Inner Retinal Oxygen Delivery and Metabolism in Streptozotocin Diabetic Rats

Justin Wanek, Pang-yu Teng, Norman P. Blair, and Mahnaz Shahidi

Department of Ophthalmology and Visual Sciences, University of Illinois at Chicago, Chicago, Illinois

Correspondence: Mahnaz Shahidi, Department of Ophthalmology and Visual Sciences, University of Illinois at Chicago, 1855 West Taylor Street, Chicago, IL 60612; mahnshah@uic.edu.

Submitted: October 30, 2013
Accepted: February 7, 2014

Citation: Wanek J, Teng P-Y, Blair NP, Shahidi M. Inner retinal oxygen delivery and metabolism in streptozotocin diabetic rats. *Invest Ophthalmol Vis Sci*. 2014;55:1588–1593. DOI: 10.1167/iovs.13-13537

Purpose. The purpose of the study is to report global measurements of inner retinal oxygen delivery (DO_{2_IR}) and oxygen metabolism (MO_{2_IR}) in streptozotocin (STZ) diabetic rats.

Methods. Phosphorescence lifetime and blood flow imaging were performed in rats 4 (STZ/4wk; $n = 10$) and 6 (STZ/6wk; $n = 10$) weeks following injection of STZ to measure retinal arterial (O_{2A}) and venous (O_{2V}) oxygen contents and total retinal blood flow (F). DO_{2_IR} and MO_{2_IR} were calculated from measurements of F and O_{2A} and of F and the arteriovenous oxygen content difference, respectively. Data in STZ rats were compared to those in healthy control rats ($n = 10$).

Results. Measurements of O_{2A} and O_{2V} were not significantly different among STZ/4wk, STZ/6wk, and control rats ($P \geq 0.28$). Likewise, F was similar among all groups of rats ($P = 0.81$). DO_{2_IR} measurements were 941 ± 231, 956 ± 232, and 973 ± 243 nL O_2/min in control, STZ/4wk, and STZ/6wk rats, respectively ($P = 0.95$). MO_{2_IR} measurements were 516 ± 175, 444 ± 103, and 496 ± 84 nL O_2/min in control, STZ/4wk, and STZ/6wk rats, respectively ($P = 0.37$).

Conclusions. Global inner retinal oxygen delivery and metabolism were not significantly impaired in STZ rats in early diabetes.

Keywords: retina, oxygen, metabolism, delivery, diabetes, rat

Diabetic retinopathy (DR) is a significant cause of blindness in developed countries.[1–3] It is thought that in some stages of DR, the ability of the retinal vasculature to deliver oxygen and of the retinal tissue to consume oxygen may be impaired. Retinal blood flow, as a marker of oxygen delivery, has been studied extensively in DR subjects using a variety of techniques such as fluorescein angiography,[4–7] laser Doppler velocimetry,[8–11] and blue field entoptic phenomenon.[12,13] Findings of these studies have been variable, with reports of reduced, unchanged, or elevated blood flow.[14–18] Due to limited technologies for measuring inner retinal oxygen consumption, abnormalities in retinal vascular oxygenation have been sought as a surrogate for impaired oxygen metabolism. In subjects with DR, retinal venous oxygen saturation (SO_2) measured by oximetry was found to be increased[19,20] while arterial SO_2 was reported to be higher than[20] or similar[19] to values in healthy subjects. Combined blood flow and oximetry can be used to estimate inner retinal oxygen metabolism (MO_{2_IR}). However, this combination has been used in only one study, and that was of neurogenic optic atrophy.[21] Therefore, in humans, the effect of diabetes on MO_{2_IR} remains unknown. Knowledge of alterations in inner retinal oxygen delivery (DO_{2_IR}) and MO_{2_IR} would be useful to better understand DR pathophysiology and potentially for development of therapeutic interventions.

The streptozotocin (STZ) diabetic rat develops retinal pathological alterations that resemble those observed in early human DR.[22] In early experimental diabetes (within 6 weeks), blood–retinal barrier breakdown,[23–26] leukostasis,[23,27,28] upregulation of endothelin-1,[29] abnormal retinal blood flow,[30–34] and reduced retinal arterial wall oxygen tension (PO_2)[35] have been reported in STZ rats, suggesting the possibility of alterations in DO_{2_IR}. However, significant reductions in tissue PO_2 may not occur even if oxygen delivery is reduced to some extent. In fact, indicators of hypoxia measured by pimonidazole[36] and hypoxia-inducible factor (HIF) levels[37] were not abnormal in excised retinal tissue of early STZ rats, though upregulation of vascular endothelial growth factor (VEGF)[24,38,39] has been reported. In addition to oxygen availability, retinal neural activity is also a determinant of the rate at which the retina consumes oxygen for energy generation. In early STZ rodents, neural changes including increased apoptosis of retinal ganglion cells,[40–43] thinning of the inner plexiform layer,[44] and reduction of amacrine cells[45] have been observed before the appearance of vascular cell changes,[22] suggesting that there may be alterations in MO_{2_IR}. However, to date, measurements of neither oxygen delivery nor oxygen metabolism in the inner retina in living STZ rats have been published. The purpose of this study was to quantitatively assess DO_{2_IR} and MO_{2_IR} in STZ rats with our previously established optical imaging method.[46,47]

Methods

Animals

Male Long Evans pigmented rats (Charles River Laboratories, Wilmington, MA) were treated in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Diabetes was induced by intravenous injection of STZ (60 mg/kg) in citrate buffer. Nonfasting blood glucose levels were measured in STZ rats weekly and immediately before imaging with the use of a commercially available

blood glucometer (FreeStyle Lite; Abbott, Alameda, CA) to confirm continued hyperglycemia. Imaging was performed either 4 weeks (STZ/4wk; $n = 10$) or 6 weeks (STZ/6wk; $n = 10$) after administration of STZ. Imaging in rats with longer duration of diabetes was precluded due to cataract formation.

Prior to imaging, rats were anesthetized with intraperitoneal injections of ketamine (100 mg/kg) and xylazine (5 mg/kg), with additional injections given to sustain anesthesia as necessary. To ensure normal systemic blood gas levels, rats were mechanically ventilated with room air (or room air and supplemental oxygen) using a small-animal ventilator (Harvard Apparatus, Inc., South Natick, MA) connected to an endotracheal tube. The femoral artery was cannulated, and a catheter was attached to draw blood and to measure the animal's physiological status with a pressure transducer. Systemic arterial oxygen tension (P_aO_2), carbon dioxide tension (P_aCO_2), and pH were measured immediately prior to imaging from arterial blood using a blood gas analyzer (Radiometer, Westlake, OH) 5 to 10 minutes after initiation of ventilation. Blood pressure (BP) and heart rate (HR) were monitored continuously with a data acquisition system (Biopac Systems, Goleta, CA) linked to the pressure transducer. Continuous BP and HR measurements obtained during imaging were averaged to derive a representative BP and HR value. Hemoglobin concentration (HgB) was also measured with a hematology system (Siemens, Tarrytown, NY) from arterial blood.

Rats were placed in an animal holder with a copper tubing water heater to maintain the body temperature at 37°C. Pupils were dilated with 2.5% phenylephrine and 1% tropicamide. A glass cover slip with 1% hydroxypropyl methylcellulose was applied to the cornea to eliminate its refractive power and prevent dehydration. For retinal vascular PO_2 imaging, an oxygen-sensitive molecular probe, Pd-porphine (Frontier Scientific, Logan, UT), was dissolved (12 mg/mL) in bovine serum albumin solution (60 mg/mL) and administered through the femoral arterial catheter (20 mg/kg). For retinal blood velocity imaging, 2-μm polystyrene fluorescent microspheres (Invitrogen, Grand Island, NY) were injected through the catheter. For vascular caliber measurement, red-free retinal imaging was performed; and in three STZ rats with low-quality red-free images, fluorescein angiography (FA) was performed by intravascular injection of 10% fluorescein sodium (5 mg/kg, AK-FLUOR; Akorn, Decatur, IL) for improved visualization of vessel diameter. Overall, the duration of the vascular PO_2 and blood flow imaging session was approximately 1 hour.

Oxygen Tension Imaging

Retinal vascular PO_2 measurements were obtained using our optical section phosphorescence lifetime imaging system.[48,49] Briefly, a laser line was projected on the retina after intravenous injection of the Pd-porphine probe. Due to the angle between the excitation laser beam and imaging path, an optical section phosphorescence image was acquired in which the retinal vessels were depth-resolved from the underlying choroid. Phosphorescence lifetimes in the retinal vessels were determined using a frequency-domain approach and converted to PO_2 measurements using the Stern-Volmer equation.[50,51] PO_2 was measured in all major retinal arteries (PO_{2A}) and retinal veins (PO_{2V}) at locations within three optic disc diameters (~600 μm) from the edge of the optic nerve head. Three repeated PO_2 measurements were averaged per blood vessel.

Blood Flow Imaging

Blood flow was measured using our previously described imaging system.[46,47] Briefly, a slit-lamp biomicroscope, equipped with a 488-nm diode laser (excitation), emission filter (560 ± 60 nm), and a high-speed charge-coupled device camera (QImaging, Surrey, Canada), was utilized for imaging of intravascular motion of the fluorescent microspheres at 108 Hz to determine retinal venous blood velocity (V). Using the same instrument, retinal venous diameter (D) was measured by red-free retinal imaging in 17 STZ rats or by FA in 3 STZ rats that had low-quality red-free images. Red-free retinal images were captured with the instrument's light source and a green filter (540 ± 5 nm), while FA images were obtained by placing a fluorescein excitation filter (488 ± 5 nm) in the illumination path and using the emission filter. Venous D was determined based on the average full width at half maximum of intensity profiles perpendicular to the blood vessel axis over a fixed length of 200 μm. Blood flow in each major vein was then calculated from V and D measurements ($V \times \pi \times D^2/4$) and summed over all veins to provide total blood flow (F) in the retinal circulation. Blood flow was determined in major retinal veins because they are less affected by pulsation, and they have higher contrast (darker) and larger diameters compared to major retinal arteries.

Global Inner Retinal Oxygen Delivery and Metabolism

The oxygen content of blood was calculated from PO_2 measurements for each major retinal artery and vein as the sum of oxygen bound to hemoglobin and dissolved in blood, as previously described.[46,47] The amount of oxygen bound to hemoglobin was calculated from the hemoglobin dissociation curve in rat using the measured vascular PO_2, pH, and HgB values,[52] while the amount of dissolved oxygen was determined from the product of the vascular PO_2 and oxygen solubility in blood. Retinal arterial (O_{2A}) and venous (O_{2V}) oxygen contents were determined by averaging the oxygen content of all major retinal arteries and veins, respectively. DO_{2_IR} was derived from the product of F and O_{2A}, while MO_{2_IR} was calculated from the product of F and the arteriovenous oxygen content difference ($O_{2A\text{-}V}$).

Data Analysis

Data obtained in STZ/4wk and STZ/6wk rats were compared to our previously published data[47] in 10 healthy control rats using analysis of variance. Mean ages of control, STZ/4wk, and STZ/6wk rats on the day of imaging were 85 ± 19, 102 ± 3, and 109 ± 12 days, respectively. Post hoc analysis using Tukey's method was performed to determine pairwise differences. Linear regression analysis was also performed to determine if F was significantly related to systemic BP or HR in each group of rats. Statistical significance was accepted at $P < 0.05$.

Results

Blood Glucose and Systemic Physiological Status

Blood glucose measurements, obtained on the day of imaging, were elevated in all STZ rats (>314 mg/dL), confirming the presence of diabetes. There was a significant difference in body weight among control (391 ± 79 g, mean ± SD, $n = 10$), STZ/4wk (339 ± 43 g, $n = 10$), and STZ/6wk (307 ± 34 g, $n = 10$) rats ($P = 0.008$), with the only significant pairwise difference between control and STZ/6wk rats ($P = 0.006$).

Table 1. Systemic Physiological Parameters (Mean ± SD) in Control, STZ/4wk, and STZ/6wk Rats

Systemic Physiologic Parameters	Control,* $n = 10$	STZ/4wk, $n = 10$	STZ/6wk, $n = 10$	*P* Value
Arterial PO_2, mm Hg	91 ± 9	85 ± 19	87 ± 14	0.64
Arterial PCO_2, mm Hg	42 ± 3	40 ± 4	43 ± 10	0.54
pH	7.36 ± 0.04	7.33 ± 0.08	7.29 ± 0.11	0.23
Blood pressure, mm Hg	101 ± 18	79 ± 23	64 ± 12	<0.001
Heart rate, beats/min	224 ± 35	193 ± 40	183 ± 33	0.04
Hemoglobin concentration, g/dL	14.2 ± 0.8	14.2 ± 1.1	14.2 ± 1.4	0.98

* Previously published data.[47]

The systemic physiological status of control and STZ rats is summarized in Table 1. P_aO_2, P_aCO_2, pH, and HgB were within normal ranges and not significantly different among control, STZ/4wk, and STZ/6wk rats ($P \geq 0.23$). However, BP was significantly different among the groups ($P < 0.001$), with both STZ/4wk and STZ/6wk rats having lower BP compared to controls ($P \leq 0.03$). Likewise, HR was significantly different among the groups ($P = 0.04$), with STZ/6wk rats having lower HR compared to control rats ($P = 0.04$).

Retinal Vascular PO_2 and Oxygen Content

Mean values of retinal vascular PO_2 and oxygen contents, compiled from measurements in all rats, are summarized in Table 2. Differences in PO_{2A} measurements among control, STZ/4wk, and STZ/6wk rats approached significance ($P = 0.06$), and no significant difference was found in PO_{2V} ($P = 0.10$). After converting PO_2 measurements to oxygen contents, there were no significant differences in O_{2A}, O_{2V}, and $O_{2A\text{-}V}$ among the groups of rats ($P \geq 0.28$).

Retinal Blood Flow

Mean values of D, V, and F, compiled from measurements in all rats, are summarized in Table 3. Venous D and V were not significantly different among control, STZ/4wk, and STZ/6wk rats ($P \geq 0.60$). Likewise, F was similar among the groups of rats ($P = 0.81$). In each group, F was not significantly correlated with either BP ($R^2 \leq 0.23$, $P \geq 0.15$, $n = 10$) or HR ($R^2 \leq 0.15$, $P \geq 0.26$, $n = 10$).

Global Inner Retinal Oxygen Delivery and Metabolism

Measurements of DO_{2_IR} and MO_{2_IR} in individual control and STZ rats are shown in the Figure. Mean and SD values of DO_{2_IR}, calculated for each group, were 941 ± 231, 956 ± 232, and 973 ± 243 nL O_2/min in control, STZ/4wk, and STZ/6wk rats, respectively. DO_{2_IR} was similar among control, STZ/4wk, and STZ/6wk rats ($P = 0.95$). Mean and SD values of MO_{2_IR} were 516 ± 175, 444 ± 103, and 496 ± 84 nL O_2/min in control, STZ/4wk, and STZ/6wk rats, respectively. Likewise, MO_{2_IR} was not significantly different among the groups of rats ($P = 0.37$).

Discussion

Although retinal alterations in early STZ rats have been observed in published studies,[23–35,38–45] corresponding changes in DO_{2_IR} and MO_{2_IR} have not been previously reported. In the present study, combined measurements of DO_{2_IR} and MO_{2_IR} are reported in living STZ rats 4 and 6 weeks after induction of diabetes, suggesting that DO_{2_IR} and MO_{2_IR} are not significantly altered in early experimental diabetes.

Systemic arterial blood gases of control and STZ rats in the current study were within normal ranges due to controlled ventilation, thereby minimizing the potentially confounding effects of systemic blood gas tensions on retinal blood flow.[17] However, BP and HR were lower in STZ rats compared to healthy control rats, similar to previously reported findings.[53,54] One may have expected reduced BP and HR in STZ rats to affect retinal blood flow. However, F was not correlated with either BP or HR; therefore the decrease of these parameters in STZ rats likely did not significantly influence measurements of DO_{2_IR} and MO_{2_IR}.

Retinal vascular PO_2 in STZ rats after 4 and 6 weeks of diabetes was comparable to that of healthy control rats. In control rats, retinal PO_{2A} and PO_{2V} measurements were in general agreement with previously reported values obtained with microelectrodes at the retinal arterial and venous walls in healthy rats, respectively.[35,47,55] Similar to findings of the current study, PO_2 measured at the retinal venous wall was not different between STZ and healthy rats, while contrary to our findings, PO_2 measured at the retinal arterial wall was lower in STZ rats.[35] This difference may possibly be attributed to reduced oxygen diffusion across the arterial wall in diabetes, such that the extraluminal PO_2 would be lower than the intraluminal PO_2. The finding of comparable O_{2A} and O_{2V} among STZ and healthy control rats is different from reported abnormalities in retinal arterial and venous SO_2 in human subjects with DR.[19,20] This may be attributed, at least in part, to differences in duration of diabetes and severity of retinopathy.

Table 2. Retinal Arterial (PO_{2A}) and Venous (PO_{2V}) Oxygen Tension, Arterial (O_{2A}) and Venous (O_{2V}) Oxygen Content, and Arteriovenous Oxygen Content Difference ($O_{2A\text{-}V}$) of Control, STZ/4wk, and STZ/6wk Rats (Mean ± SD)

Retinal Oxygenation Parameters	Control,* $n = 10$	STZ/4wk, $n = 10$	STZ/6wk, $n = 10$	*P* Value
PO_{2A}, mm Hg	44 ± 4	49 ± 8	53 ± 11	0.06
PO_{2V}, mm Hg	26 ± 3	30 ± 5	31 ± 8	0.10
O_{2A}, mL O_2/dL	11.9 ± 1.4	12.7 ± 2.9	13.0 ± 1.9	0.53
O_{2V}, mL O_2/dL	5.4 ± 1.3	6.8 ± 2.3	6.5 ± 2.3	0.28
$O_{2A\text{-}V}$, mL O_2/dL	6.5 ± 1.5	5.9 ± 1.2	6.5 ± 1.4	0.49

* Previously published data.[47]

Investigative Ophthalmology & Visual Science

Table 3. Retinal Venous Diameter (D), Velocity (V), and Blood Flow (F) of Rats (Mean ± SD) in Control, STZ/4wk, and STZ/6wk Rats

Blood Flow Parameters	Control,* $n = 10$	STZ/4wk, $n = 10$	STZ/6wk, $n = 10$	*P* Value
D, μm	51 ± 6	50 ± 9	52 ± 6	0.85
V, mm/s	11.8 ± 2.8	12.6 ± 2.4	11.5 ± 2.7	0.60
F, μL/min	7.9 ± 1.7	7.7 ± 1.8	7.4 ± 1.1	0.81

* Previously published data.[47]

Blood flow and DO_{2_IR} were not altered in STZ rats after 4 or 6 weeks of diabetes as compared to healthy control rats. Retinal blood flow measurements in control rats were comparable to values reported using Doppler OCT[56] and fluorescent microsphere impaction.[57] Similar to the findings of the current study, retinal blood flow has been reported to be unchanged in STZ rats at 3 weeks[58] and STZ mice at 2.5 months,[59] while other published studies have shown reductions or elevations of blood flow in STZ rodents.[30–34] Inner retinal oxygen delivery in control rats was previously reported by us,[47] but to our knowledge there have been no other published data in healthy or diabetic animals. The finding of comparable DO_{2_IR} between STZ and healthy rats suggests that previously reported vascular abnormalities[23–29] were not sufficiently severe or widespread within 6 weeks of experimental diabetes to significantly impair retinal hemodynamics.

Inner retinal oxygen metabolism measurements were similar among STZ and healthy control rats in the current study. Oxygen metabolism measurements of whole retinas removed from alloxan-induced diabetic rats were higher than in healthy control retinas,[60] though glucose metabolism measurements in STZ rats were not elevated by hyperglycemia.[61] Although outer retina oxygen consumption of long-term diabetic cats has been reported,[62] it is difficult to compare these results to our findings of inner retina oxygen consumption of short-term diabetic rats because of differences in species, duration of diabetes, and retinal cell layers. Since DO_{2_IR} was not reduced due to diabetes, MO_{2_IR} was not limited by oxygen supply. Furthermore, the finding of unaltered MO_{2_IR} in STZ rats suggests that previously reported loss of inner retinal neurons[40–45] may not have significantly impacted the overall neuronal activity in early experimental diabetes.

Since retinal tissue oxygen levels reflect the balance between oxygen delivery and metabolism, the findings of unaltered DO_{2_IR} and MO_{2_IR} in the current study suggest similar retinal tissue PO_2 in STZ and control rats. In agreement with our findings, inner retinal tissue PO_2 measured by oxygen microelectrodes was comparable between 4-week STZ and healthy rats (Lau JC, et al. *IOVS* 2010;51:ARVO E-Abstract 5644). Similarly, hypoxia indicators using pimonidazole in the retinal tissue were not altered in 3-week STZ rats, though the HIF-2α level was increased in some peripheral retinal layers.[36] Furthermore, HIF-1α and HIF-2α levels in the retinal tissue of 4-week STZ rodents were not increased.[37] In later stages of experimental diabetes between 3 and 6 months, hypoxia indicators and VEGF expression were abnormal in the retinal tissue of STZ rodents,[37,63] while other studies reported that hypoxia indicators (Reeves D, et al. *IOVS* 2002;43:ARVO E-Abstract 785) and the ratio of nicotinamide adenine dinucleotide (NAD^+) to NADH[64] were not altered.

There were limitations in the current study. Imaging of STZ rats beyond 6 weeks of diabetes was impeded due to cataract formation, thereby prohibiting measurements with our optical methods. Although Pd-porphine has been shown to be phototoxic to the retina under high light levels and long exposures,[65] given the retinal irradiance levels used for vascular PO_2 imaging, it is unlikely that the results of the current study were affected. The small sample size reduced the statistical power to detect subtle differences among STZ and healthy rats, given the interanimal variability of the measurements. Blood glucose was not measured in control rats, though it was expected to be significantly lower than in STZ rats because the two groups were maintained under identical conditions with the exception of STZ administration. Since sham injections were not administered in control rats, and since control and diabetic rats were not litter matched, these factors may have affected the results. Oxygen delivered by the retinal circulation supplies approximately 7% of the metabolic needs of the photoreceptors under light-adapted conditions[66];

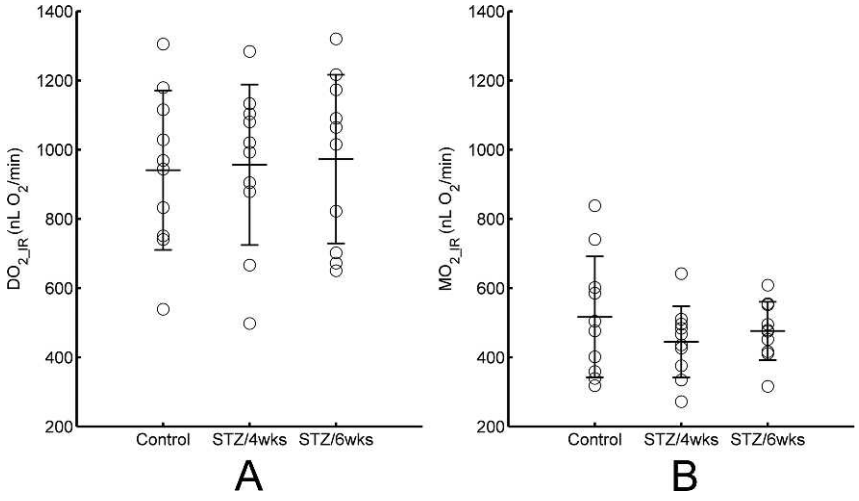

Figure. Measurements of (**A**) inner retinal oxygen delivery (DO_{2_IR}) and (**B**) inner retinal oxygen metabolism (MO_{2_IR}) in individual control ($n = 10$), STZ/4wk ($n = 10$), and STZ/6wk ($n = 10$) rats. *Solid horizontal lines* indicate the mean and standard deviation of measurements in each group.

thus a small fraction of the outer retinal oxygen consumption is reflected in the reported MO_{2_IR} measurements. Since DO_{2_IR} and MO_{2_IR} measurements represented global values derived for the inner retina of the whole eye, it is possible that oxygen delivery and consumption in local retinal areas may have been impaired in early experimental diabetes. In future studies, our alternative method[46] to measure DO_2 and MO_2 in local regions of the retina may be utilized to investigate multifocal abnormalities in STZ rats.

In conclusion, inner retinal oxygen delivery and metabolism were not significantly altered within 6 weeks of experimental diabetes. Findings from this study may imply minimal alterations in global inner retinal oxygen delivery and metabolism in early human diabetes.

Acknowledgments

Supported by National Eye Institute Grants EY017918 and EY001792; and a Senior Scientific Investigator Award (MS) and an unrestricted departmental award from Research to Prevent Blindness, New York, New York.

Disclosure: **J. Wanek**, None; **P.-Y. Teng**, None; **N.P. Blair**, None; **M. Shahidi**, P

References

1. Kempen JH, O'Colmam BJ, Leske C, et al. The prevalence of diabetic retinopathy among adults in the United States. *Arch Ophthalmol*. 2004;122:552–563.
2. Rossing P. The changing epidemiology of diabetic microangiopathy in type 1 diabetes. *Diabetologia*. 2005;48:1439–1444.
3. Congdon N, O'Colmain B, Klaver CCW, et al. Causes and prevalence of visual impairment among adults in the United States. *Arch Ophthalmol*. 2004;122:477–485.
4. Bertram B, Wolf S, Fiehofer S, Schulte K, Arend O, Reim M. Retinal circulation times in diabetes mellitus type 1. *Br J Ophthalmol*. 1991;75:462–465.
5. Blair NP, Feke GT, Morales-Stoppello J, et al. Prolongation of the retinal mean circulation time in diabetes. *Arch Ophthalmol*. 1982;100:764–768.
6. Cunha-Vaz JG, Fonseca JR, de Abreu JR, Lima JJ. Studies on retinal blood flow. II. Diabetic retinopathy. *Arch Ophthalmol*. 1978;96:809–811.
7. Yoshida A, Feke GT, Morales-Stoppello J, Collas GD, Goger DG, McMeel JW. Retinal blood flow alterations during progression of diabetic retinopathy. *Arch Ophthalmol*. 1983;101:225–227.
8. Feke GT, Tagawa H, Yoshida A, et al. Retinal circulatory changes related to retinopathy progression in insulin-dependent diabetes mellitus. *Ophthalmology*. 1985;92:1517–1522.
9. Grunwald JE, Riva CE, Sinclair SH, Brucker AJ, Petrig BL. Laser Doppler velocimetry study of retinal circulation in diabetes mellitus. *Arch Ophthalmol*. 1986;104:991–996.
10. Patel V, Rassam S, Newsom R, Wiek J, Kohner E. Retinal blood flow in diabetic retinopathy. *BMJ*. 1992;305:678–683.
11. Feke GT, Buzney SM, Ogasawara H, et al. Retinal circulatory abnormalities in type 1 diabetes. *Invest Ophthalmol Vis Sci*. 1994;35:2968–2975.
12. Fallon TJ, Chowiencyzk P, Kohner EM. Measurement of retinal blood flow in diabetes by the blue-light entoptic phenomenon. *Br J Ophthalmol*. 1986;70:43–46.
13. Rimmer T, Fallon TJ, Kohner EM. Long-term follow-up of retinal blood flow in diabetes using the blue light entoptic phenomenon. *Br J Ophthalmol*. 1989;73:1–5.
14. Kur J, Newman EA, Chan-Ling T. Cellular and physiological mechanisms underlying blood flow regulation in the retina and choroid in health and disease. *Prog Retin Eye Res*. 2012;31:377–406.
15. Pemp B, Schmetterer L. Ocular blood flow in diabetes and age-related macular degeneration. *Can J Ophthalmol*. 2008;43:295–301.
16. Pournaras CJ, Riva CE. Retinal blood flow evaluation. *Ophthalmologica*. 2013;229:61–74.
17. Pournaras CJ, Rungger-Brandle E, Riva CE, Hardarson SH, Stefansson E. Regulation of retinal blood flow in health and disease. *Prog Retin Eye Res*. 2008;27:284–330.
18. Schmetterer L, Wolzt M. Ocular blood flow and associated functional deviations in diabetic retinopathy. *Diabetologia*. 1999;42:387–405.
19. Hammer M, Vilser W, Riemer T, et al. Diabetic patients with retinopathy show increased retinal venous oxygen saturation. *Graefes Arch Clin Exp Ophthalmol*. 2009;247:1025–1030.
20. Hardarson SH, Stefansson E. Retinal oxygen saturation is altered in diabetic retinopathy. *Br J Ophthalmol*. 2012;96:560–563.
21. Sebag J, Delori FC, Feke GT, Weiter JJ. Effects of optic atrophy on retinal blood flow and oxygen saturation in humans. *Arch Ophthalmol*. 1989;107:222–226.
22. Robinson R, Barathi VA, Chaurasia SS, Wong TY, Kern TS. Update on animal models of diabetic retinopathy: from molecular approaches to mice and higher mammals. *Dis Model Mech*. 2012;5:444–456.
23. Joussen AM, Poulaki V, Mitsiades N, et al. Nonsteroidal anti-inflammatory drugs prevent early diabetic retinopathy via TNF-alpha suppression. *FASEB J*. 2002;16:438–440.
24. Qaum T, Xu Q, Joussen AM, et al. VEGF-initiated blood-retinal barrier breakdown in early diabetes. *Invest Ophthalmol Vis Sci*. 2001;42:2408–2413.
25. Xu X, Zhu Q, Xia X, Zhang SJ, Gu Q, Luo DW. Blood-retinal barrier breakdown induced by activation of protein kinase C via vascular endothelial growth factor in streptozotocin-induced diabetic rats. *Curr Eye Res*. 2004;28:251–256.
26. Zhang JF, Wu YL, Jin Y, et al. Intravitreal injection of erythropoietin protects both retinal vascular and neuronal cells in early diabetes. *Invest Ophthalmol Vis Sci*. 2008;49:732–742.
27. Kinoshita N, Kakehashi A, Inoda S, et al. Effective and selective prevention of retinal leukostasis in streptozotocin-induced diabetic rats using gliclazide. *Diabetologia*. 2002;45:735–739.
28. Nonaka A, Kiryu J, Tsujikawa A, et al. PKC-beta inhibitor (LY333531) attenuates leukocyte entrapment in retinal microcirculation of diabetic rats. *Invest Ophthalmol Vis Sci*. 2000;41:2702–2706.
29. Deng D, Evans T, Mukherjee K, Downey D, Chakrabarti S. Diabetes-induced vascular dysfunction in the retina: role of endothelins. *Diabetologia*. 1999;42:1228–1234.
30. Bursell SE, Clermont AC, Shiba T, King GL. Evaluating retinal circulation using video fluorescein angiography in control and diabetic rats. *Curr Eye Res*. 1992;11:287–295.
31. Cringle SJ, Yu DY, Alder VA, Su EN. Retinal blood-flow by hydrogen clearance polarography in the streptozotocin-induced diabetic rat. *Invest Ophthalmol Vis Sci*. 1993;34:1716–1721.
32. Lee S, Morgan GA, Harris NR. Ozagrel reverses streptozotocin-induced constriction of arterioles in rat retina. *Microvasc Res*. 2008;76:217–223.
33. Sutera SP, Chang K, Marvel J, Williamson JR. Concurrent increases in regional hematocrit and blood flow in diabetic rats: prevention by sorbinil. *Am J Physiol*. 1992;263:H945–H950.

34. Tilton RG, Chang K, Pugliese G, et al. Prevention of hemodynamic and vascular albumin filtration changes in diabetic rats by aldose reductase inhibitors. *Diabetes*. 1989; 38:1258–1270.
35. Alder VA, Yu DY, Cringle SJ, Su EN. Changes in vitreal oxygen-tension distribution in the streptozotocin diabetic rat. *Diabetologia*. 1991;34:469–476.
36. Wright WS, McElhatten RM, Harris NR. Increase in retinal hypoxia-inducible factor-2alpha, but not hypoxia, early in the progression of diabetes in the rat. *Exp Eye Res*. 2011;93:437–441.
37. Wright WS, McElhatten RM, Messina JE, Harris NR. Hypoxia and the expression of HIF-1alpha and HIF-2alpha in the retina of streptozotocin-injected mice and rats. *Exp Eye Res*. 2010; 90:405–412.
38. Kusari J, Zhou SX, Padillo E, Clarke KG, Gil DW. Inhibition of vitreoretinal VEGF elevation and blood-retinal barrier breakdown in streptozotocin-induced diabetic rats by brimonidine. *Invest Ophthalmol Vis Sci*. 2010;51:1044–1051.
39. Masuzawa K, Goto K, Jesmin S, et al. An endothelin type A receptor antagonist reverses upregulated VEGF and ICAM-1 levels in streptozotocin-induced diabetic rat retina. *Curr Eye Res*. 2006;31:79–89.
40. Barber AJ, Lieth E, Khin SA, Antonetti DA, Buchanan AG, Gardner TW. Neural apoptosis in the retina during experimental and human diabetes. Early onset and effect of insulin. *J Clin Invest*. 1998;102:783–791.
41. Kern TS. Contributions of inflammatory processes to the development of the early stages of diabetic retinopathy. *Exp Diabetes Res*. 2007;2007:95103.
42. Martin PM, Roon P, Van Ells TK, Ganapathy V, Smith SB. Death of retinal neurons in streptozotocin-induced diabetic mice. *Invest Ophthalmol Vis Sci*. 2004;45:3330–3336.
43. Ning X, Baoyu Q, Yuzhen L, Shuli S, Reed E, Li QQ. Neuro-optic cell apoptosis and microangiopathy in KKAY mouse retina. *Int J Mol Med*. 2004;13:87–92.
44. Aizu Y, Oyanagi K, Hu JG, Nakagawa H. Degeneration of retinal neuronal processes and pigment epithelium in the early stage of the streptozotocin-diabetic rats. *Neuropathology*. 2002;22: 161–170.
45. Seki M, Tanaka T, Nawa H, et al. Involvement of brain-derived neurotrophic factor in early retinal neuropathy of streptozotocin-induced diabetes in rats: therapeutic potential of brain-derived neurotrophic factor for dopaminergic amacrine cells. *Diabetes*. 2004;53:2412–2419.
46. Wanek J, Teng PY, Albers J, Blair NP, Shahidi M. Inner retinal metabolic rate of oxygen by oxygen tension and blood flow imaging in rat. *Biomed Opt Express*. 2011;2:2562–2568.
47. Wanek J, Teng PY, Blair NP, Shahidi M. Inner retinal oxygen delivery and metabolism under normoxia and hypoxia in rat. *Invest Ophthalmol Vis Sci*. 2013;54:5012–5019.
48. Shahidi M, Shakoor A, Blair NP, Mori M, Shonat RD. A method for chorioretinal oxygen tension measurement. *Curr Eye Res*. 2006;31:357–366.
49. Shahidi M, Wanek J, Blair NP, Mori M. Three-dimensional mapping of chorioretinal vascular oxygen tension in the rat. *Invest Ophthalmol Vis Sci*. 2009;50:820–825.
50. Lakowicz JR, Szmacinski H, Nowaczyk K, Berndt KW, Johnson M. Fluorescence lifetime imaging. *Anal Biochem*. 1992;202: 316–330.
51. Shonat RD, Kight AC. Oxygen tension imaging in the mouse retina. *Ann Biomed Eng*. 2003;31:1084–1096.
52. Cartheuser CF. Standard and pH-affected hemoglobin-O2 binding curves of Sprague-Dawley rats under normal and shifted P50 conditions. *Comp Biochem Physiol Comp Physiol*. 1993;106:775–782.
53. Hicks KK, Seifen E, Stimers JR, Kennedy RH. Effects of streptozotocin-induced diabetes on heart rate, blood pressure and cardiac autonomic nervous control. *J Auton Nerv Syst*. 1998;69:21–30.
54. Katovich MJ, Hanley K, Strubbe G, Wright BE. Effects of streptozotocin-induced diabetes and insulin treatment on blood pressure in the male rat. *Proc Soc Exp Biol Med*. 1995;208:300–306.
55. Yu DY, Cringle SJ, Alder V, Su EN. Intraretinal oxygen distribution in the rat with graded systemic hyperoxia and hypercapnia. *Invest Ophthalmol Vis Sci*. 1999;40:2082–2087.
56. Zhi ZW, Cepurna W, Johnson E, Shen T, Morrison J, Wang RKK. Volumetric and quantitative imaging of retinal blood flow in rats with optical microangiography. *Biomed Opt Express*. 2011;2:579–591.
57. Shih YY, Wang L, De La Garza BH, et al. Quantitative retinal and choroidal blood flow during light, dark adaptation and flicker light stimulation in rats using fluorescent microspheres. *Curr Eye Res*. 2013;38:292–298.
58. Granstam E, Granstam SO. Regulation of uveal and retinal blood flow in STZ-diabetic and non-diabetic rats; involvement of nitric oxide. *Curr Eye Res*. 1999;19:330–337.
59. Muir ER, Renteria RC, Duong TQ. Reduced ocular blood flow as an early indicator of diabetic retinopathy in a mouse model of diabetes. *Invest Ophthalmol Vis Sci*. 2012;53:6488–6494.
60. Deroetth A Jr, Pei YF. Metabolism of the alloxan diabetic rat retina. *Arch Ophthalmol*. 1964;71:73–76.
61. Ola MS, Berkich DA, Xu Y, et al. Analysis of glucose metabolism in diabetic rat retinas. *Am J Physiol Endocrinol Metab*. 2006;290:E1057–E1067.
62. Linsenmeier RA, Braun RD, McRipley MA, et al. Retinal hypoxia in long-term diabetic cats. *Invest Ophthalmol Vis Sci*. 1998;39:1647–1657.
63. de Gooyer TE, Stevenson KA, Humphries P, Simpson DAC, Gardiner TA, Stitt AW. Retinopathy is reduced during experimental diabetes in a mouse model of outer retinal degeneration. *Invest Ophthalmol Vis Sci*. 2006;47:5561–5568.
64. Diederen RM, Starnes CA, Berkowitz BA, Winkler BS. Reexamining the hyperglycemic pseudohypoxia hypothesis of diabetic oculopathy. *Invest Ophthalmol Vis Sci*. 2006;47: 2726–2731.
65. Stepinac TK, Chamot SR, Rungger-Brandle E, et al. Light-induced retinal vascular damage by Pd-porphyrin luminescent oxygen probes. *Invest Ophthalmol Vis Sci*. 2005;46:956–966.
66. Lau JC, Linsenmeier RA. Oxygen consumption and distribution in the Long-Evans rat retina. *Exp Eye Res*. 2012;102:50–58.

Investigative Ophthalmology & Visual Science