Diabetic retinopathy (DR) is a major cause of blindness. The major clinical features of DR include microaneurysms, hemorrhages, exudates, venous changes, intraretinal microvascular abnormalities, neovascularization, vitreous hemorrhage, fibrous proliferation, traction retinal detachment, and macular edema. Strong evidence indicates that retinal hypoxia, which inhibits energy production and cell survival, is a major component of the pathophysiology in the advanced, sight-threatening stages of DR. Fluorescein angiograms in patients with substantial DR frequently demonstrate areas of retinal capillary nonperfusion. Also, VEGF which is stimulated by hypoxia, is elevated. However, no information is available in human DR on the rate of inner retinal oxygen metabolism (MO2) that is imposed on the tissue by hypoxia. Furthermore, in patients with diabetes there are no measurements of the rate that oxygen is delivered to the retina (DO2) which depends on both the retinal blood flow and the oxygen content of the central retinal artery. The retinal arterial oxygen content tends to increase in the more severe stages of DR, but there have been conflicting results of retinal blood flow measurements.

We have developed imaging methods to measure DO2 and MO2 and applied them in rats with diabetes induced by streptozotocin (STZ). We found no change in either DO2 or MO2 at 4 and 6 weeks of diabetes. However, we were not able to make measurements at longer durations of diabetes because of cataract formation. The Ins2Akita mouse (Akita) has a dominant point mutation in the insulin-2 gene that induces spontaneous type 1 diabetes by approximately 4 weeks of age. In contrast to STZ-diabetic rats, Akita mice do not develop cataract, allowing evaluation of the retina by imaging methods at longer durations of diabetes. These mice have been studied extensively, revealing a variety of neural, vascular, and biochemical alterations in the retina. However, the clinical correlates of DR are absent in Akita mice. The purpose of the present study was to test the hypothesis that abnormalities in DO2, MO2, and the oxygen extraction fraction (OEF), which is the ratio of MO2/DO2, are present in Akita diabetic mice at 12 and 24 weeks of age.

**Methods**

**Animals**

The study was performed on age-matched nondiabetic C57BL/6j (n = 22) and diabetic Ins2Akita (Akita) C57BL/6j (n = 22) mice.
Oxygen Delivery and Metabolism in Diabetic Mice

from the Jackson Laboratory (Bar Harbor, ME, USA) at 12 weeks (nondiabetic: n = 12, diabetic: n = 11) and 24 weeks (nondiabetic: n = 10, diabetic: n = 11) of age. The mice were treated in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Prior to imaging, mice were anesthetized with intraperitoneal injections of ketamine (100 mg/kg) and xylazine (5 mg/kg) with additional injections given to maintain anesthesia as necessary. Non-fasting blood glucose levels in blood from a tail puncture were measured with a commercially available blood glucometer (FreeStyle Lite; Abbott, Alameda, CA, USA). The femoral artery was cannulated and a catheter was attached. Mice were then placed in an animal holder and their pupils were dilated with 2.5% phenylephrine and 1% tropicamide. A glass cover slip with 1% hydroxypropyl methylcellulose was applied to the cornea to minimize its refractive power and prevent dehydration.

For retinal vascular oxygen tension (PO2) imaging, an oxygen-sensitive molecular probe, Pd-porphine (Frontier Scientific, Logan, UT, USA), was dissolved (12 mg/ml) in bovine serum albumin solution (60 mg/ml) and administered through the femoral arteriovenous catheter (20 mg/kg). For retinal blood vessels were determined using a frequency-domain approach.25 Typically, 20 to 30 microsphere velocity measurements were averaged per blood vessel. An average arterial (PO2A) and venous (PO2V) PO2 value was calculated from the individual artery and vein measurements in each animal, respectively.

Blood Flow Imaging

Our previously described prototype blood flow imaging system15 was used for fluorescent microsphere imaging to assess retinal venous blood velocity and for performing FA to measure retinal arterial and venous vessel diameters. A slit-lamp biomicroscope with the standard light illumination (Carl Zeiss, Oberkochen, Germany) was equipped with a 488-nm diode laser (Melles Griot, Carlsbad, CA, USA) and an emission filter (560 ± 60 nm; Spectrotech, Inc., Saugus, MA, USA) for fluorescent microsphere imaging. Image sequences of the intravascular motion of the microspheres were captured at 105 Hz using an electron multiplier charge coupled device camera (QuantEM; Photometrics, Tucson, AZ, USA). The camera sensor was binned to maximize the frame rate, allowing the motion of the microspheres to be resolved in time. Multiple image sequences, each 5 seconds in duration, were recorded over several minutes immediately following the injection of the microspheres. After microsphere imaging, FA retinal images were captured using the slit-lamp white light illumination with a narrow band optical filter (480 ± 5 nm; Edmund Optics, Barrington, NJ, USA) and the above emission filter. Fluorescein angiography retinal images were obtained using the full resolution of the camera (512 × 512 pixels) to maximize the spatial resolution for vessel diameter measurements.

Diameters of all individual major retinal arteries (D Aind) and veins (D Vind) were measured from the FA images over a fixed vessel length (~100 μm), spanning approximately 150 to 250 μm from the center of the optic disk, as shown in Figure 1B. D Aind and D Vind were determined by the average full width at half maximum of seven intensity profiles perpendicular to the blood vessel axis. A mean arterial (D A) and venous diameter (D V) was calculated from all D Aind and D Vind values in each mouse, respectively.

As shown in Figure 1C, blood velocity in all individual veins was measured by manually tracking displacements of the microspheres over time, following our previously reported method.15 Typically, 20 to 30 microsphere velocity measurements were obtained in each individual vein and averaged to derive a velocity measurement per vessel (Vind). A mean velocity (V) in each mouse was calculated based on all Vind measurements. V was measured in veins because they are less affected by pulsation and have larger diameters as compared with arteries.

Blood flow in each major vein was calculated based on D Vind and Vind measurements: (Vind × π × D Vind² / 4). These blood flow measurements were summed over all veins to determine the total venous blood flow in the retinal circulation (F) for that animal. Because the retinal circulation is an end-artery system, F was equivalent to the total retinal blood flow. Measurements of F were obtained within 15 minutes after PO2 imaging.

**Figure 1.** Examples of images used to evaluate oxygen delivery, metabolism, and oxygen extraction fraction in a 12-week-old diabetic mouse. (A) Cross-sectional retinal vascular PO2 maps (rectangles) overlaid on a fluorescein angiogram. PO2 values are depicted in four arteries and four veins. Color bar shows PO2 in millimeters of mercury. (B) Fluorescein angiogram on which the boundaries of retinal vessels (red lines) have been detected and outlined within a circumpapillary region (green circles). (C) Four successive image frames acquired at 105 Hz superimposed on a red free image that display locations of a single fluorescent microsphere over time, which were used to determine blood velocity.

**Oxygen Tension Imaging**

Retinal vascular PO2 measurements were obtained using our optical section phosphorescence lifetime imaging system.15 Briefly, a laser line was projected onto the retina after intravenous injection of the Pd-porphine probe. Due to the angle between the excitation laser beam and imaging path, optical section phosphorescence images were 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.25,26 Phosphorescence lifetimes were converted to PO2 measurements using the Stern-Volmer equation. PO2 was measured in individual major retinal arteries (PO2Aind) and retinal veins (PO2Vind) at locations within 3 optic disc diameters from than edge of the optic nerve head, as shown in Figure 1A. Four repeated PO2Aind and PO2Vind measurements were averaged per blood vessel. An average arterial (PO2A) and venous (PO2V) PO2 value was calculated from the individual artery and vein measurements in each animal, respectively.

**Blood Flow Imaging**

Our previously described prototype blood flow imaging system15 was used for fluorescent microsphere imaging to assess retinal venous blood velocity and for performing FA to measure retinal arterial and venous vessel diameters. A slit-lamp biomicroscope with the standard light illumination (Carl Zeiss, Oberkochen, Germany) was equipped with a 488-nm diode laser (Melles Griot, Carlsbad, CA, USA) and an emission filter (560 ± 60 nm; Edmund Optics, Barrington, NJ, USA) and the above emission filter. Fluorescein angiography retinal images were obtained using the full resolution of the camera (512 × 512 pixels) to maximize the spatial resolution for vessel diameter measurements.

Diameters of all individual major retinal arteries (D Aind) and veins (D Vind) were measured from the FA images over a fixed vessel length (~100 μm), spanning approximately 150 to 250 μm from the center of the optic disk, as shown in Figure 1B. D Aind and D Vind were determined by the average full width at half maximum of seven intensity profiles perpendicular to the blood vessel axis. A mean arterial (D A) and venous diameter (D V) was calculated from all D Aind and D Vind values in each mouse, respectively.

As shown in Figure 1C, blood velocity in all individual veins was measured by manually tracking displacements of the microspheres over time, following our previously reported method.15 Typically, 20 to 30 microsphere velocity measurements were obtained in each individual vein and averaged to derive a velocity measurement per vessel (Vind). A mean velocity (V) in each mouse was calculated based on all Vind measurements. V was measured in veins because they are less affected by pulsation and have larger diameters as compared with arteries.

Blood flow in each major vein was calculated based on D Vind and Vind measurements: (Vind × π × D Vind² / 4). These blood flow measurements were summed over all veins to determine the total venous blood flow in the retinal circulation (F) for that animal. Because the retinal circulation is an end-artery system, F was equivalent to the total retinal blood flow. Measurements of F were obtained within 15 minutes after PO2 imaging.
Global Inner Retinal Oxygen Delivery, Metabolism, and Extraction Fraction

The oxygen content of blood in each retinal artery (O2Aind) and vein (O2Vind) was calculated as the sum of oxygen bound to hemoglobin and dissolved in blood: O2ind = SO2 × C × HgB + PO2ind × k, where SO2 is the oxygen saturation (%), C is the oxygen-carrying capacity of hemoglobin (1.39 mL O2/g),27 HgB is the hemoglobin concentration, and k is the oxygen solubility in blood (0.003 mL O2/dL mm Hg).29 SO2 was calculated from the hemoglobin oxygen dissociation curve in mice29 by using the measured PO2ind and an assumed pH value of 7.4. A constant Hgb value of 13.8 mg/dL, derived by averaging the Hgb concentration of blood samples from three separate nondiabetic mice, was used for O2ind calculations in all mice. In each animal, a mean arterial (O2A) and venous (O2V) oxygen content was determined from O2ind measurements and the arteriovenous oxygen content difference was calculated as: O2AVA = O2A - O2V.

DO2, defined as the rate that oxygen becomes available to the inner retinal tissue supplied by the retinal circulation, was calculated as the product of F and O2A. MO2, defined as the rate that oxygen is extracted from the retinal circulation and metabolized by the inner retinal tissue, was calculated as the product of F and O2AVA. The OEF equals the ratio of MO2 to DO2. It varies between 0 and 1, and it can be calculated as: O2AVA/DO2.

Data Analysis

Twelve continuous outcome variables (PO2A, PO2V, O2A, O2V, O2AVA, D, Dm, V, F, DO2, MO2, and OEF) were evaluated to assess the relationship of each with age and the presence of diabetes. The distributions of the variables were evaluated for data normalcy and to identify outliers. Regression diagnostics including Cook’s distance were performed on DO2 and MO2 and OEF to identify data points that were outliers, had leverage, or were influential. Two outliers were identified, which were removed from further analyses, both from the 24-week diabetic group. One mouse was an outlier due to abnormally high DO2 and MO2 values, and another mouse was identified as an outlier due to an abnormally high MO2 value. The effects of diabetes (absence or presence) and age (12 or 24 weeks) on body weight, blood glucose, and the above outcome variables were determined using 2-way ANOVA. In 1 of the 12-week and 4 of the 24-week diabetic mice, blood glucose exceeded the maximum level (600 mg/dL) that could be measured by the glucometer. To compare values in nondiabetic with diabetic mice on the day of imaging were 151 ± 19 and 159 ± 19 mg/dL at 12 and 24 weeks of age, respectively. The highest value at either time was 198 mg/dL. Mean blood glucose measurements (assigning the value of 600 mg/dL to measurements above the glucometer maximum) in diabetic mice on the day of imaging were 444 and 505 mg/dL at 12 and 24 weeks of age, respectively. The lowest value at either time was 269 mg/dL. There was a significant difference in blood glucose levels between diabetic and nondiabetic mice in both age groups (P < 0.001).

Retinal Vascular PO2 and Oxygen Content

Mean values of retinal vascular PO2 and O2 content in nondiabetic and diabetic mice at 12 and 24 weeks of age are summarized in Table 1. There was a significant main effect of diabetes on PO2V and O2V, such that diabetic mice had a 15% reduction in PO2V and a 30% reduction in O2V as compared with nondiabetic mice (P ≤ 0.01). There were no significant interactions between diabetes and age or significant main effects of age on PO2V or O2V. There were no significant interactions between diabetes and age or main effects of diabetes or age on PO2A, O2A, or O2AVA.

Retinal Vessel Diameter, Blood Velocity, and Blood Flow

As expected, Dx was larger than Dm in both nondiabetic and diabetic mice (P < 0.001). Mean values of Dm, Dx, V, and F in nondiabetic and diabetic mice in both age groups are summarized in Table 2. There was a significant interaction effect between diabetes and age on F (P = 0.01), and their simple main effects are presented in Table 3. There was no significant simple main effect of diabetes on F at 12 weeks, but at 24 weeks, F was 36% higher in the diabetic than in the diabetic mice (P = 0.005). F increased by 37% between 12 and 24 weeks in the nondiabetic mice (P = 0.01), but did not change significantly over that time interval in the diabetic

| Variable | Age | No Diabetes | Diabetes | P Value |
|----------|-----|-------------|----------|---------|
| PO2A, mmHg | 12 | 36 ± 7 | 34 ± 4 | 0.12 |
| PO2V, mmHg | 24 | 37 ± 5 | 33 ± 6 | 0.01* |
| O2A, mL O2/dL | 24 | 22 ± 5 | 19 ± 4 | 0.001* |
| O2V, mL O2/dL | 12 | 8 ± 2 | 7 ± 1 | 0.06 |
| O2AVA, mL O2/dL | 24 | 8 ± 2 | 7 ± 2 | 0.002* |
| O2AVA, mL O2/dL | 12 | 3 ± 1 | 2 ± 1 | 0.07 |
| O2AVA, mL O2/dL | 24 | 4 ± 1 | 3 ± 1 | 0.57 |
| PO2A, mmHg | 24 | 12 ± 8 | 9 ± 3 | 0.04 |
| PO2V, mmHg | 24 | 5 ± 2 | 3 ± 1 | 0.07 |
| O2A, mL O2/dL | 24 | 4 ± 1 | 3 ± 2 | 0.09 |
| O2V, mL O2/dL | 24 | 4 ± 1 | 3 ± 2 | 0.09 |

* Statistical significance.

Table 1. Retinal Arterial (PO2A) and Venous (PO2V) Oxygen Tension, and Arterial (O2A) and Venous (O2V) Oxygen Content, and Arteriovenous Oxygen Content Difference (O2AVA) of Nondiabetic and Diabetic (Akita) Mice at 12 and 24 Weeks of Age (Mean ± SD)
Oxygen Delivery and Metabolism in Diabetic Mice

Global Inner Retinal Oxygen Delivery, Metabolism, and Extraction Fraction

Mean values of DO₂, MO₂, and OEF in nondiabetic and diabetic mice at 12 and 24 weeks of age are summarized in Table 4. There was a significant interaction effect between diabetes and age on DO₂, and their simple main effects are presented in Table 5. No effect of diabetes on DO₂ was found at 12 weeks, but at 24 weeks DO₂ was 43% higher in the nondiabetic mice (P < 0.001). Also, although DO₂ did not change significantly between 12 and 24 weeks within the diabetic mice, it increased by 32% in the nondiabetic mice (P = 0.03). There was no significant interaction between diabetes and age or main effect of diabetes or age on MO₂.

Values of OEF in individual mice are displayed in Figure 2, illustrating that the mean OEF was higher in the diabetic mice than in the nondiabetic mice at both ages. There was a significant main effect of diabetes on OEF such that OEF in diabetic mice exceeded that in nondiabetic mice by 17% (P = 0.01). There was no significant interaction between diabetes and age or a main effect of age on OEF (P ≥ 0.28).

Table 2. Retinal Arterial Diameter (DA), Venous Diameter (DV), Venous Velocity (V), and Total Retinal Blood Flow (F) of Nondiabetic and Diabetic (Akita) Mice at 12 and 24 Weeks of Age (Mean ± SD)

| Outcome Variable | Age, wk | No Diabetes | Diabetes | P Value |
|------------------|---------|-------------|----------|---------|
| DA, μm           | 12      | 25 ± 3      | 25 ± 2   | 0.82    |
|                  | 24      | 26 ± 2      | 26 ± 2   | 0.70    |
| DV, μm           | 12      | 27 ± 3      | 28 ± 3   | 0.11    |
|                  | 24      | 29 ± 3      | 27 ± 3   | 0.01*   |
| V, mm/s          | 12      | 7 ± 2       | 7 ± 1    | 0.03*   |
|                  | 24      | 8 ± 1       | 6 ± 2    | 0.14    |
| F, μL/min         | 12      | 1.4 ± 0.4   | 1.4 ± 0.3| 0.01*   |
|                  | 24      | 1.9 ± 0.5   | 1.2 ± 0.4| 0.01*   |

* Statistical significance.

Table 3. Simple Main Effects of Total Retinal Blood Flow (F) and Inner Retinal Oxygen Delivery (DO₂) in Nondiabetic and Diabetic (Akita) Mice at 12 and 24 Weeks of Age (Mean ± SD)

| Age, wk | No Diabetes | Diabetes | P Value |
|---------|-------------|----------|---------|
| F, μL/min | 1.4 ± 0.4 | 1.4 ± 0.3 | 0.9     |
|         | 1.9 ± 0.5  | 1.2 ± 0.4 | 0.003*  |

* Statistical significance.

Table 4. No effect of diabetes on DO₂ was found at 12 weeks, but at 24 weeks DO₂ was 43% higher in the nondiabetic mice (P < 0.001). Also, although DO₂ did not change significantly between 12 and 24 weeks within the diabetic mice, it increased by 32% in the nondiabetic mice (P = 0.03). There was no significant interaction between diabetes and age or main effect of diabetes or age on MO₂.

Table 5. Simple Main Effects of Total Retinal Blood Flow (F) and Inner Retinal Oxygen Delivery (DO₂) in Nondiabetic and Diabetic (Akita) Mice at 12 and 24 Weeks of Age (Mean ± SD)

| Age, wk | No Diabetes | Diabetes | P Value |
|---------|-------------|----------|---------|
| F, μL/min | 1.4 ± 0.4 | 1.4 ± 0.3 | 0.9     |
|         | 1.9 ± 0.5  | 1.2 ± 0.4 | 0.003*  |
| DO₂, μL O₂/min | 112 ± 40 | 97 ± 29  | 0.34    |
|         | 148 ± 31   | 85 ± 37  | 0.001*  |

* Statistical significance.

Discussion

The present study revealed an increase in OEF in diabetic Akita mice at both 12 and 24 weeks of age, due to reductions in PO₂ and O₂. Furthermore, there was a lack of normal increase in DO₂ with age in diabetic mice, driven by failure of F to increase with age in the diabetic group. These changes in OEF and DO₂ were in the context of no discernible abnormalities in MO₂ in the diabetic mice.

There are two major ways for the retina to maintain adequate tissue oxygenation and MO₂. First, DO₂ can be increased by adjusting F. At 24 weeks of age, DO₂ (and F) was significantly lower in the diabetic group than in the nondiabetic group because the normal increase in DO₂ (and F) from 12 to 24 weeks did not occur. DO₂, which is based on both F and O₂, has not been measured previously in diabetic mice, but there are several reports of F. Studies by both Wright et al. and Muir and colleagues found decreases in F of 40% and 28%, respectively, in Akita mice after approximately 6 months of diabetes. These decreases are very similar to the 37% decrease in F at 24 weeks in the current study. They are also similar to other reported reductions in F values in STZ-diabetic mice. Had these investigators measured O₂, it is likely that they would have found decreases in DO₂ similar to the decrease of 43% in the current study. These defective DO₂ and F responses in diabetes likely correspond to the well-known impaired autoregulation seen in humans with diabetes.

The second way for the retina to maintain adequate tissue oxygenation and MO₂ is by extracting a greater fraction of the oxygen supplied by the blood, that is, by increasing OEF. Because MO₂ did not differ between nondiabetic and diabetic mice, it was not limited by the availability of oxygen. However, in the diabetic mice, the retina had to increase OEF to 0.63 at 24 weeks as opposed to 0.54 in the nondiabetic mice in order to maintain MO₂. This means that much of the oxygen supply reserve was already spent solely by being diabetic. We note that OEF increased similarly from 0.55 to 0.63 in a previous study in rats in which the inspired gas was reduced from 21% to 10% oxygen, and MO₂ could not be maintained. It appears that the retina of the Akita diabetic mouse exists in a state of vulnerability to superimposed metabolic stress due to its limited reserve of oxygen supply.

The current study is the first report of OEF in animal models of diabetes. However, it is now possible to calculate OEF from data acquired in two previous studies on STZ-diabetic rats from our laboratory. In one study, OEF at 4 weeks of diabetes was 0.47, whereas it was 0.53 in the nondiabetic rats. In another study, the OEF values were 0.46 and 0.51 in the diabetic rats at 4 and 6 weeks of diabetes, respectively, and 0.55 in the nondiabetic rats. The results of these past studies differ from those of the current study. These differences may, at least in part, be attributable to species differences. Obrosova et al. showed a number of biochemical abnormalities were more prominent in STZ-diabetic rats than in STZ-diabetic mice, but they did not measure PO₂. Another possible explanation for the difference may be the longer duration of diabetes in the mice, because cataract prevented us from making measurements in diabetic rats with more than 6 weeks of diabetes. In humans OEF tends not to increase in diabetes (unpublished data, 2016), so there may be differences in the pathogenesis of DR between humans, rats, and mice.

We reported MO₂ in diabetic rodents for the first time in rats with STZ diabetes, and no abnormality was found, as in the current study. Illing et al. and Sutherland and colleagues found total retinal oxygen consumption to be decreased in diabetic rabbits in vitro, but most of the inner retina is avascular in this species. Increased total retinal oxygen consumption in excised alloxan-diabetic rat retinas was reported by de Roetth.

It is expected that MO₂ will be maintained unless the inner retinal tissue PO₂ is reduced enough for hypoxic energy failure to supervene and threaten cell survival. Using oxygen microelectrodes, Lau and Linsenmeier found no decrease in inner retinal PO₂ in rats with STZ-induced diabetes for 4 to 12 weeks.
weeks. Furthermore, the inner retinal PO₂ actually was elevated relative to the choroidal PO₂ at 12 weeks of diabetes. These findings are consistent with the finding of no difference between MO₂ in mice with and without diabetes in the current study. On the other hand, Linsenmeier et al. found foci of hypoxia with oxygen microelectrodes in cats with diabetes of 6 years duration. Evaluation of retinal tissue hypoxia using pimonidazole has yielded conflicting results.22,46,47 Reduced retinal PO₂ stimulates hypoxia-inducible factors, but assessments of these factors in diabetic rodents have not yielded consistent abnormalities.48–53 Unfortunately, these methods do not permit rigorous estimation of MO₂ in the inner retina.

Taken together, these results suggest that inner retinal hypoxia is not a major abnormality in these rodent models of diabetes. Hypoxia could be a factor at longer durations of diabetes or if there are localized areas of hypoxia. The extent to which the findings of the current study resemble the conditions in human DR remains to be elucidated. The safest extrapolation of our results to the clinic would be that MO₂ may not be limited by hypoxia in the absence of significant nonproliferative or proliferative DR.

Akita diabetic mice are known to have reduced body weight and this appears, at least in part, to be related to leptin. Similarly, STZ-diabetic rats have reduced body weight and humans who become diabetic prior to puberty may have retarded growth. We do not know if differences in body weight alone are a cause for higher sensitivity of Akita mice toward metabolic stress and subsequent longitudinal alterations in retinal oxygen delivery. However, including body weight as a covariate did not change the results of the statistical analysis, thus differences in body weight did not substantially influence the conclusions.

The limitations of this work include image clarity, but we excluded eyes in which the images could not be evaluated with confidence. The number of mice in the four groups was relatively small so that we may not have been able to identify certain differences as statistically significant. Furthermore, our measurements represent global assessment of retinal physiology. Substantial areas of normal function may obscure localized areas of abnormality. Our measurement of MO₂ assumes that the volumes of tissue supplied by the retinal vessels and choroid are the same in the nondiabetic and diabetic mice. The retinal PO₂ profiles obtained from microelectrodes in normal and STZ-diabetic rats suggest that this assumption is reasonable.

In conclusion, our finding of normal inner retinal oxygen metabolism in diabetic Akita mice indicates that elevation of the oxygen extraction fraction adequately compensates for reduced oxygen delivery and prevents oxidative metabolism from being limited by hypoxia. However, these retinas may have inadequate capacity to compensate for common superimposed stresses so that pathologic changes may develop.

**Acknowledgments**

Supported by the National Eye Institute, Bethesda, Maryland, EY017918 and EY001792, and Research to Prevent Blindness, New York, New York, Senior Scientific Investigator award (MS) and an unrestricted departmental award.

Disclosure: N.P. Blair, None; J. Wanek, None; A.E. Felder, None; K.C. Brewer, None; C.E. Joslin, None; M. Shahidi, P.

**References**

1. Congdon N, O’Colmain B, Klaver CC, et al. Causes and prevalence of visual impairment among adults in the United States. *Arch Ophtalmol*. 2004;122:477–485.

2. Kempen JH, O’Colmain BJ, Leske MC, et al. The prevalence of diabetic retinopathy among adults in the United States. *Arch Ophtalmol*. 2004;122:552–563.

3. Aiello LP, Cavallerano J, Prakash M, Aiello LM. Diagnosis, management and treatment of nonproliferative diabetic retinopathy. In: DM Albert, JW Miller, Azar DT, Blodi BA, eds. *Albert & Jacobiec’s Principles and Practice of Ophtalmology*. 3rd ed. Philadelphia, PA: Saunders Elsevier; 2008:1775–1792.

4. Danis RP. Diabetic macular edema. In: DM Albert, JW Miller, Azar DT, Blodi BA, eds. *Albert & Jacobiec’s Principles and Practice of Ophtalmology*. 3rd ed. Philadelphia, PA: Saunders Elsevier; 2008:1793–1806.

5. Sun JK, Miller JW, Aiello LP. Proliferative diabetic retinopathy. In: DM Albert, JW Miller, Azar DT, Blodi BA, eds. *Albert &
Oxygen Delivery and Metabolism in Diabetic Mice

**Jacobi's Principles and Practice of Ophthalmology**: 3rd ed. Philadelphia, PA: Saunders Elsevier. 2008:1807–1828.

6. Early Treatment Diabetic Retinopathy Study Research Group. Fluorescein angiographic risk factors for progression of diabetic retinopathy. ETDRS report number 13. *Ophthalmology*. 1991:854–860.

7. Early Treatment Diabetic Retinopathy Study Research Group. Classification of diabetic retinopathy from fluorescein angiograms. ETDRS report number 11. *Ophthalmology*. 1991:807–822.

8. Adams AP, Miller JW, Bernal MT, et al. Increased vascular endothelial growth factor levels in the vitreous of eyes with proliferative diabetic retinopathy. *Am J Ophthalmol*. 1994;118:445–450.

9. Aciolo LP, Avery RI, Arrigg PG, et al. Vascular endothelial growth factor in ocular fluid of patients with diabetic retinopathy and other retinal disorders. *Neurol J Med*. 1994;331:1480–1487.

10. Hardarson SH, Stefansson E. Retinal oxygen saturation is altered in diabetic retinopathy. *Br J Ophthalmol*. 2012:96:560–563.

11. Jorgensen C, Bek T. Increasing oxygen saturation in larger retinal vessels after photocoagulation for diabetic retinopathy. *Invest Ophthalmol Vis Sci*. 2014;55:5365–5369.

12. Khooobehi B, Finn K, Thompson H, Reinoso M, Beach J. Retinal arterial and venous oxygen saturation is altered in diabetic patients. *Invest Ophthalmol Vis Sci*. 2013;54:7103–7106.

13. Pemp B, Schmetterer L. Ocular blood flow in diabetes and age-related macular degeneration. *Can J Ophthalmol*. 2008;43:295–301.

14. Pournaras CJ, Runnger-Brandle E, Riva CE, Hardarson SH, Stefansson E. Regulation of retinal blood flow in health and disease. *Prog Retin Eye Res*. 2008;27:284–350.

15. 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. *Biomedical Optics Exp*. 2011;2:2562–2568.

16. 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.

17. Wanek J, Teng PY, Blair NP, Shahidi M. Inner retinal oxygen delivery and metabolism in streptozotocin diabetic rats. *Invest Ophthalmol Vis Sci*. 2014;55:1588–1593.

18. Barber AJ, Antonetti DA, Kern TS, et al. The Ins2Akita mouse as a model of early retinal complications in diabetes. *Invest Ophthalmol Vis Sci*. 2005;46:2210–2218.

19. Gastinger MJ, Kunselman AR, Conboy EB, Bronson SK, Barber AJ. Dendrite remodeling and other abnormalities in the retinal ganglion cells of Ins2 Akita diabetic mice. *Invest Ophthalmol Vis Sci*. 2008;49:2635–2642.

20. Gastinger MJ, Singh RS, Barber AJ. Loss of cholinergic and dopaminergic amacrine cells in streptozotocin-diabetic rat and Ins2Akita-diabetic mouse retinas. *Invest Ophthalmol Vis Sci*. 2006;47:3143–3150.

21. Han Z, Guo J, Conley SM, Naash MI. Retinal angiogenesis in the Ins2(Akita) mouse model of diabetic retinopathy. *Invest Ophthalmol Vis Sci*. 2013;54:574–584.

22. Wright WS, Yadav AS, McElhatten RM, Harris NR. Retinal blood flow abnormalities following six months of hyperglycemia in the Ins2(Akita) mouse. *Exp Eye Res*. 2012:98:9–15.

23. McLenachan S, Magno AL, Ramos D, et al. Angiography reveals novel features of the retinal vasculature in healthy and diabetic mice. *Exp Eye Res*. 2015;138:6–21.

24. McLenachan S, Chen X, McMenamin PG, Rakocy EP. Absence of clinical correlates of diabetic retinopathy in the Ins2Akita retina. *Clin Exp Ophthalmol*. 2013;41:582–592.

25. 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.

26. Shahidi M, Shakoor A, Blair NP, Mori M, Shonat RD. A method for chorioretinal oxygen tension measurement. *Curr Eye Res*. 2006;31:357–366.

27. Nathan AT, Singer M. The oxygen trail: tissue oxygenation. *Br Med Bull*. 1999;55:96–108.

28. Pittman RN. Regulation of Tissue Oxygenation. San Rafael, CA: Morgan & Claypool Life Sciences; 2011.

29. Gray LH, Steadman JM. Determination of the oxyhaemoglobin dissociation curves for mouse and rat blood. *Journal Physiol*. 1964;175:161–171.

30. Teng PY, Wanek J, Blair NP, Shahidi M. Inner retinal oxygen extraction fraction in rat. *Invest Ophthalmol Vis Sci*. 2013;54:647–651.

31. 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.

32. Wang Z, Yadav AS, Leskova W, Harris NR. Attenuation of streptozotocin-induced microvascular changes in the mouse retina with the endothelin receptor A antagonist atrasentan. *Exp Eye Res*. 2010;91:670–675.

33. Wang Z, Yadav AS, Leskova W, Harris NR. Inhibition of 20-HETE attenuates diabetes-induced decreases in retinal hemodynamics. *Exp Eye Res*. 2011;95:108–115.

34. Wright WS, Harris NR. Ozagrel attenuates early streptozotocin-induced constriction of arterioles in the mouse retina. *Exp Eye Res*. 2008;86:528–536.

35. Wright WS, Messina JE, Harris NR. Attenuation of diabetes-induced retinal vasoconstriction by a thromboxane receptor antagonist. *Exp Eye Res*. 2009;88:106–112.

36. Yadav AS, Harris NR. Effect of tempol on diabetes-induced decreases in retinal blood flow in the mouse. *Curr Eye Res*. 2011;36:456–461.

37. 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.

38. Blair NP, Wanek JM, Mori M, Shahidi M. Abnormal retinal vascular oxygen tension response to light flicker in diabetic rats. *Invest Ophthalmol Vis Sci*. 2009;50:5444–5448.

39. Obrosova IG, Drel VR, Kumagai AK, Szabo C, Pacher P, Stevens MJ. Early diabetes-induced biochemical changes in the retina: comparison of rat and mouse models. *Diabetologia*. 2006;49:2525–2533.

40. Felder AE, Wanek J, Blair NP, et al. The effects of diabetic retinopathy stage and light flicker on inner retinal oxygen extraction fraction. *Invest Ophthalmol Vis Sci*. 2016;57:5586–5592.

41. Illing EK, Gray CH. Retinal metabolism in diabetes; the metabolism of retinoids of normal and alloxandiabetic rabbits. *J Endocrinol*. 1951;7:242–247.

42. Sutherland FS, Stefansson E, Hatchell DL, Reiser H. Retinal oxygen consumption in vitro. The effect of diabetes mellitus oxygen and glucose. *Acta Ophthalmol*. 1990;68:715–720.

43. de Roeth AF. Metabolism of the alloxan diabetic rat retina. *Trans Am Ophthalmol Soc*. 1963;61:429–458.

44. Lau JC, Linsenmeier RA. Increased intraretinal PO2 in short-term diabetic rats. *Diabetes*. 2014;63:4538–4542.

45. Linsenmeier RA, Braun RD, McRipley MA, et al. Retinal hypoxia in long-term diabetic cats. *Invest Ophthalmol Vis Sci*. 1998;39:1647–1657.

46. de Gooyer TE, Stevenson KA, Humphries P, Simpson DA, Gardiner TA, Sitt AW. Retinopathy is reduced during experimental diabetes in a mouse model of outer retinal degeneration. *Invest Ophthalmol Vis Sci*. 2006;47:5561–5568.
47. Ly A, Yee P, Vessey KA, Phipps JA, Jobling AI, Fletcher EL. Early inner retinal astrocyte dysfunction during diabetes and development of hypoxia, retinal stress, and neuronal functional loss. *Invest Ophthalmol Vis Sci*. 2011;52:9316–9326.

48. Kondo T, Kahn CR. Altered insulin signaling in retinal tissue in diabetic states. *J Biol Chem*. 2004;279:37997–38006.

49. Li C, Xu Y, Jiang D, et al. The expression of HIF-1 in the early diabetic NOD mice [in Chinese]. *Yan ke xue bao*. 2006;22:107–111.

50. Poulaki V, Joussen AM, Mitsiades N, Mitsiades CS, Iliaki EF, Adamis AP. Insulin-like growth factor-I plays a pathogenetic role in diabetic retinopathy. *Am J Pathol*. 2004;165:457–469.

51. Poulaki V, Qin W, Joussen AM, et al. Acute intensive insulin therapy exacerbates diabetic blood-retinal barrier breakdown via hypoxia-inducible factor-1alpha and VEGF. *J Clin Invest*. 2002;109:805–815.

52. 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;93:457–441.

53. 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.

54. Naito M, Fujikura J, Ebihara K, et al. Therapeutic impact of leptin on diabetes, diabetic complications and longevity in insulin-deficient diabetic mice. *Diabetes*. 2011;60:2265–2273.

55. Tattersall RB, Pyke DA. Growth in diabetic children. Studies in identical twins. *Lancet*. 1973;2:1105–1109.