Inner Retinal Oxygen Delivery, Metabolism, and Extraction Fraction in Ins2^{Akita} Diabetic Mice

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Purpose. Retinal nonperfusion and hypoxia are important factors in human diabetic retinopathy, and these presumably inhibit energy production and lead to cell death. The purpose of this study was to elucidate the effect of diabetes on inner retinal oxygen delivery and metabolism in a mouse model of diabetes.

Methods. Phosphorescence lifetime and blood flow imaging were performed in spontaneously diabetic Ins2^{Akita} ($n = 22$) and nondiabetic ($n = 22$) mice at 12 and 24 weeks of age to measure retinal arterial (O_{2A}) and venous (O_{2V}) oxygen contents and total retinal blood flow (F). Inner retinal oxygen delivery (DO_2) and metabolism (MO_2) were calculated as $F * O_{2A}$ and $F * (O_{2A} - O_{2V})$, respectively. Oxygen extraction fraction (OEF), which equals MO_2/DO_2, was calculated.

Results. DO_2 at 12 weeks were 112 ± 40 and 97 ± 29 nL O_2/min in nondiabetic and diabetic mice, respectively (NS), and 148 ± 31 and 85 ± 37 nL O_2/min at 24 weeks, respectively ($P < 0.001$). MO_2 were 65 ± 31 and 66 ± 27 nL O_2/min in nondiabetic and diabetic mice at 12 weeks, respectively, and 79 ± 14 and 54 ± 28 nL O_2/min at 24 weeks, respectively (main effects = NS). At 12 weeks OEF were 0.57 ± 0.17 and 0.67 ± 0.09 in nondiabetic and diabetic mice, respectively, and 0.54 ± 0.07 and 0.63 ± 0.08 at 24 weeks, respectively (main effect of diabetes: $P < 0.01$).

Conclusions. Inner retinal MO_2 was maintained in diabetic Akita mice indicating that elevation of the OEF adequately compensated for reduced DO_2 and prevented oxidative metabolism from being limited by hypoxia.

Keywords: Akita diabetic mice, oxygen delivery, oxygen metabolism, oxygen extraction fraction

Diabetic retinopathy (DR) is a major cause of blindness.[1,2] 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.[3–5] 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.[6,7] Also, VEGF, which is stimulated by hypoxia, is elevated.[8,9] However, no information is available in human DR on the rate of inner retinal oxygen metabolism (MO_2) 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 (DO_2), 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,[10–12] but there have been conflicting results of retinal blood flow measurements.[13,14]

We have developed imaging methods to measure DO_2 and MO_2[15,16] and applied them in rats with diabetes induced by streptozotocin (STZ).[17] We found no change in either DO_2 or MO_2 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 Ins2^{Akita} mouse (Akita) has a dominant point mutation in the insulin-2 gene that induces spontaneous type 1 diabetes by approximately 4 weeks of age.[18] 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.[18–23] However, the clinical correlates of DR are absent in Akita mice.[24] The purpose of the present study was to test the hypothesis that abnormalities in DO_2, MO_2, and the oxygen extraction fraction (OEF), which is the ratio of MO_2 to DO_2, 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 Ins2^{Akita} (Akita) C57BL/6J ($n = 22$) mice

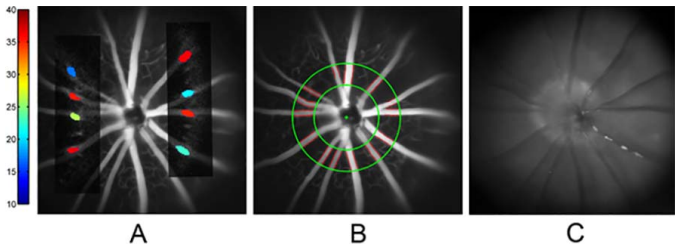

Figure 1. Examples of images used to evaluate oxygen delivery, metabolism, and oxygen extraction fraction in a 12-week-old diabetic mouse. (**A**) Cross-sectional retinal vascular PO_2 maps (*rectangles*) overlaid on a fluorescein angiogram. PO_2 values are depicted in four arteries and four veins. *Color bar* shows PO_2 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.

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. Nonfasting 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 (PO_2) 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 arterial catheter (20 mg/kg). For retinal blood velocity imaging, 2-μm polystyrene fluorescent microspheres (Invitrogen, Grand Island, NY, USA) were injected through the catheter. Typically, two to three injections of the microspheres were given, and the volume of each injection was approximately 0.4 mL (10^5 microspheres/mL). For retinal vascular caliber measurement, fluorescein angiography (FA) was performed by the intravascular injection of 10% fluorescein sodium (5 mg/kg, AK-FLUOR; Akorn, Decatur, IL, USA).

Oxygen Tension Imaging

Retinal vascular PO_2 measurements were obtained using our optical section phosphorescence lifetime imaging system.[15,25] 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 PO_2 measurements using the Stern-Volmer equation. PO_2 was measured in individual major retinal arteries (PO_{2Aind}) and retinal veins (PO_{2Vind}) at locations within 3 optic disc diameters from than edge of the optic nerve head, as shown in Figure 1A. Four repeated PO_{2Aind} and PO_{2Vind} measurements were averaged per blood vessel. An average arterial (PO_{2A}) and venous (PO_{2V}) PO_2 value was calculated from the individual artery and vein measurements in each animal, respectively.

Blood Flow Imaging

Our previously described prototype blood flow imaging system[15] 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 (V_{ind}). A mean velocity (V) in each mouse was calculated based on all V_{ind} 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 V_{ind} measurements: $(V_{ind} \times \pi \times D^2_{Vind}/4)$. 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 PO_2 imaging.

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The oxygen content of blood in each retinal artery (O_{2Aind}) and vein (O_{2Vind}) was calculated as the sum of oxygen bound to hemoglobin and dissolved in blood: $O_{2ind} = SO_2 \times C \times HgB + PO_{2ind} \times k$, where SO_2 is the oxygen saturation (%), C is the oxygen-carrying capacity of hemoglobin (1.39 mL O_2/g),[27] HgB is the hemoglobin concentration, and k is the oxygen solubility in blood (0.003 mL O_2/dL·mm Hg).[28] SO_2 was calculated from the hemoglobin oxygen dissociation curve in mice[29] by using the measured PO_{2ind} 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 O_{2ind} calculations in all mice. In each animal, a mean arterial (O_{2A}) and venous (O_{2V}) oxygen content were determined from O_{2ind} measurements and the arteriovenous oxygen content difference was calculated as: $O_{2A\text{-}V} = O_{2A} - O_{2v}$.

DO_2, 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 O_{2A}. MO_2, 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 $O_{2A\text{-}V}$. The OEF equals the ratio of MO_2 to DO_2. It varies between 0 and 1, and it can be calculated as $O_{2A\text{-}V}/O_{2A}$.[30]

Data Analysis

Twelve continuous outcome variables (PO_{2A}, PO_{2V}, O_{2A}, O_{2V}, $O_{2A\text{-}V}$, D_A, D_V, V, F, DO_2, MO_2, 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 DO_2, MO_2, 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 DO_2 and MO_2 values, and another mouse was identified as an outlier due to an abnormally high MO_2 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 we assigned the value of 600 mg/dL to any measurement that exceeded the maximal level. Two-sided statistical significance was accepted at P less than 0.05. When a significant interaction was found, simple main effects were determined by the independent samples t-test. Because the weights of the diabetic mice were lower than those of the nondiabetic mice, we also performed two-way analysis of covariance with weight as the covariate on all outcome variables. However, the statistical results did not change, except a marginally significant reduction in $O_{2A\text{-}V}$ present in the diabetic mice. Herein, we present only the results of the 2-way ANOVA.

Results

Body Weight and Blood Glucose Concentration

The body weights of the nondiabetic mice were 28 ± 3 and 32 ± 3 g (mean ± SD) at 12 and 24 weeks of age, respectively. The body weights of the diabetic mice were 24 ± 2 g at both 12 and 24 weeks of age. There was a significant interaction effect between the presence of diabetes and age ($P = 0.029$). The simple main effect of diabetes (presence or absence) on weight was significant at both 12 and 24 weeks of age ($P \leq 0.001$). The simple main effect of age (12 or 24 weeks) on weight was significant in nondiabetic mice ($P = 0.008$), but it was not significant in diabetic mice ($P = 1$).

In nondiabetic mice, blood glucose measurements obtained on the day of imaging were 151 ± 19 and 139 ± 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 ($P < 0.001$).

Table 1. Retinal Arterial (PO_{2A}) and Venous (PO_{2V}) Oxygen Tension, and Arterial (O_{2A}) and Venous (O_{2V}) Oxygen Content, and Arteriovenous Oxygen Content Difference ($O_{2A\text{-}V}$) of Nondiabetic and Diabetic (Akita) Mice at 12 and 24 Weeks of Age (Mean ± SD)

Outcome Variable	Age, wk	No Diabetes	Diabetes	*P* Value: Diabetes	*P* Value: Age	*P* Value: Diabetes × Age
PO_{2A}, mm Hg	12	36 ± 7	34 ± 4	0.12	0.94	0.81
	24	37 ± 5	33 ± 6			
PO_{2V}, mm Hg	12	22 ± 5	19 ± 4	0.01*	0.19	0.70
	24	24 ± 3	20 ± 3			
O_{2A}, mL O_2/dL	12	8 ± 2	7 ± 1	0.06	0.88	0.95
	24	8 ± 2	7 ± 2			
O_{2V}, mL O_2/dL	12	3 ± 1	2 ± 1	0.002*	0.31	0.76
	24	4 ± 1	3 ± 1			
$O_{2A\text{-}V}$, mL O_2/dL	12	5 ± 2	5 ± 1	0.97	0.42	0.90
	24	4 ± 1	4 ± 2			

* Statistical significance.

Retinal Vascular PO_2 and Oxygen Content

Mean values of retinal vascular PO_2 and O_2 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 PO_{2V} and O_{2V}, such that diabetic mice had a 15% reduction in PO_{2V} and a 30% reduction in O_{2V} as compared with nondiabetic mice ($P \leq 0.01$). There were no significant interactions between diabetes and age or significant main effects of age on PO_{2V} or O_{2V}. There were no significant interactions between diabetes and age or main effects of diabetes or age on PO_{2A}, O_{2A}, or $O_{2A\text{-}V}$.

Retinal Vessel Diameter, Blood Velocity, and Blood Flow

As expected, D_V was larger than D_A in both nondiabetic and diabetic mice ($P < 0.001$). Mean values of D_A, D_V, 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 nondiabetic than in the diabetic mice ($P = 0.003$). 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

Table 2. Retinal Arterial Diameter (D_A), Venous Diameter (D_V), 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: Diabetes	*P* Value: Age	*P* Value: Diabetes × Age
D_A, μm	12	25 ± 3	25 ± 2	0.82	0.19	0.55
	24	26 ± 2	26 ± 2			
D_V, μm	12	27 ± 3	28 ± 3	0.70	0.73	0.06
	24	29 ± 3	27 ± 3			
V, mm/s	12	7 ± 2	7 ± 1	0.11	0.37	0.17
	24	8 ± 1	6 ± 2			
F, μL/min	12	1.4 ± 0.4	1.4 ± 0.3	0.01*	0.14	0.01*
	24	1.9 ± 0.5	1.2 ± 0.4			

* Statistical significance.

mice. No significant effect of age, diabetes, or their interaction was found on D_A, D_V, or V.

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Mean values of DO_2, MO_2, 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 DO_2, and their simple main effects are presented in Table 3. No effect of diabetes on DO_2 was found at 12 weeks, but at 24 weeks DO_2 was 43% higher in the nondiabetic mice ($P < 0.001$). Also, although DO_2 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 MO_2.

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 ($P \geq 0.28$).

Table 3. Simple Main Effects of Total Retinal Blood Flow (F) and Inner Retinal Oxygen Delivery (DO_2) 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			
12	1.4 ± 0.4	1.4 ± 0.3	0.9
24	1.9 ± 0.5	1.2 ± 0.4	0.003*
P value	0.01*	0.34	
DO_2, nL O_2/min			
12	112 ± 40	97 ± 29	0.34
24	148 ± 31	85 ± 37	0.001*
P value	0.03*	0.4	

* 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 PO_{2V} and O_{2V}. Furthermore, there was a lack of normal increase in DO_2 with age in diabetic mice, driven by failure of F to increase with age in the diabetic group. These changes in OEF and DO_2 were in the context of no discernible abnormalities in MO_2 in the diabetic mice.

There are two major ways for the retina to maintain adequate tissue oxygenation and MO_2. First, DO_2 can be increased by adjusting F. At 24 weeks of age, DO_2 (and F) was significantly lower in the diabetic group than in the nondiabetic group because the normal increase in DO_2 (and F) from 12 to 24 weeks did not occur. DO_2, which is based on both F and O_{2A}, has not been measured previously in diabetic mice, but there are several reports of F. Studies by both Wright et al.[22] and Muir and colleagues[31] 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.[32–36] Had these investigators measured O_{2A}, it is likely that they would have found decreases in DO_2 similar to the decrease of 43% in the current study. These defective DO_2 and F responses in diabetes likely correspond to the well-known impaired autoregulation seen in humans with diabetes.[14,37]

The second way for the retina to maintain adequate tissue oxygenation and MO_2 is by extracting a greater fraction of the oxygen supplied by the blood, that is, by increasing OEF. Because MO_2 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 MO_2. 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 MO_2 could not be maintained.[16] 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.[38] 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.[17] 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.[39] showed a number of biochemical abnormalities were more prominent in STZ-diabetic rats than in STZ-diabetic mice, but they did not measure PO_2. 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[40] (Blair NP, unpublished data, 2016), so there may be differences in the pathogenesis of DR between humans, rats, and mice.

We reported MO_2 in diabetic rodents for the first time in rats with STZ diabetes, and no abnormality was found, as in the current study.[17] Illing et al.[41] and Sutherland and collegues[42] 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.[43]

It is expected that MO_2 will be maintained unless the inner retinal tissue PO_2 is reduced enough for hypoxic energy failure to supervene and threaten cell survival. Using oxygen microelectrodes, Lau and Linsenmeier[44] found no decrease in inner retinal PO_2 in rats with STZ-induced diabetes for 4 to 12

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Table 4. Inner Retinal Oxygen Delivery (DO_2), Oxygen Metabolism (MO_2), and Oxygen Extraction Fraction (OEF) in Nondiabetic and Diabetic (Akita) Mice at 12 and 24 Weeks of Age (Mean ± SD)

				P Value		
Outcome Variable	**Age, wk**	**No Diabetes**	**Diabetes**	**Diabetes**	**Age**	**Diabetes × Age**
DO_2, nL O_2/min	12	112 ± 40	97 ± 29	0.001*	0.28	0.03*
	24	148 ± 31	85 ± 37			
MO_2, nL O_2/min	12	65 ± 31	66 ± 27	0.16	0.91	0.10
	24	79 ± 14	54 ± 28			
OEF	12	0.57 ± 0.17	0.67 ± 0.09	0.01*	0.28	0.92
	24	0.54 ± 0.07	0.63 ± 0.08			

* Statistical significance.

weeks. Furthermore, the inner retinal PO_2 actually was elevated relative to the choroidal PO_2 at 12 weeks of diabetes. These findings are consistent with the finding of no difference between MO_2 in mice with and without diabetes in the current study. On the other hand, Linsenmeier et al.[45] 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 PO_2 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 MO_2 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 MO_2 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.[54] Similarly, STZ-diabetic rats have reduced body weight[17] and humans who become diabetic prior to puberty may have retarded growth.[55] 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.

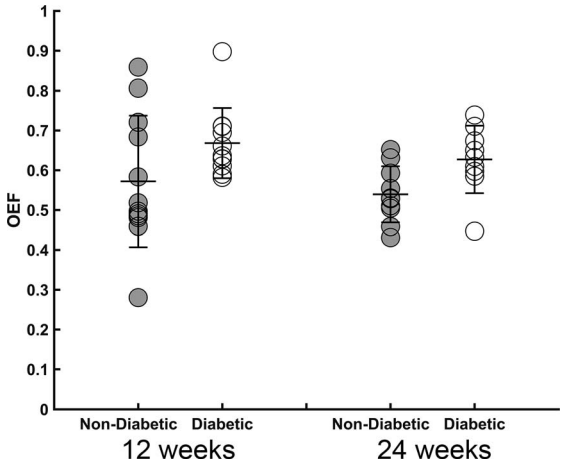

Figure 2. Oxygen extraction fraction (OEF) in nondiabetic (*gray circles*) and diabetic (*white circles*) mice at 12 and 24 weeks of age. *Horizontal bars* denote mean and SD.

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 MO_2 assumes that the volumes of tissue supplied by the retinal vessels and choroid are the same in the nondiabetic and diabetic mice. The retinal PO_2 profiles obtained from microelectrodes in normal and STZ-diabetic rats suggest that this assumption is reasonable.[44]

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.

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