20 milliseconds; max fill MS/MS, to 60 milliseconds; and the underfill ratio was set to 0.1%. Peptides were then identified as previously described. Peptide hit totals were normalized to total peptide hits per sample, protein intensities were scaled to logarithmic base 10, and statistically significant protein differences (analysis of variance, P < 0.05) were determined using Geonomics Suite software version 6.6. Significantly altered protein lists were used to find relevant pathway representation due to diabetic disease progression (Ingenuity Pathway Analysis).

TEM Analysis

Human and rat tissues were processed for TEM imaging by embedding in Epon 812 resin (Ted Pella, Redding, CA). Small sample sizes in the rat limited the present analysis of the ciliary body to ultrastructural analysis, which was prioritized because of the known ultrastructural vascular and mitochondrial changes attributable to T2DM. TEM images were obtained from the aqueous-facing edges of ciliary processes, adjacent to the site of aqueous humor secretion. The imaging was performed on a JEM 1230 microscope (Jeol USA Inc., Peabody, MA) equipped with the K2 digital camera (Gatan, Pleasanton, CA).

At least five images (depending on tissue quality and grid position) per rat eye were collected for vascular endothelial cell mitochondrial morphometrics analysis. Using ImageJ software version 1.51w (NIH, Bethesda, MD; http://imagej.nih.gov/ij), mitochondrial areas were measured in both diabetic and control rats. Areas were compared using the t-test. A similar analysis in human tissues was previously published.
<table>
<thead>
<tr>
<th>Table 4</th>
<th>Morphologic Grading Criteria for Rat Ciliary Body TEM Images</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grading scale</td>
<td>1</td>
</tr>
<tr>
<td>Pericyte Coverage</td>
<td>Circumference of vessel almost entirely covered by pericytes</td>
</tr>
<tr>
<td>EC Activation</td>
<td>Smooth appearance, even thickness of ECs around the vessel</td>
</tr>
<tr>
<td>BM</td>
<td>Thin, even layer of BM all of the way around the vessel</td>
</tr>
<tr>
<td>Autophagy</td>
<td>Few vacuoles and autophagocytic inclusions</td>
</tr>
<tr>
<td>Edema</td>
<td>Collagen fibers tightly packed around vessel perimeter</td>
</tr>
</tbody>
</table>

BM, basement membrane; EC, endothelial cell.

**Figure 1** Changes in endothelial cell density associated with T2DM in rat corneas. A and B: Representative image of group mean endothelial cell density in control (A) and diabetic (B) rat corneas; density is decreased in diabetic rats compared to controls. The boxed regions correspond to C and D at higher magnification. C and D: Higher-magnification images of control (C) and diabetic (D) corneas demonstrating central density. $n = 8$ (B); $n = 10$ (A). Scale bar = 750 μm.
Wang et al

Figure 2  Metabolic changes associated with streptozotocin i.p. in a rat model of severe T2DM. Dashed line, \( P = 0.05 \). DOPA, dihydroxyphenylalanine; QM, N/C, no change.

Qualitative TEM analysis of both human and rat cornea and ciliary body tissues was performed by an observer who was masked to diabetic versus control sample status.

Human and rat eye TEM images of the microvasculature of ciliary processes in the diabetic and ND groups were analyzed quantitatively for vascular endothelium morphology. A total of 10 human donor eyes from five donors with diabetes (mean age, 74.6 years) and 16 human donor eyes from eight control donors (mean age, 74.88 years) were analyzed by TEM. In rats, 6 HFD-STZ eyes and 10 control eyes were analyzed. Morphologic analysis was performed on at least three ciliary body blood vessel cross sections per sample. For consistency, sections were analyzed only if they appeared to show the vessel in cross section rather than in longitudinal section. Each sample was scored on its morphologic appearance in a handful of criteria generated from a review of current literature on both normal ciliary body morphology and observed changes in the ciliary body, blood–aqueous barrier, and aqueous in diabetes. In human samples, the categories included mitochondrial morphology, pericyte dropout, perivascular edema, vascular endothelial cell activation, vascular endothelial cell vacuolization and autophagy, and basement membrane thickness (Table 3). In rat samples, mitochondrial morphology was unable to be evaluated at the magnification and resolution of the TEM images, but rats were graded on pericyte dropout, perivascular edema, vascular endothelial cell activation, vascular endothelial cell vacuolization and autophagy, and basement membrane thickness (Table 4). Categories were scored from 1 to 5, with 1 indicating a completely normal, nonpathologic appearance, and 5 indicating an extreme pathologic.

Table 5 Top 15 Statistically Significant Alterations in Metabolomics Data for Diabetic Rat versus Control Rat Aqueous Comparison

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>( P )</th>
<th>Ratio (diabetic versus control)</th>
<th>Fold change (diabetic versus control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inositol</td>
<td>1.04 ( \times 10^{-7} )</td>
<td>0.371654</td>
<td>-2.69067</td>
</tr>
<tr>
<td>Glutamine</td>
<td>2.48 ( \times 10^{-7} )</td>
<td>0.463891</td>
<td>-2.15568</td>
</tr>
<tr>
<td>Undecanoic acid</td>
<td>2.80 ( \times 10^{-7} )</td>
<td>0.383175</td>
<td>-2.60978</td>
</tr>
<tr>
<td>Fructose 6-phosphate</td>
<td>2.91 ( \times 10^{-7} )</td>
<td>8.74372</td>
<td>8.74372</td>
</tr>
<tr>
<td>Dihydroxyphenylalanine</td>
<td>2.91 ( \times 10^{-7} )</td>
<td>0.395121</td>
<td>-2.53087</td>
</tr>
<tr>
<td>Adonitol</td>
<td>5.20 ( \times 10^{-7} )</td>
<td>0.659</td>
<td>-1.51745</td>
</tr>
<tr>
<td>Tryptamine</td>
<td>1.44 ( \times 10^{-6} )</td>
<td>5.62764</td>
<td>5.62764</td>
</tr>
<tr>
<td>Arachidonate</td>
<td>1.64 ( \times 10^{-6} )</td>
<td>2.81545</td>
<td>2.81545</td>
</tr>
<tr>
<td>Lauric acid</td>
<td>3.09 ( \times 10^{-6} )</td>
<td>0.267065</td>
<td>-3.7444</td>
</tr>
<tr>
<td>Serotonin</td>
<td>3.68 ( \times 10^{-6} )</td>
<td>9.4835</td>
<td>9.4835</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>4.50 ( \times 10^{-6} )</td>
<td>0.439734</td>
<td>-2.2741</td>
</tr>
<tr>
<td>Glucose</td>
<td>1.01 ( \times 10^{-5} )</td>
<td>2.86683</td>
<td>2.86683</td>
</tr>
<tr>
<td>Glycine</td>
<td>1.26 ( \times 10^{-5} )</td>
<td>0.652747</td>
<td>-2.20874</td>
</tr>
<tr>
<td>Indolealdehyde</td>
<td>4.18 ( \times 10^{-5} )</td>
<td>5.9154</td>
<td>5.9154</td>
</tr>
<tr>
<td>Gluconic acid</td>
<td>5.67 ( \times 10^{-5} )</td>
<td>3.36142</td>
<td>3.36142</td>
</tr>
</tbody>
</table>
**Figure 3** T2DM-related morphologic changes in human anterior chamber. **A:** Smooth endothelial appearance, close proximity of pericytes (arrows). **B:** Pericytes (arrows) surround most of the vessel circumference. **C:** Thin, defined basement membranes (arrows) around an endothelial cell and pericyte. **D:** Oblong mitochondria with many rows of cristae. **E:** Endothelial cells with many reactive filaments. **F:** Thick basement membrane (red arrow) and ring of edema (white arrow) separate the vessel from the stroma. **G:** Endothelial cell (red arrow) with multiple layers of thick, reduplicated basement membrane (black arrow). **H:** Distended mitochondria with autophagocytic vesicles (arrows) and loss of normal cristae architecture. Scale bars: 1 \( \mu \text{m} \) (**A**, **B**, **E**, and **F**); 0.5 \( \mu \text{m} \) (**C** and **G**); 0.2 \( \mu \text{m} \) (**D** and **H**).

**Figure 4** T2DM-related morphologic changes in rat anterior chamber modeling. **A:** Smooth endothelial appearance with tightly packed stroma surrounding the vessel. **B:** Pericytes (arrows) closely line most of the vessel circumference. **C:** Endothelial cells appear smooth and even in thickness. **D:** Higher magnification of **C** demonstrates single layer of basement membrane (white arrow) with pericytes (red arrows) closely lining the vessel. **E:** Highly reactive appearance of endothelial cells with activated filaments extending into the lumen. **F:** Vessel with endothelial cell activation and thick basement membrane. **G:** Higher magnification of **F** demonstrates many layers of reduplicated basement membrane (arrows). **H:** Ring of edema (arrow) separates vessel from surrounding stroma. Scale bars: 1 \( \mu \text{m} \) (**A**–**C**, **F**, and **H**); 0.2 \( \mu \text{m} \) (**D**, **E**, and **G**).
appearance. An aggregate score combining all categories was compiled for each sample.

Statistical Analysis

Comparisons between diabetic and control groups of human and rat samples were conducted using the pairwise t-test, where \( P < 0.05 \) was considered significant.

Results

Diabetic Rat Eyes Have Cataracts and Reduced Corneal Endothelial Cell Density, Similar to Diabetic Human Eyes

Diabetic HFD-STZ rat eyes \((n = 10)\) and control rat eyes \((n = 10)\) were analyzed by light microscopy for the presence of crystalline lens opacification or discoloration indicative of cataract formation. All but one of the diabetic rat eyes (90%) had cataracts including lens opacities or discoloration, while none of the control rat eyes (0%) had lens opacities or discoloration. Diabetic and control HFD-STZ rat eyes were also imaged by confocal microscopy and analyzed on a MicroBrightField stereology platform to determine total corneal endothelial cell counts. Diabetic rat eyes \((n = 8)\) had lower corneal endothelial cell density compared to control eyes \((n = 10)\) (mean, 3162 versus 2462 cells/mm\(^2\); difference, −700 cells/mm\(^2\); \( P < 0.05 \)) (Figure 1), a finding that is directly analogous to the decrease in corneal endothelial cell density in human donor corneas published previously (difference, −102 cells/mm\(^2\); \( P < 0.05 \)).

Aqueous Humor Metabolomics and Proteomics Indicate Markers of Increased Oxidative Stress and Poor Glucose Control during Diabetic Disease Progression

Metabolomic analysis by gas chromatography/mass spectrometry was performed on pooled aqueous humor samples from diabetic rat eyes \((n = 8)\), with each sample consisting of three pooled aqueous samples to obtain necessary volume, and control rat eyes \((n = 8)\), with each sample consisting of three pooled aqueous samples to obtain necessary volume, to evaluate for metabolites and changes associate with diabetes (Figure 2). Of the top 95 metabolites, the concentrations of 57 were found to be significantly different between diabetics and controls \((P < 0.05)\). The top 10 metabolites in order of greatest significance were inositol, glutamine, undecanoic acid, dihydroxyphenylalanine, adonitol, and lauric acid. Of these, the metabolites that showed a significant increase in diabetics compared to controls were fructose 6-phosphate, tryptoamine, arachidonate, and serotonin, and those that showed a significant decrease were inositol, glutamine, undecanoic acid, dihydroxyphenylalanine, adonitol, and lauric acid.

Statistical analysis of diabetic rat aqueous metabolites versus controls demonstrated markers of poor glucose control, which is associated with increased oxidative stress and damage (Table 5 and Supplemental Tables S1–S3). The aqueous samples from the diabetic rats demonstrated a 2.87-fold increase in glucose and an 8.74-fold increase in fructose 6-phosphate compared to controls (both, \( P < 0.0001 \)). Additionally, aqueous samples from diabetic rats demonstrated a 2.69-fold decrease in inositol compared to controls (\( P < 0.0001 \)).

Proteomic analysis by UHPLC-MS/MS was performed on pooled aqueous humor samples from AD and control donor cornea eyes to evaluate for proteins and changes associated with diabetes. Aqueous humor samples were collected from a total of 16 donor eyes, including 6 from the AD group, 5 from the NAD group, and 5 from the ND group (Table 1). The top 15 significantly altered protein pathways associated with diabetic disease progression demonstrated changes in proteins associated with known pathologic mechanisms of diabetes related to cell—cell junction dysfunction and VEGF signaling. A complete list of proteins identified, the top significantly changed proteins (analysis of variance, \( P < 0.05 \)), and pathways are shown in Supplemental Tables S4–S6.

Diabetic Human Eyes Show Signs of Vascular Endothelial Cell Activation, Blood—Aqueous Barrier Breakdown, and Disorganized Vascular Endothelial Cell Mitochondria at the Ciliary Processes

Histologic ultrastructural analysis by TEM was performed on human donor eyes to evaluate the microvasculature of the ciliary process for changes associated with diabetes. A total of 10 human donor eyes from five donors with diabetes and 16 human donor eyes from eight control donors were analyzed by TEM (Table 2 and Figure 3). In the control eyes, vascular endothelial cells formed a thin, even layer around the circumference of the vessel. In contrast, vascular endothelial cells in diabetic eyes demonstrated focal proliferation and cytoplasmic protrusions at various points along the vessel wall. Additionally, the vascular endothelial cells in the diabetic eyes demonstrated increased numbers of vacuoles and autophagic inclusions compared to the control eyes. In the most pathologic samples, vascular endothelial cells appeared to be undergoing apoptosis and breaking off from the vessel wall into the lumen, leaving large sections of the vessel wall missing.

While basement membranes in the control group often adhered closely to the vascular endothelium in an even, well-defined single layer surrounding the vessel, basement membranes in the diabetic group were thick and uneven, often proliferating into multiple layers. Indicators
of compromised blood–aqueous barrier integrity in the microvasculature of ciliary processes were found in the diabetic human eyes, including expansion of the basement membrane generating greater separation of vascular endothelial cells from pericytes, and perivascular edema.

Additionally, the vascular endothelial cell mitochondria in the diabetic human eyes had a pathologic appearance, with disorganization or absence of the normal cristae formation, in contrast to the tightly packed and neatly layered cristae in healthy mitochondria in the control eyes. Vascular endothelial cell mitochondria in the diabetic human eyes were frequently observed to be completely circular in shape, not oblong as in the control eyes, due to distension.

Diabetic Rat Eyes Have Vascular Endothelial Cell Activation, Blood—Aqueous Barrier Breakdown, and Mitochondrial Disorganization, Mirroring Changes in Diabetic Human Eyes

Histologic ultrastructural analysis by TEM was performed on rat eyes to evaluate the microvasculature of ciliary processes for changes associated with diabetes. In rats, a total of 6 HFD-STZ eyes and 10 control eyes were analyzed morphologically. Qualitative analysis of rat eye TEM (Figure 4) demonstrated remarkable similarity to the findings observed in human eyes. As in humans, rat diabetic samples differed from controls by demonstrating thicker, reduplicated basement membranes and vascular endothelial cell activation. Diabetic rat eyes also demonstrated perivascular and stromal edema compared to controls. The association between diabetes and pericyte separation and dropout from around the blood vessels seemed less clear in rats than in humans, as pericytes were often absent from control vessels as well as diabetic vessels.

The level of autophagocytic activity and the morphology of the mitochondria were more difficult to evaluate in rat eyes than in human eyes. Mitochondrial ultrastructural features were not able to be identified well enough on TEM in the diabetic eyes to be analyzed qualitatively due to limited resolution and magnification of the images, which may have been attributable to processing such small eyes. Analysis of mitochondrial areas by ImageJ software in diabetic (n = 5) and control (n = 7) rat eyes demonstrated that diabetic rat eyes had a 33% greater mean mitochondrial area compared to control eyes (P < 0.05), a finding that is directly analogous to the increased mitochondrial area found previously in human diabetic corneal endothelial cells (AD group).25

Overall, the endothelial cells, basement membranes, and perivascular stroma associated with the ciliary body microvasculature in diabetic rats showed signs of blood–aqueous barrier breakdown that mirrored the changes in diabetic humans.

Discussion

In this study, the HFD-STZ rat model of T2DM recapitulated key findings of human T2DM pathology in the anterior segment of the eye. The majority of diabetic rats had developed cataracts by 48 weeks of age, while none of the age-matched controls had developed cataracts. Diabetic rats were also found to have decreased corneal endothelial cell density compared to controls. The results of the light-microscopy study strengthened the evidence for using rats as a model of T2DM, reproducing a known association between hyperglycemia and cataract formation in humans.75–78 The reduced density of corneal endothelial cells seen in humans79–81 was reproduced in rats, making HFD-STZ rats a promising model for studying surgical outcomes in diabetic cornea donors or recipients. Metabolomic analysis of rat aqueous found significant disturbances in metabolite levels previously correlated with poor glucose control and increased oxidative stress. Proteomic analysis of human aqueous samples demonstrated altered levels of proteins in pathways associated with diabetes progression, blood–aqueous barrier breakdown, and increased angiogenic drive. Analysis of mitochondrial size found that diabetic rats have an increase in mean cross section area that mirrors the changes seen in diabetic human mitochondria. Although markers of mitochondrial function were not analyzed in this study, the morphologic changes observed may suggest mechanistic similarities in rats and humans that future research will elucidate. Additionally, both human and rat eyes demonstrated ultrastructural evidence of blood–aqueous barrier breakdown in the ciliary body on TEM analysis. Overall, the similarity of changes observed on both rat and human TEM supported the present hypothesis that ciliary body blood–aqueous barrier compromise as an essential component in the development of diabetes-related pathology in the anterior segment.

Developing the understanding of how diabetes affects the anterior segment has a wide range of implications, from better risk stratification of surgical candidates and donors to understanding the impact of diabetes on other anterior-segment pathologies, including glaucoma. Furthermore, establishing the HFD-STZ rat as an animal model of T2DM expands the possibilities for development in therapeutics and surgical practice.

In prior diabetes investigations, the STZ T1DM model has been used to demonstrate ultrastructural changes in the cornea, including epithelial vacuoles, keratocyte apoptosis, swollen mitochondria in corneal endothelial cells, and morphologic changes in Descemet membrane.26,82 While multiple animal models of T1DM have proved incredibly useful in expanding the knowledge of T1DM in humans, none of these models have been able to reproduce the insulin resistance and hyperinsulinemia that characterize T2DM, and the disease process in T1DM models shows a delayed onset of corneal and peripheral nerve changes.
compared to T2DM models. Given that 90% of diabetes cases are type 2, research establishing the mechanisms at work in T2DM is necessary to characterize the risk profiles of both the patient and donor pools in corneal surgery. The HFD-STZ model appears to be a strong fit for studying ultrastructural changes in the setting of DM. The model’s greatest strength is that it reproduces the natural history of T2DM using a HFD to develop initial obesity, resultant insulin resistance, and compensatory hyperinsulinemia in relatively older rats, mirroring the prediabetic state in humans. The low-dose STZ subsequently given to induce pancreatic β-cell toxicity generates the progression to relative hypoinsulinemia and frank T2DM, and the entire process simulates the lifestyle conditions, indolent course, and age ranges in which T2DM usually develops in the general population. This model is easy to manage and widely available, and has been well-characterized to demonstrate common complications of long-term T2DM, such as diabetic neuropathy, decreased corneal nerve fiber length, epithelial innervation, sensitivity, and wound healing. There have been attempts to generate rodent models of T2DM that provide varying approximation of human disease, but many have significant weaknesses, including models with hypertension as a confounding factor, models that induce obesity but do not replicate decreased pancreatic β-cell function, and models with a short life span that develop pathology at a relatively young age, making them unsuitable for studying a disease that primarily occurs in middle-aged or elderly humans. The HFD-STZ model replicates the simultaneous hyperglycemia and hyperlipidemia unique to T2DM that accelerate capillary cell apoptosis, pericyte loss, oxidative stress, and mitochondrial damage in vascular and perivascular tissues.

A proposed mechanism for the pathophysiologic diabetic changes observed in the anterior segment of the eye is through increased vascular permeability in the ciliary body secondary to pericyte loss and vascular endothelial activation, similar to what is seen in the retinal microvasculature in diabetic retinopathy. This investigation provides substantive support for the hypothesis that breakdown of the blood–aqueous barrier and vascular leakage lead to aqueous humor aberrations that drive the multitude of changes seen in the avascular cornea and subsequent corneal endothelial dysfunction. The TEM results demonstrated that signs of blood–aqueous barrier breakdown classically found in the retina were also seen in the ciliary bodies of both rats and humans. Increased vascular endothelial cell cytoplasmic filaments, as well as basement membrane reduplication, indicate the activation of endothelial cells and pericytes; these were the strongest trends conserved between the rat and human TEM tissues analyzed. Cell loss was apparent in many samples, with pericyte dropout as well as vascular endothelial cell loss observed in severe cases. While pericyte dropout was common to both the rat and human samples, frank vascular endothelial cell loss was more characteristic of pathologic human samples than rat. Both human and rat TEM demonstrated signs of perivascular edema in pathologic samples. While signs of cell activation were indicative of vascular pathology, the areas of cell loss and edema surrounding microvessels in the ciliary body processes were clear evidence of blood–aqueous barrier breakdown.

Aqueous humor analyses also support the hypothesis of a causal relationship between anterior segment blood–aqueous barrier breakdown and anterior segment structural disease in T2DM. Elevated glucose levels in the aqueous of diabetic rats demonstrate that the anterior segment is not completely protected by the blood–aqueous barrier from elevated glucose levels present in the serum. In humans, hyperglycemia has been shown to activate pathways in the retina that lead to activation of the protein kinase C pathway, polyol pathway, hexosamine pathway, and formation of advanced glycation end-products. Each of these pathways is associated with the generation of excess reactive oxygen species, especially superoxide dismutase in mitochondria, and increased oxidative stress, and they are implicated in a host of pathologic changes in the retinal microvasculature, including thickened basement membranes, pericyte and endothelial apoptosis, loss of intercellular junctions, increased vascular permeability, and capillary ischemia. Some of these pathways, including the association of increased superoxide anion with blood–retinal barrier breakdown, increased formation of advanced glycation end-products, and increased vascular permeability, have been demonstrated in diabetic rats as well. The high levels of glucose in the aqueous suggest that similar mechanisms of blood–aqueous barrier breakdown may be occurring in the anterior segment. The most significant metabolite change in rat aqueous analysis was inositol, which demonstrated a 2.69-fold decrease in diabetic rats compared to controls (P < 0.0001). In humans, inositol is known to have insulin-like properties and play a role in lowering blood glucose levels. The glucose-regulatory properties of inositol have been demonstrated in STZ rats, and abnormally low levels of inositol or inositol-containing second messengers have been seen in both human and animal models of diabetes, including T2DM Goto-Kakizaki rats. The disturbance in inositol level is further evidence that the anterior chamber is not protected from the pathophysiological mechanisms of diabetes.

When comparing the top metabolic pathways impacted by diabetes in rat aqueous (Supplemental Table S3) to the top metabolic pathways impacted by diabetes in human aqueous using data from Fortenbach et al, both data sets demonstrated that many of the top pathways altered were involved in glycolysis and gluconeogenesis. Some of the common pathways that were in the top 20 pathways of both data sets included the iRNA charging pathway, glycine betaine degradation, citrulline biosynthesis, glycine biosynthesis, cysteine degradation, and tyrosine biosynthesis. While Fortenbach found elevated levels of the branched-chain
Table 5), only glucose and phenylalanine were shared in elevations in branched-chain amino acids in this model. Of resulting in increased oxidative stress, there were no similar amino acids in the most severe diabetics and postulated that insulin resistance was achieved by showing elevated levels of glucose accumulating in the aqueous chamber, a crucial finding in establishing the validity of the model.

The quantity of rat aqueous available in this study was insufficient to conduct both metabolic and proteomic analyses and, therefore, proteomic analysis was not done. Proteomic analysis of increasing diabetic severity in human aqueous samples specifically pointed to cell—cell junction disruption and increased drive for angiogenesis, complementing the rat metabolomics data and adding support to the hypothesis of blood—aqueous barrier breakdown. It will be important in future studies validating this model to perform proteomic analysis in rats for comparison to human data.

Limitations of the study included the range in severity of diabetes among the human samples. While diabetes was induced in the rats by a standardized method, the natural history of each human donor’s disease was more varied, including time since diagnosis, medication status, and insulin-dependence status. The difficulty of trying to compare a model generated in a standardized, controlled environment with a heterogenous population of human donors was especially highlighted in certain areas of this study, such as comparing metabolomics data between rats and humans. Many of the barriers that the study investigators experienced in attempting to validate this rat model as a surrogate for human disease ironically highlight the necessity of developing a reliable rat model. Human studies involving diabetes, particularly retrospective studies, are difficult to control and thus generate conclusive findings, particularly regarding the corneal endothelium; animal models such as this rat model and prospective, multicenter, randomized clinical trials such as the Diabetes Endothelial Keratoplasty Study (ClinicalTrials.gov identifier NCT05134480) are best poised to demonstrate the effects of T2DM on corneal endothelial cell density. Another limitation was the fact that only male rats were used in the study. In previous attempts to reproduce a model of T2DM in female Sprague-Dawley rats, there was considerable difficulty in inducing obesity and impaired glucose tolerance using the same protocol that reliably reproduced a model of T2DM in male rats. Although using only male rats made conducting the study much easier, future investigation into generating a reproducible female rat model of T2DM may be necessary to validate the generalizability of some of these results.

Conclusion

Numerous correlations between rat and human pathologic alterations attributable to T2DM disease support the HFD-STZ rat as a reliable animal model of T2DM for use in studies of anterior segment ocular structures. Changes observed in the lens, cornea, aqueous humor, and ciliary body microvasculature in this model implicate blood—aqueous barrier breakdown and vascular leakage into aqueous as a key pathophysiological mechanism of diabetes in the anterior segment of the eye, analogous to the vascular leakage observed in the posterior segment of the eye and other end organs damaged in T2DM.

Acknowledgments

We thank our cornea donors and their families.

Disclosure Statement

None declared.

Supplemental Data

Supplemental material for this article can be found at http://doi.org/10.1016/j.ajpath.2024.02.004.


34. Sato E, Mori F, Igarashi S: Corneal advanced glycation end products increase in patients with proliferative diabetic retinopathy. Diabetes Care 2001, 24:479–482


37. Lass JH, Benetza BA, Patel SV: Donor, recipient, and operative factors associated with increased endothelial cell loss in the cornea preservation time study. JAMA Ophthalmol 2019, 137:185–193


76. Lee D, Park S: Hyperglycemia and hypo-HDL-cholesterolemia are primary risk factors for age-related cataract, and a Korean-style balanced diet has a negative association, based on the Korean genome and epidemiology study. J Korean Med Sci 2021, 36:e155


84. Yorek M, Obrosov A, Shevalye H: Effect of diet-induced obesity or type 1 or type 2 diabetes on corneal nerves and peripheral neuropathy in C57Bl/6J mice. J Peripher Nerv Syst 2015, 20:24–31


