Experimental Anterior Ischemic Optic Neuropathy in Diabetic Mice Exhibited Severe Retinal Swelling Associated With VEGF Elevation

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PURPOSE. Diabetes mellitus (DM) is one of the most important risk factors for nonarteritic anterior ischemic optic neuropathy (AION). In this study, we investigated for the first time the impact of experimental AION in a DM model.

METHODS. We induced a photochemical thrombosis model of AION after streptozotocin-induced DM and performed serial optical coherence tomography (OCT), morphometric analyses, and VEGF levels in the retina and sera.

RESULTS. Compared with non-DM animals, experimental AION in DM mice led to significantly greater retinal swelling on day 1 and worse thinning at week 3 on OCT measurements. Greater retinal swelling on OCT in DM-AION eyes was associated with significantly increased loss of brain-specific homeobox/POU domain protein 3A (Brn3A+) retinal ganglion cells at week 3. In acute AION, there was greater inflammation as seen by an increase in ionized calcium-binding adapter molecule 1 (Iba1+)-activated microglia. On day 1, there was an increase in vascular endothelial growth factor (VEGF) level in nondiabetic AION retinas and sera, but the VEGF level was the highest in the diabetic AION group, which decreased to nondiabetic levels after insulin treatment. The decrease in retinal and serum VEGF levels after insulin treatment correlated with a reduction in retinal swelling.

CONCLUSIONS. In the setting of hyperglycemia, AION led to greater acute, postischemic microglial activation and elevation of VEGF levels, which likely contributed to greater retinal swelling acutely and worse retinal thinning and loss of retinal ganglion cells chronically. Treatment of hyperglycemia with insulin reduced VEGF levels and retinal swelling, consistent with the idea that VEGF is an important factor in postischemic swelling and that good glycemic control following AION may lead to better visual outcome.

Keywords: diabetes mellitus, ischemic optic neuropathy, ischemia, anterior ischemic optic neuropathy, optic nerve, retinal ganglion cells, optical coherence tomography

Diabetes is the leading cause of vision loss in the working population, most commonly due to macular edema and diabetic retinopathy. Having diabetes also increases the risk of vision loss in general as diabetes is one of the most common risk factors for central nervous system (CNS) ischemia due to stroke and nonarteritic anterior ischemic optic neuropathy (AION), the most common acute optic neuropathy in those older than 50 years. Having diabetes not only increases the risk of first eye involvement in AION but also second eye, and 30% to 36% of AION patients have concurrent diabetic retinopathy, which increases disease burden. In large retrospective studies, diabetic patients with AION are found to have greater vascular changes, more peripapillary retinal hemorrhages at onset, and longer time until resolution of optic disc edema, although not worse long-term visual outcome. Because of the inherent limitations of retrospective human studies, the specific effects of diabetes on AION have remained elusive. Central nervous system ischemia in the setting of diabetes is attributed to macro- and microvascular damage, activation of immune pathways, hematologic abnormality, and metabolic effects of hyperglycemia. We and others have characterized retinal changes after experimental AION. In acute AION, there are significant retinal swelling and early upregulation of inflammatory markers. Chronically, retinal thinning and loss of retinal ganglion cells (RGCs) occur within 3 to 4 weeks in rodent experimental AION, and the severity of the acute retinal swelling seems to correlate with the extent of chronic thinning. In this study, we investigated the retinal changes after experimental AION in diabetic mice and assessed the effects of insulin treatment.

MATERIALS AND METHODS

Animals

We used more than 200 wild-type adult C57BL/6 mice (Charles River Laboratories International, Inc., Hollister, CA, USA), which were housed in a temperature-controlled room and maintained on a 12-hour light-dark schedule with free access to food and water. All animals were treated in accordance with the ARVO Statement for the Use of Animals.
in Ophthalmic and Vision Research. Mice were anesthetized by intraperitoneal injection of ketamine 50 to 100 mg/kg (Hospira, Inc., Lake Forest, IL, USA); xylazine 2 to 5 mg/kg (Bedford Laboratories, Bedford, OH, USA); and buprenorphine 0.05 mg/kg (Bedford Laboratories) under sedation. The pupils of anesthetized mice were dilated with 1% tropicamide (Alcon Laboratories, Inc., Fort Worth, TX, USA) and 2.5% phenylephrine hydrochloride (Akorn, Inc., Lake Forest, IL, USA). Many animals underwent serial manipulations (e.g., diabetes then AION induction, optical coherence tomography [OCT] measurements), then killed for various histologic or tissue analyses. Only animals that satisfied criteria for each step were included in subsequent steps, and only high-quality data were included in analysis.

Diabetes Induction

We induced diabetes in our mice using an intraperitoneal injection of streptozotocin (STZ; Sigma-Aldrich Corp., St. Louis, MO, USA) in citrate buffer (pH 4.5).30 Three weeks after diabetes induction, we obtained blood from the tail vein to test glucose levels using a blood glucose monitoring system (One Touch Basic Accu-Check; Aviva Plus, Roche, Pleasanton, CA, USA), and mice with blood glucose levels consistently >250 mg/dL were considered diabetic. Data in Figure 1 compared measurements in three groups: (1) mice injected with STZ in citrate buffer; (2) mice injected with citrate buffer only; and (3) un.injected, normal mice. As there were no difference in glucose level or weight between citrate buffer–injected control animals and uninjected controls in a sufficiently large number of animals per group, for the rest of the experiments, we no longer used citrate buffer–injected animals as controls. Instead, controls were un.injected normal mice.

Experimental AION and Experimental Groups

Three weeks after diabetes induction, which is considered sufficient time to induce chronic diabetes,31 we induced experimental AION using photocoagulation-induced thrombosis,23,25,26 following injection of rose bengal (1.25 mM in phosphate-buffered saline, 5 µL/g body weight) into the tail vein using transpupillary laser light with a frequency doubled Nd:YAG laser (Pascal; OptiMedica, Santa Clara, CA, USA) at 400-µm spot diameter (50 mW power, 1 second duration, 15 spots). To ensure consistency of studies, the same person performed AION induction in all experiments, and one eye in each mouse had AION induction, whereas the contralateral (fellow) eye served as control.

Spectral-Domain Optical Coherence Tomography

We performed OCT scans at baseline and at 1 day, 1 week, and 3 weeks after AION using an OCT device (Spectralis OCT; Heidelberg Engineering, GmbH, Heidelberg, Germany).23,25,26 The number of eyes imaged at various time points was different because some animals were imaged at day 1 and immediately killed (for Iba-1 staining), whereas some animals were sacrificed by heart puncture at 3 weeks.

Figure 1. Streptozotocin (STZ)-induced hyperglycemia and experimental AION. (A) Experimental protocol. (B) Blood glucose levels at baseline, 3 weeks, and 8 weeks in STZ injected, citrate buffer–injected, and control normal mice. *P < 0.001. (C) Body weight at baseline, 3 weeks, and 8 weeks STZ injected, citrate buffer–injected, and control normal mice. *P < 0.001 for week 3 and P = 0.005 for week 8.
were imaged serially and killed at week 3 (for Brn3A staining). Measurements of OCT with poor image quality were not included in the analysis. Imaging quality was most challenging at day 1 due to the impact of consecutive days of anesthesia and manipulations, cataract formation, and severity of optic disc edema. Every effort was made to ensure consistency of the study over time, including AION induction by the same person for all the experiments and inclusion of a sufficiently large number of AION and control eyes from both diabetic and nondiabetic animals in every experiment. The measurements of OCT at each time point came from an equal number of pooled experiments, and the greatest number of animals was imaged at day 1 and the smallest number at week 3.

We performed the circular scan (scan angle 12°, retinal nerve fiber [RNFL] scan) using the enhanced depth imaging (EDI) and high resolution mode, with each B-scan consisting of 1536 A-scans centered around the optic disc and average of 16 B-scans per image. For the circular RNFL scans, we manually segmented the thickness of the ganglion cell complex (GCC), which was defined as the combined thicknesses of the RNFL, ganglion cell layer, and the inner plexiform layer (global average measurement).25–26 The segmentation was performed under magnification (×200) and was visually distinguished from the adjacent vitreous on one side and the inner nuclear layer on the other side due to an obvious change in signal intensities. The segmentation was performed by one well-trained masked investigator to reduce variability. The segmentation was visually confirmed by a second investigator as needed. OCT studies with poor image quality or segmentation were not included. We performed posterior pole scans (scan angle 30° × 25°) using EDI at high-speed mode, (B-scan consisted of 768 A-scans, average 9 frames/B-scan) and 25-line scans (scan angle 25° × 15°) at high-resolution mode (average 16 frames/B-scan). The total retinal thickness, defined as the RNFL to Bruch’s membrane, was automatically segmented by the OCT software (Heidelberg Engineering) and measured using the 1- or 3-mm diameter concentric circle grid with the optic disc in the center.

Immunohistochemistry and Morphometric Analyses

At day 1 and at week 3 after AION, we performed intracardiac perfusion using 4% paraformaldehyde in phosphate-buffered saline, whole mount retinal dissection, immunohistochemistry, and fluorescence microscopy (Nikon Eclipse TE300 microscope; Nikon Corp., Tokyo, Japan) with ×4, ×10, and ×20 objectives (Nikon Corp.) and commercial software (Meta-morph; Molecular Devices, LLC, Sunnyvale, CA, USA). To measure activated microglia at day 1, we performed morphometric analyses of fluorescence signal after immunohistochemistry of whole mount retina using primary rabbit polyclonal anti-Iba1 antibody, which labeled-activated microglia (1:200 dilution; Wako Chemicals, Richmond, VA, USA) and secondary goat anti-rabbit IgG AlexaFluor 568–labeled antibody (1:200 dilution; Life Technologies, Waltham, MA, USA). To count Brn3A+ RGCs at week 3, we performed whole mount retinal dissection 3 weeks after AION and then stained with primary mouse monoclonal anti-Brn3A antibody (1:200 dilution; Santa Cruz Biototechnology, Dallas, TX, USA) and secondary goat anti-mouse IgG AlexaFluor 568–labeled antibody (1:200 dilution; Life Technologies). All retinal whole mount preparations were mounted with DAPI-containing media (Vectashield; Vector Laboratories, Burlingame, CA, USA). We then used ImageJ (http://rsweb.nih.gov/ij/; provided in the public domain by the National Institutes of Health, Bethesda, MD, USA) in a masked setting to quantify Iba1+ signal in images that were taken using the same parameters under a masked setting. We used a 300-pixel diameter circular region of interest, isolated all particles of interest using the plugin function to analyze particles, and then measured the fluorescence signal for each eye. To quantify Brn3A+ signal, we took eight images (four quadrants, two images of 0.14 mm² each, taken at magnification of ×200) and used a custom-written ImageJ script to quantify and calculate the number of Brn3A+ cells/mm². Only eyes with high-quality imaging at all four quadrants were included in the analysis.

ELISA Analysis for Expression of VEGF After AION

To measure retinal VEGF levels, we prepared retinal tissue by harvesting day 1 AION eyes and control fellow eyes from diabetic and nondiabetic mice (n = 6–9 eyes per condition) immediately after euthanasia in radioimmunoprecipitation assay lysis buffer (Abcam, Cambridge, MA, USA); homogenized the tissue in a sonicator; and then stored in −20°C. Protein concentration was measured using a protein assay kit (Pierce BCA; Thermo Fisher Scientific, Waltham, MA, USA) in a commercial kit (mouse VEGF Quantikine ELISA kit; R&D Systems, Inc., Minneapolis, MN, USA) and standardized. We measured VEGF level/mg protein using a commercial kit (mouse VEGF Quantikine ELISA kit; R&D Systems, Inc., Minneapolis, MN, USA) and protocol. Serum VEGF levels were measured in a small number of mice by collecting 50 μL of blood, and then were measured using the same ELISA kit and calculated in pg/mL sera. All absorbance measurements were done at a wavelength of 450 nm using a microplate reader (Infinite M1000; Tecan Group Ltd, Männedorf, Switzerland) with wavelength correction to 540 nm. After constructing a standard curve by plotting absorbance for each standard against the concentration, the VEGF level in each sample was measured according to the best-fit curve.

Insulin Treatment

To treat hyperglycemia immediately after AION induction, we injected a combination of intermediate-acting and short-acting insulin, insulin lispro (0.5 IU/kg Humalog 75/25; Lilly USA LLC, Indianapolis, IN, USA), twice a day and glargine (0.5 IU/kg Lantus; Sanofi-Aventis U.S. LLC, Bridgewater, NJ, USA) once a day, and measured blood glucose as frequently as every hour as needed. If the blood glucose was lower than 100 mg/dL, which occurred in 30% of mice, we performed intraperitoneal injection of 20% D-glucose in 0.9% saline (4 g/kg). If the blood glucose level was higher than 300 mg/dL, which was less common, we injected insulin lispro (Lilly USA LLC) according to an insulin sliding scale. For each mouse, multiple blood glucose measurements were performed to ensure there was good glycemic control.

Statistical Analysis

All data were presented as mean ± SEM. We performed statistical analysis using commercial software (SPSS 15.0; SPSS, Inc., Chicago, IL, USA), and statistical significance was defined as P < 0.05. We used Wilcoxon signed-rank test for paired data and Mann-Whitney U test for unpaired data. Correlation was performed using Pearson correlation coefficient.

RESULTS

Diabetes Induction with STZ

Three weeks after diabetes induction using STZ, the average blood glucose level in STZ-injected mice was 499.3 ± 20.5 mg/dL (n = 25), which was significantly higher than that of the citrate buffer–injected controls (150.0 ± 6.0 mg/dL, n = 21, P
Diabetic AION Eyes Exhibited Twice as Much Swelling Acutely and at Least Twice as Much Thinning Chronically as Nondiabetic AION Eyes

Once hyperglycemia was confirmed, we induced AION using photochemical thrombosis (Fig. 1A). Comparing AION and control eyes, there was significant swelling of the GCC as measured using OCT in the AION eyes compared with contralateral control eyes 1 day after ischemia in both diabetic (DM-AION: 108.3 ± 3.4 μm, DM-control: 81.0 ± 0.7 μm, n = 28 mice, P < 0.001) and nondiabetic animals (non–DM-AION: 97.7 ± 2.4 μm, non–DM-control: 82.3 ± 1.0 μm, n = 17 mice, P = 0.003; See "Methods" section; Figs. 2A, 2B). Relative to baseline measurements, there was almost twice as much (13 μm) retinal swelling in the DM eyes than non–DM eyes 1 day after AION (DM-AION eyes: 28.2 ± 3.2 μm, n = 25 mice; non–DM-AION eyes: 15.5 ± 3.1 μm, n = 15 mice; P = 0.005; Fig. 2D). At week 3 after AION, there was significant thinning of the GCC in the AION eyes compared with control eyes in both DM (DM-AION: DM-control = 68.4 ± 2.3 μm: 77.5 ± 1.3 μm, n = 11 mice, P = 0.003) and non–DM animals (non–DM-AION: non–DM-control = 77.1 ± 1.4 μm: 81.4 ± 0.9 μm, n = 7 mice, P = 0.034; Fig. 2B). Relative to baseline measurements, there was more than twice as much thinning (6.6 μm) of the GCC in the DM-AION eyes compared with non–DM-AION eyes (DM-AION: 12.4 ± 2.3 μm, n = 10 mice; non–DM-AION: 5.8 ± 1.0 μm, n = 6 mice; P = 0.05; Fig. 2D).

Segmentation of the total retinal thickness also showed a similar pattern of greater acute swelling and chronic thinning in DM-AION eyes compared with non–DM-AION eyes (Fig. 2C). On day 1, there was significant swelling in the AION eyes compared with control eyes in DM (DM-AION: 305.8 ± 10.7 μm, DM-control: 245.3 ± 2.0 μm, n = 27 mice each, P < 0.001) and non–DM animals (non–DM-AION: 290.1 ± 8.8 μm, non–DM-control: 246.7 ± 2.5 μm, n = 16 mice each, P = 0.002). Relative to baseline, there was twice as much swelling (28.2 μm) in the DM-AION eyes (DM-AION: increased 62.5 ± 11.8 μm, n = 22 mice; non–DM-AION increased 34.1 ± 8.6 μm, n = 10 mice; P = 0.05). At week 3, there was significant thinning of the total retinal thickness in AION eyes compared with control eyes in both DM (DM-AION: 211.1 ± 9.7 μm, DM-control: 245.6 ± 4.2 μm, n = 12 mice, P = 0.002) and non–DM animals (non–DM-AION: 220.5 ± 13.0 μm, non–DM-control: 249.0 ± 2.8 μm, n = 7 mice, P = 0.043). Relative to baseline, there was more than four times greater thinning (29.7 μm) of the total retinal thickness in the DM-AION eyes compared with non–DM-AION eyes (DM-AION: thinning 36.6 ± 8.7 μm, n = 14 mice; non–DM-AION: thinning 6.9 ± 4.3 μm, n = 9 mice; P = 0.054; Fig. 2E).

After AION in Diabetic Mice, Greater Thinning on OCT Correlated With Greater Loss of Brn3A⁺ RGCs

We counted Brn3A⁺ RGCs in the retinal whole mount prepared from the same eyes as OCT studies at 3 weeks after AION, which is considered chronic AION. There was significant loss of Brn3A⁺ cells in the AION eyes compared with control eyes in both DM (DM-AION: 1556 Brn3A⁺ cells/ mm², n = 8 eyes, DM-control: 3017 Brn3A⁺ cells/mm², n = 11 eyes; P = 0.012) and non–DM animals (non–DM-AION: 2059 Brn3A⁺ cells/mm², n = 14 eyes; non–DM-control: 3102 Brn3A⁺ cells/mm², n = 14 eyes; P = 0.002, Fig. 3B). Comparing AION eyes, there was significantly greater loss (14%) of Brn3A⁺ cells in the DM compared with non–DM animals (DM-AION: 48% loss, non–DM-AION: 34% loss, P = 0.03). There was a linear correlation between Brn3A⁺ cell counts and OCT GCC measurements (r = 0.428, P = 0.015; Fig. 3C). Similarly, there was a linear correlation between Brn3A⁺ cell counts and total retinal thickness measurements using the 1 mm diameter circle (r = 0.650, P < 0.001, Fig. 3D) and the 3 mm diameter circle (r = 0.482, P = 0.007, Fig. 3E). These findings confirmed that greater retinal thinning measured on OCT correlated with greater RGC loss in diabetic AION eyes.

Greater Iba1⁺-Activated Microglia After Acute AION in Diabetic Mice

We investigated the factors in diabetic AION eyes that may be responsible for greater swelling acutely and worse thinning chronically by looking for evidence of microglial activation using anti-Iba1 antibody. Immunohistochemistry and morphometric analyses of retinal whole mounts revealed there was significantly greater increase in Iba1⁺ signal after AION in both DM (DM-AION: 3.5 ± 0.5 × 10⁶ arbitrary fluorescence units [AFU], n = 7 eyes; DM-control: 1.3 ± 0.2 × 10⁶ AFU, n = 6 eyes; P = 0.028) and non–DM eyes (non–DM-AION: 2.1 ± 0.2 × 10⁶ AFU, n = 4 eyes; non–DM-control: 1.2 ± 1.0 × 10⁶ AFU, n = 6 eyes; P = 0.068; Fig. 4). We found DM-AION eyes had significantly greater Iba1⁺ signal (66.7%) compared with non–DM-AION eyes (P = 0.038), consistent with greater inflammation in DM animals. Although not significantly different between DM and non–DM eyes, some control diabetic eyes had slightly greater Iba1⁺ expression (Fig. 4A), suggesting there may be mild chronic inflammation even without AION in diabetic mice.

Significant Elevation of VEGF Level in Diabetic Mice after AION

Next, we used ELISA to measure the changes in the retinal and serum level of VEGF, a factor well known to be associated with retinal edema and swelling on OCT. We found AION in nondiabetic animals led to a small increase (12%) in the retinal VEGF level (non–DM-AION: 74.5 ± 4.2 pg/mg protein, n = 9 eyes; non–DM-control: 61.7 ± 7.1 pg/mg protein, n = 9 eyes, P = 0.05; Fig. 5A). In diabetic animals, AION led to a large increase (241%) in retinal VEGF level (DM-AION: 154.3 ± 45.6 pg/mg protein, n = 6 eyes; DM-control eyes: 63.9 ± 6.6 pg/mg protein, n = 7 eyes, P = 0.028). Similarly, there was a significant increase (14%) in serum VEGF level in DM mice with AION compared with non–DM mice with AION (DM-AION: 90.37 ± 1.88 pg/μL, n = 5 mice; non–DM-AION: 60.78 ± 8.35 pg/μL, n = 4 mice, P = 0.054; Fig. 5B).

Insulin Treatment Immediately After AION in Diabetic Mice Decreased Retinal and Serum VEGF Levels and Retinal Swelling

We assessed the effects of insulin treatment immediately after AION induction in DM animals and found that insulin treatment significantly decreased the retinal VEGF level (insulin-treated DM-AION: 84.9 ± 8.0 pg/mg protein, n = 8; insulin-untreated DM-AION: 154.3 ± 45.6 pg/mg protein, n = 6, P = 0.05) to a level similar to that of the non–DM-AION eyes (74.5 ± 4.2 pg/mg protein, n = 9; P = 0.29) and insulin-treated...
FIGURE 2. Diabetic AION eyes had twice as much swelling acutely and twice as much thinning chronically as nondiabetic mice on OCT GCC, and total retinal thickness measurements. (A) Representative images of OCT (line scan through optic disc) of DM-AION and non-DM-AION eyes on day 0 (before AION) and on day 1 and week 3 after AION. White asterisk indicates thickening of peripapillary retina in day 1 AION and more subretinal fluid in DM mice. (B) Serial GCC measurements in AION and control eyes in non-DM (left) and DM (right) mice. (C) Serial total retinal thickness measurements in AION and control eyes in non-DM (left) and DM (right) mice. (D) Change in GCC thickness relative to baseline in day 1 and week 3 in non–DM-AION and DM-AION eyes. (E) Change in total retinal thickness relative to baseline in day 1 and week 3 in non–DM-AION and DM-AION eyes. *P < 0.05.
DM-control eyes (70.6 ± 6.9 pg/mg protein, n = 8, P = 0.07; Fig. 5A). Similarly, in the DM-AION mice, insulin treatment decreased the serum VEGF level (insulin-treated DM-AION mice: 59.4 ± 13.7 pg/mL, n = 3; insulin-untreated DM-AION mice: 90.4 ± 1.9 pg/mL, n = 3, P = 0.05) to a level similar to that of the non-DM-AION mice (60.8 ± 8.3 pg/mL, n = 4, P = 0.7; Fig. 5B). Such decrease in retinal VEGF levels with insulin treatment indicated that treatment of hyperglycemia in acute AION could ameliorate the acute rise in VEGF, and similar decrease in changes in the retinal and serum VEGF levels suggested that serum VEGF level may be a useful approxima-
tion of the changes in the retinal VEGF level with insulin treatment (Fig. 5B). With insulin treatment (and decrease in VEGF level), there was a significant decrease in retinal swelling in acute AION in the same eyes. On day 1, swelling of GCC in insulin-treated DM-AION eyes decreased by 23 μm compared with untreated DM-AION eyes (90.6 ± 1.6 μm, n = 12; 113.6 ± 15.4 μm, n = 7, respectively; P = 0.009; Fig. 5C). Similarly, insulin treatment led to a significant decrease in swelling of total retinal thickness by 26.7 μm (insulin-treated DM-AION eyes: 263.1 ± 5.6 μm, n = 12; insulin-untreated DM-AION eyes: 289.8 ± 10.7 μm, n = 7; P = 0.035; Fig. 5C). The decrease in VEGF level in insulin treatment correlated with the decrease in GCC (r = 0.818, n = 25, P < 0.001) and total retinal thickness (r = 0.608, n = 25, P = 0.001; Fig. 5D).

**DISCUSSION**

The hallmark of acute AION is optic disc edema associated with ischemic damage to the proximal optic nerve, leading to irreversible optic atrophy and RGC loss. Using serial OCT measurements, we showed that in diabetic animals, experimental AION was associated with greater optic nerve head swelling acutely and greater thinning and loss of Brn3A+ RGCs chronically. Greater swelling following AION in diabetic animals was associated with significantly greater Iba1+ microglia.
microglial activation and significantly increased retinal and serum VEGF levels. Insulin treatment in acute AION normalized both retinal and serum VEGF elevation and decreased retinal swelling. Our data showed that the combination of hyperglycemia and AION led to worse prognosis and that treatment of hyperglycemia with insulin in our model reduced retinal swelling and decreased both levels of retinal and serum VEGF that could result in improved visual outcome in humans with nonarteritic AION.

Hyperglycemia has multiple effects that can potentially impact not only AION onset \(^{11-13}\) and second eye involvement, but also visual outcome once optic nerve ischemia occurs. Other than the well-known effects of hyperglycemia on blood vessels, \(^{31-35}\) there is also evidence that diabetes increases cellular inflammation, \(^{36-39}\) which can be directly attributed to hyperglycemia. \(^{40}\) In hyperglycemia, there is known upregulation of levels of retinal VEGF, which is secreted by Müller cells in vivo and in vitro, \(^{41-45}\) which can prime the hyperglycemic retinal milieu to develop greater swelling and more prominent inflammation in ischemia. \(^{31,35}\) Although we still do not understand the impact of diabetes on human AION, and stroke is not the same as AION, there are some interesting studies in diabetic stroke that may be relevant to diabetic AION. In the setting of diabetes, stroke is associated with greater cerebral edema, larger stroke size, and worse morbidity and mortality following ischemia in human studies. \(^{5}\) In animal models of diabetes and stroke, hyperglycemia is associated with greater breakdown of the blood–brain barrier \(^{19,44}\) and some of these changes can be reversed with glucose control. \(^{45}\)

Our data show for the first time that glucose control in our model of acute AION reduces retinal swelling and both retinal and serum VEGF levels. Feit-Leichman and coworkers \(^{34}\) show that the retinal vascular changes in diabetic mice were not seen until 6 months after STZ injection using the same mice strain (C57BL/6) that we used in our experiment. Therefore, we believe that the effect of insulin on retinal and serum VEGF levels and retinal swelling (diabetes at 3 weeks, AION at 1 day) results from its effects on hyperglycemia rather than on diabetic vascular changes. Previous studies have shown that treatment with systemic or local insulin can reduce retinal neuronal cell death in diabetic rats. \(^{46,47}\) Although the benefit of insulin treatment in AION patients has not been well studied, many studies have tried to determine if treatment of hyperglycemia can improve stroke outcome. Unfortunately, despite many large clinical trials, the benefit of strict glucose control in stroke has remained unclear, which may be related to the complexity of the postischemic cascade and known risk of hypoglycemia on stroke outcome. \(^{6,18}\) Although there is currently no effective therapy for human AION, one commonly considered treatment option to dampen retinal swelling is systemic corticosteroids. \(^{16}\) If corticosteroid treatment increases blood glucose level, its benefit can be negatively impacted based on our data showing the harmful effects of hyperglycemia in acute AION and retinal and serum VEGF levels.

Given the increase in VEGF in diabetic animals after AION, anti-VEGF therapy is another approach to treat AION in diabetes. Although this treatment has not been tested in diabetic AION, anti-VEGF therapy has not been effective in promoting functional or histologic improvement in a nonhuman primate model of nonarteritic AION \(^{49}\) and has even been associated with greater RGC loss in experimental diabetes without AION. \(^{80}\) In the last decade, anti-VEGF therapy has been used to treat diabetic macular edema \(^{52}\) and other conditions associated with persistent retinal edema, including in a small number of patients with acute AION. \(^{50-52}\) Unfortunately, there are reported cases of anti-VEGF treatment leading to AION, \(^{55-57}\) including one case with diabetic macular edema, \(^{58}\) so the efficacy of anti-VEGF therapy in acute AION remains unclear.

Limitations to our study include the use of animal models of diabetes and AION, which is not the same as the human disease. Also, the STZ-induced diabetes model relatively rapidly induces hyperglycemia and loss of insulin-secreting cells, so it does not simulate the typical patient with chronic diabetes who develops AION. Our baseline OCT measurements before AION induction can potentially be impacted by changes related to several weeks of hyperglycemia. A novel mouse model of diabetes using specific strain has shown that there is a reduction in RGCs in peripheral retina as early as 7 days, \(^{53}\) which can potentially impact retinal thickness measurements. Another study using same strain of mice as our study demonstrates no detectable loss of RGCs up to 1 year after induction of diabetes. \(^{54}\) In our study, we did not find a significant difference in the pre-AION, baseline OCT measurements between diabetic and nondiabetic mice just before AION induction, consistent with a relatively modest impact of baseline diabetes-related changes in our pre-AION, baseline measurements. Our study provides a good starting point to investigate the impact of hyperglycemia and DM on AION, which will hopefully lead to effective treatment to improve visual outcome in AION.

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