Reduced Ocular Blood Flow as an Early Indicator of Diabetic Retinopathy in a Mouse Model of Diabetes

Eric R. Muir,¹ René C. Rentería,^{2,3} and Timothy Q. Duong^{1,2,4-6}

PURPOSE. To investigate ocular blood flow and visual function in the Ins2^{Akita} diabetic retinopathy mouse model at early and late time points after onset of hyperglycemia.

METHODS. Mice heterozygous for the Ins2^{Akita} mutation, which become hyperglycemic at approximately 4 weeks old, were studied at 2.5 and 7.5 months of age, with age-matched wild-type littermates used as controls. Retinal and choroidal blood flows were noninvasively imaged at 42 × 42 × 400 μm using magnetic resonance imaging. Visual function was measured using optokinetic tracking to determine spatial frequency and contrast thresholds from the same mice.

RESULTS. At 2.5 months, choroidal blood flow was significantly reduced ($P < 0.01$) by 20% in Ins2^{Akita} mice ($n = 13$) compared with age-matched controls ($n = 16$), whereas retinal blood flow and visual function were not significantly affected ($P > 0.05$). At 7.5 months, both choroidal and retinal blood flow were significantly reduced ($P < 0.05$) by 27% and 28%, respectively, in Ins2^{Akita} mice ($n = 11$) compared with age-matched controls ($n = 15$). Visual functions were also significantly worse ($P < 0.05$) in Ins2^{Akita} mice at 7.5 months, as indicated by a 19% decreased spatial frequency threshold and 135% increased contrast threshold compared with age-matched controls. The magnitudes of the blood flow and vision deficits, however, were not correlated.

CONCLUSIONS. Although both choroidal and retinal blood flow and vision were altered after prolonged diabetes in the Ins2^{Akita} mouse, choroidal blood flow was reduced even in young diabetic animals, suggesting ocular blood flow deficit could be an early pathological change in diabetic retinopathy. (*Invest Ophthalmol Vis Sci.* 2012;53:6488–6494) DOI:10.1167/iov.12-9758

From the ¹Research Imaging Institute, the departments of ²Physiology, ⁴Ophthalmology, and ⁵Radiology, and the ³Center for Biomedical Neuroscience, University of Texas Health Science Center, San Antonio, Texas; and ⁶South Texas Veterans Health Care System, San Antonio, Texas.

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Corresponding author: Timothy Q. Duong, Research Imaging Center, UTHSCSA, 8403 Floyd Curl Drive, San Antonio, TX 78229; duongt@uthscsa.edu.

Diabetic retinopathy (DR) is the leading cause of new blindness in adults between 20 and 74 years of age.¹ Dysfunction of the retinal vasculature is the most prominent aspect of DR, including thickening of the vascular layers, capillary nonperfusion, plasma protein leakage, ischemia, and hypoxia. Eventually proliferative growth of new vessels, tractional retinal detachment, and macular edema can occur, leading to severe vision loss.² Although many studies have sought to identify predictive parameters for the development of DR in the early stages,^{3–5} no clinically significant single parameter has been identified. New methods to identify diabetic patients at risk to develop DR are needed.

Changes in retinal blood flow (RBF) and hemodynamics, and less commonly in choroidal blood flow (ChBF), have been reported in DR,⁶ but the ocular blood flow (BF) changes remain controversial. Decreased BE, increased BE, and no changes in BF have all been found in diabetic patients with early and nonproliferative DR^{4,7–12} and animal models.^{13–20} Because changes in BF may be an early indicator of DR, more work is necessary to clarify the discrepancies in previous studies. Another early indicator of DR could be subtle visual deficits, such as in contrast sensitivity and color vision, which may precede clinical signs of vascular lesions.^{5,21} Currently, it is unclear whether early visual deficits are caused by early vascular dysfunction or through other mechanisms independent of vascular changes, such as direct effects of hyperglycemia on neurons.²²

The Ins2^{Akita} (Akita) mouse is a genetic model of diabetes that develops retinal complications of early, nonproliferative DR.^{23–25} The Akita mouse has a mutation that leads to misfolding of the insulin 2 protein,²⁶ causing pancreatic β-cell death and resulting in hypoinsulinemia and hyperglycemia. Male mice heterozygous for the Ins2^{Akita} mutation develop hyperglycemia at 4 weeks of age²⁶ and begin to show signs of DR 1 to 3 months after hyperglycemia onset, including apoptosis in the retina and reduced retinal ganglion and amacrine cell number.^{23,24} Vascular abnormalities also occur, including increased retinal vascular permeability and acellular capillaries.^{23,24}

The aims of this study were to assess whether RBF and ChBF changes are present early after hyperglycemia onset in the Akita mouse and whether BF correlates with visual deficits. We used a magnetic resonance imaging (MRI) method we recently developed to noninvasively image layer-specific RBF and ChBF.²⁷ Spatial frequency threshold and contrast sensitivity were assessed in the Akita mice, which have optomotor deficits,²⁸ by monitoring the optokinetic tracking response (OKT) to rotating gratings, which induces reflexive turning of the head and neck in rodents.^{29,30}

METHODS

Animal Preparation

The protocols were approved by the local Institutional Animal Care and Use Committee and adhered to the ARVO Statement for the Use of

Animals in Ophthalmic and Vision Research. Experiments were performed on male mice heterozygous for the *Ins2^{Akita}* mutation on a C57BL/6J background (Jackson Laboratory, Bar Harbor, ME). Age-matched, male littermates wild type for *Ins2* were used for controls. Mice were housed in standard mouse cages open to room air with a 12/12 light/dark cycle and fed a typical rodent chow with 5.7% fat and 20% protein (Teklad LM-485; Harlan, Houston, TX). The mice were generally housed with their hyperglycemic and control littermates (1–5 mice per cage); several of the hyperglycemic Akita mice were used for breeding and were housed singly subsequently. Mice used in this study were bred in our animal facilities by crossing heterozygous males with normal C57BL/6J females. Genotyping was used to confirm heterozygous and wild-type genotypes (Transnetyx, Inc., Cordova, TN). Nonfasting blood glucose was measured in late morning using a blood glucose meter (AlphaTrak; Abbott Labs, Abbott Park, IL) after 4.5 weeks of age,³¹ which may lead to higher blood glucose values than in fasted mice.

Optomotor responses of awake, freely moving animals were determined at 9 to 11 weeks of age (2.5-month group, $n = 12$ wild type, $n = 10$ Akita) and 27 to 32 weeks of age (7.5-month group, $n = 14$ wild type, $n = 10$ Akita). MRI was performed on anesthetized mice at 10 to 12 weeks of age (2.5-month group, $n = 16$ wild type, $n = 13$ Akita) and 30 to 37 weeks of age (7.5-month group, $n = 15$ wild type, $n = 11$ Akita). Visual tests and MRI were performed on most of the same animals. Some of the OKT results were part of a previously published data set.²⁸ One extreme outlier in spatial frequency threshold was excluded, a 7.5-month wild-type mouse with a value of 0.71 cycles/degree, which is more than 9 SDs higher than the mean; we believe this data point was an operator data entry error, given our experience with OKT in mice. BF data and contrast threshold from this animal were still included in the analysis. Two mice had damage to their right eye, a 2.5-month Akita with micro-ophthalmia and a 2.5-month wild-type mouse with a cloudy right eye, but were not excluded because only the left eye was studied here. One 2.5-month old Akita mouse had poor physiology under anesthesia, leading to the animal being recovered before MRI data were acquired. The Akita mice otherwise did not have notable signs of poor health or distress.

Magnetic Resonance Imaging

Animals were placed into a head holder with ear and tooth bars. Imaging was performed under 1.0% to 1.4% isoflurane, 30% oxygen balanced with nitrogen, and spontaneous breathing. A circulating warm water pad was used to maintain rectal temperature at $37 \pm 0.5^\circ\text{C}$. Mice were prepared in a lit room before being transferred to the MRI room, in which lights were turned off but which was not completely dark. Respiration rate, heart rate, and oxygen saturation were monitored (MouseOx; STARR Life Science Corp., Oakmont, PA) and maintained by adjusting the isoflurane level.

MRI was performed on a 7-T, 30-cm horizontal magnet with a 1500-mT/m gradient (Bruker, Billerica, MA). For imaging, a small circular surface eye coil with active decoupling (diameter = 6 mm) was placed over the left eye. A circular labeling coil (diameter = 8 mm) was placed at the heart position for cardiac spin labeling.³²

BF MRI was acquired using two-coil continuous arterial spin labeling (ASL) with an echo planar imaging (EPI) sequence.²⁷ Paired images, one with and one without labeling, were acquired in an interleaved fashion. ASL used a 2.6-second square radiofrequency pulse to the labeling coil in the presence of a 2.0 G/cm gradient along the posterior-anterior direction with a postlabel delay of 350 ms. Images were acquired in a coronal orientation with a single slice passing through the optic nerve head and angled perpendicular to the retina. Two-segment, gradient-echo EPI was used with field of view = $6 \times 6 \text{ mm}^2$, matrix = 144×144 ($42 \times 42 \mu\text{m}^2$ resolution), a 0.4-mm slice, repetition time = 3.0 seconds per segment, and echo time = 12.6 ms. For each scan, 100 pairs of images were acquired in time-series with a total acquisition time of 20 minutes.

MRI Analysis

Image analysis was done using Matlab (Math-Works, Natick, MA) and Statistical Parametric Mapping 5 (SPM5; University College London, London, United Kingdom) software. Images were zero-padded to 256×256 before subsequent processing. Images were acquired as time series, aligned using the spatial realignment function in SPM5, and averaged off line. Blood flow images (S_{BF}) in units of (mL blood)/(g tissue)/min were calculated pixel-by-pixel using $S_{\text{BF}} = \lambda/T_1 ([S_{\text{NL}} - S_{\text{L}}]/[S_{\text{L}} + (2\alpha - 1)S_{\text{NL}}])$,³³ where S_{NL} and S_{L} are signal intensities of the nonlabeled and labeled images, respectively. The water tissue-blood partition coefficient λ was taken to be 0.9, the same as the brain.³⁴ The retina and choroid T_1 at 7 T were taken to be 1.8 seconds.³⁵ The labeling efficiency α was previously measured to be approximately 0.7.³²

BF intensity profiles across the retinal thickness were obtained from BF images by projecting lines perpendicular to the retina with profiles obtained at $4\times$ spatial interpolation.^{36,37} Further motion correction was performed on the profiles.³⁷ BF profiles were averaged along the length of the retina, excluding a small region surrounding the optic nerve head. Peak values of RBF and ChBF were measured from the average BF profiles for each animal. The lengths of the retina used for profile analysis in the 2.5-month group were $2363 \pm 116 \mu\text{m}$ in wild-type mice and $2340 \pm 139 \mu\text{m}$ in Akita mice. The retinal lengths in the 7.5-month group were $2541 \pm 88 \mu\text{m}$ in wild-type mice and $2504 \pm 51 \mu\text{m}$ in Akita mice.

Optomotor Response

Spatial frequency threshold and contrast threshold were assessed in mice by measuring optomotor responses to drifting gratings^{29,30} using the OptoMotry system (Cerebral Mechanics, Inc., Lethbridge, Canada). The mouse was placed on a platform in the center of a chamber made of computer monitors with a virtual cylinder of sinusoidal gratings rotating at 12 degrees/second displayed. The mouse was monitored via an overhead video camera for a trained observer to center the virtual cylinder at the animal's head and to score smooth head turns in response to the rotating gratings. These tracking responses are robust at middle spatial frequencies and contrasts and diminish until they cease at threshold.²⁸ Responses were measured for the left eye in which BF was measured by MRI. This test assays the eyes individually because the response is driven by temporal to nasal movement in the visual field (i.e., clockwise rotation tests the left eye and counterclockwise rotation tests the right eye, even in the presence of a stimulus that illuminates the entire visual field).³⁰

To determine the spatial frequency threshold, the spatial frequency of gratings (in cycles per degree [cpd]) presented at maximum contrast (98.6%) was varied using a staircase protocol, with a minimum step size of 0.003 cpd. Spatial frequencies were stepped through until the highest frequency still eliciting a response was determined. This determination was made by the software when the observer indicated that the given spatial frequency elicited a tracking response at least four times with no more than three indications of nontracking. Contrast threshold was assessed at a spatial frequency of 0.103 cpd with a minimum step size of 0.1%. Contrast was calculated as $(\text{max} - \text{min})/(\text{max} + \text{min})$, where max and min are the maximum and minimum values of the sinusoidal gratings. These data are presented as contrast thresholds, although it also is sometimes expressed as contrast sensitivity, which is the reciprocal of the contrast threshold.

Statistical Analysis

Statistical analysis was performed using SPSS (SPSS Inc., Chicago, IL) and Matlab. Group-average data are expressed as mean $\pm$ SD. Physiological data were analyzed using 2-tailed *t*-tests to compare age-matched Akita and wild-type mice. For BF and visual data, Levene's test was used to determine equality of variances between groups. Variances were unequal for ChBF, spatial frequency threshold, and contrast

TABLE 1. Physiological Parameters in Wild-Type and Akita Mice (Mean $\pm$ SD)

	2.5 Months		7.5 Months	
	Wild Type	Akita	Wild Type	Akita
Blood glucose, mg/dL, after 4.5 wk	218 $\pm$ 59	408 $\pm$ 76*	182 $\pm$ 34	473 $\pm$ 104*
Age, wk, at time of MRI	11.0 $\pm$ 0.6	11.0 $\pm$ 0.9	32.0 $\pm$ 1.7	33.0 $\pm$ 2.2
Age, wk, at time of visual tests	10.0 $\pm$ 0.4	10.0 $\pm$ 0.7	30.0 $\pm$ 0.7	30.0 $\pm$ 1.7
Weight, g†	24 $\pm$ 1.9	17 $\pm$ 1.2*	29 $\pm$ 3.4	23 $\pm$ 2.2*
Respiration rate, breaths/min†	107 $\pm$ 12	100 $\pm$ 9	105 $\pm$ 9	99 $\pm$ 9
Heart rate, beats/min†	408 $\pm$ 52	375 $\pm$ 33	445 $\pm$ 54	396 $\pm$ 42*
Oxygen saturation, %†	98.5 $\pm$ 0.8	98.7 $\pm$ 0.4	98.4 $\pm$ 0.8	98.3 $\pm$ 0.4

* $P < 0.05$ between Akita and age-matched wild-type mice.

† Obtained during MRI under isoflurane anesthesia.

threshold ($P < 0.05$). BF data and spatial frequency threshold were analyzed using two-way ANOVA and two-tailed t -tests (for equal or unequal variances as appropriate) for multiple comparisons. The Wilcoxon rank-sum test was used to perform pairwise comparison of contrast thresholds, which were expected to be non-normal. Multiple comparisons were made between aged-matched Akita and wild-type mice, 2.5- and 7.5-month Akita mice, and 2.5- and 7.5-month wild-type mice. To account for multiple comparisons, the Bonferroni-Holm correction was used to adjust α -values for t -tests and for rank-sum tests with an uncorrected α of 0.05. Correlations between ChBF, RBF, spatial frequency threshold, and contrast threshold were analyzed in each group of animals using Spearman's correlation coefficient.

RESULTS

The physiological parameters are summarized in Table 1. The Akita mice, which were not treated with insulin, developed severe hyperglycemia, consistent with previous studies.^{23,24,31} BF maps calculated from ASL MRI for each mouse eye had two distinct BF layers in the retina, separated by a region of low BF (Fig. 1), as previously reported.²⁷ The outer BF layer, which corresponds to the choroid, had high BF. The inner BF layer, which corresponds to the retinal vasculature, had lower BF than the choroid. The middle layer with little BF contrast corresponds to the avascular region of the retina, made up of the outer nuclear layer and the outer and inner photoreceptor segments. BF values of the retina and choroid in Akita and control mice are summarized in Figure 2. Overall, BF was lower in the diabetic Akita retina compared with age-matched wild-type controls. ChBF was significantly lower in Akita mice at both 2.5 and 7.5 months compared with age-matched wild-type mice, whereas RBF was significantly lower in Akita mice only at 7.5 months. There were no significant differences in ChBF or RBF between 2.5- and 7.5-month Akita mice or 2.5- and 7.5-month wild-type mice ($P > 0.05$).

FIGURE 1. MR image of the mouse eye and the corresponding BF map from a 7.5-mo wild-type mouse at $42 \times 42 \times 400 \mu\text{m}^3$ showing the retinal and choroidal BF. ON, optic nerve.

Measurements of spatial frequency and contrast thresholds are summarized in Figure 3. The spatial frequency threshold of Akita mice at 7.5 months was significantly lower compared with age-matched wild-type mice and with 2.5-month Akita mice. Contrast threshold was significantly higher in the Akita mice at 7.5 months compared with age-matched wild-type mice. Both of these results indicate poorer visual performance of the Akita mice. At 2.5 months, neither spatial frequency nor contrast thresholds were significantly different between Akita and wild-type mice, which agrees with previous data suggesting progressive decline of OKT visual parameters in diabetes.²⁸

Correlations among ChBF, RBF, spatial frequency threshold, contrast threshold, and blood glucose are shown in Table 2. Blood glucose was measured at 4 to 5 weeks of age, whereas MRI and OKT were performed at 2.5 and 7.5 months. Although ChBF and RBF tended to be significantly correlated and spatial frequency threshold and contrast threshold tended to be significantly correlated, BF generally was not strongly correlated with vision. There was no clear difference in correlations between the Akita and wild-type mice, although correlation coefficients between BF and vision measurements tended to be lower in the Akita mice. ChBF and RBF were not significantly correlated only in the 7.5-month Akita mice, suggesting reduced correspondence between the two vasculatures after extended hyperglycemia, although the correlation coefficient remained moderate. BF and visual tests were not significantly correlated with blood glucose in any groups except for contrast threshold and glucose in 2.5-month Akita mice.

FIGURE 2. Group averaged choroidal and retinal blood flow values show age-dependent BF reductions in hyperglycemic Akita mice. Choroidal and retinal BF in wild-type ($n = 16$ and 15 at 2.5 and 7.5 months, respectively) and Akita ($n = 13$ and 11 at 2.5 and 7.5 months, respectively) mice (mean $\pm$ SD). Significant differences from t -tests with Bonferroni-Holm correction are indicated with brackets, and P -values are from uncorrected t -tests.

FIGURE 3. Optokinetic tests show loss of visual function in hyperglycemic Akita mice. A loss of vision is manifested as a lower spatial frequency threshold or a higher contrast threshold. Spatial frequency threshold and contrast threshold in wild-type ($n = 12$ and 14 at 2.5 and 7.5 months, respectively) and Akita mice ($n = 10$ and 10 at 2.5 and 7.5 months, respectively) (mean $\pm$ SD). Significant differences from t -tests (spatial frequency threshold) or Wilcoxon rank-sum tests (contrast threshold) are indicated with brackets with P values from the corresponding uncorrected tests.

DISCUSSION

In Akita mice, ChBF was reduced early after onset of hyperglycemia (2.5 months), whereas RBF and spatial frequency and contrast thresholds to rotating gratings were significantly affected only at 7.5 months of age. Thus, reduced ocular BF, specifically of the choroid, may be an early pathological change in DR. Choroidal hemodynamics are difficult to assess with traditional techniques and have not been frequently studied in DR, but they can be measured with the MRI method used here. In human DR, vascular damage can eventually lead to neovascularization and macular edema. Early reduction in choroidal BF could provide a way to assess onset of early DR before severe damage or progression to proliferative retinopathy occurs.

Retinal and Choroidal BF

RBF, which nourishes the inner retina, was significantly lower in Akita mice at 7 to 8 months of age; however, by 2 to 4 months of age, Akita mice have neural damage in the inner retina and retinal vascular leakage.²³ RBF tended to be slightly reduced in 2.5-month Akita mice and was not significantly affected by age, so it remains possible that RBF is slightly reduced by 2.5 months. Decreased RBF is reported in many

other animal models of diabetes, often soon after onset of hyperglycemia. In nonobese diabetic (NOD) mice and streptozotocin (STZ)-injected mice, retinal BF, blood velocity, or vessel diameter was lower after 3 to 8 weeks of hyperglycemia.^{13,14} In STZ rats, measures of retinal vessel BF^{15,16} or diameter¹⁶ were reduced by 1 to 3 weeks after STZ injection. Increased RBF is also reported in diabetic rodents, including the STZ rat 5 to 6 weeks after injection^{19,20} and the db/db mouse after 10 weeks of hyperglycemia.¹⁸

ChBF, which mainly nourishes the outer retina, was significantly reduced by 2.5 months in Akita mice. Choroidal angiopathy, including capillary dropout and neovascularization, could be an early pathological change, having been found in diabetic patients with mild or no retinopathy.³⁸ In the choriocapillaris of STZ rats, blood velocity and flux were decreased, whereas vessel diameter was not changed 7 to 8 weeks after STZ injection.¹⁷ However, ChBF is also reported to increase in STZ rats 6 weeks after STZ injection.¹⁹ Neuronal damage is found in the inner retina in human DR³⁹ and in Akita mice,^{23,24} and changes in the electroretinogram (ERG) in early DR are believed to originate from the inner retina.⁴⁰ It is unclear what relationship early ChBF reduction could have with inner retinal dysfunction, but in severe DR, choroidal damage has been found to be related to photoreceptor damage and reduced ERG.⁴¹

A few mechanisms for BF changes in DR have been proposed, including capillary nonperfusion, capillary dropout, or vasoconstriction by various mechanisms, such as protein kinase C or renin-angiotensin.⁴² In Akita mice, however, acellular capillaries have not been reported until 9 months of age and pericyte ghosts have not been detected.²³ Another possibility is leukocyte adhesion in the retinal vasculature, which occurs in Akita mice by 3 months of age,²³ but reduced BF may not be dependent on leukocyte adhesion.⁴² Lower metabolic demand in the retina due to neuronal death could also cause a regulatory reduction of RBF and could result in the choroidal nutrient supply reaching more of the retina, further reducing the need for RBF. Another factor could be glucose, which may acutely increase RBF,⁴³ although the reported effects of glucose are inconsistent.¹² Thus, ocular BF may be even lower in Akita mice if blood glucose was controlled with supplemental insulin during the BF measurement.

Heart rate in this study was lower in the anesthetized Akita mice compared with controls, which could potentially reduce BF, dependent on other factors such as blood pressure and local vessel tone. In awake and lightly isoflurane-anesthetized Akita mice, heart rate was reported to not be altered.^{44,45} Isoflurane, which reduces heart rate and is a vasodilator that

TABLE 2. Correlation between Ocular BF and Visual Function (Spearman's ρ)

	2.5 Months				7.5 Months			
	ChBF	RBF	SFT	CT	ChBF	RBF	SFT	CT
Wild type								
RBF	0.73*				0.74*			
SFT	0.32	0.42			0.52	0.43		
CT	−0.38	−0.62*	−0.61*		−0.49	−0.47	−0.49	
GLC	0.02	0.16	−0.02	−0.06	0.04	0.22	0.29	−0.15
Akita								
RBF	0.65*				0.55			
SFT	−0.16	−0.11			0.20	−0.22		
CT	0.16	0.21	−0.49		−0.57	−0.10	−0.71*	
GLC	−0.03	0.03	0.16	0.64*	0.40	−0.06	0.51	−0.21

CT, contrast threshold; GLC, blood glucose; SFT, spatial frequency threshold.

* Correlation is significant at $P < 0.05$.

dose-dependently increases BF,⁴⁶ may be more potent in diabetes.⁴⁷ A higher sensitivity of Akita mice to the deeper isoflurane anesthesia we used could possibly cause the lower heart rate but could also cause a larger BF increase relative to wild-type mice. Thus, we do not expect the lower heart rate in Akita mice is the cause of reduced ocular BF. Finally, inner retinal thickness is decreased by 15% to 20% or 10 to 15 μm after 22 weeks of hyperglycemia in Akita mice.²³ Thinning of the vascular inner retina could increase partial volume effects in the RBF layer (i.e., a pixel located in the RBF layer is more likely to contain a larger proportion of avascular tissue as the inner retina thins), causing lower RBF values. Given that the inner retina thickness ($\sim 60\ \mu\text{m}$) remains larger than a pixel²³ and that the peak BF value was used, the small change in thickness is unlikely to have a major impact on the RBF value. Choroid thickness does not change in Akita mice,²³ and ChBF measurements should not be affected by changes in retinal thickness.

A spectrum of diabetic patients, ranging from no retinopathy to severe retinopathy, has been shown to exhibit no changes in blood velocity, BF, and arteriolar diameter of the retinal vasculature.^{7,12} Other studies report RBF is increased in diabetic patients with nonproliferative or no retinopathy,^{4,8,9} whereas blood velocity in retinal veins is unchanged^{4,9} or decreased.¹⁰ In the choroid, blood volume and BF is lower in proliferative DR, whereas blood velocity was unchanged.¹¹

Possible reasons for the inconsistent BF findings in DR are the following: type or model of diabetes (type 1 or 2 diabetes, toxin-induced or genetic animal models), duration and stage of DR (e.g., preclinical, mild, proliferative), treatment to maintain normal blood glucose, techniques for measuring BF (e.g., laser Doppler velocimetry, fluorescein angiography, microspheres), measured hemodynamic parameters (e.g., blood velocity/flow/volume, circulation time of dyes, vessel size), and the location of measurement (e.g., arteries, capillaries, tissue). BF studies in animals are commonly performed only 1 to 8 weeks after induction of diabetes, with few studies after long durations.^{14–17} Most optical techniques are nonquantitative or can measure BF in retinal surface vessels only. By contrast, BF MRI can measure volumetric BF in mL/g/min of both the retina and choroid without depth limitation and with a large field of view. MRI can also be used to acquire functional data, which will be useful to study altered responses to physiologic or visual stimuli in disease.^{36,48}

Visual Function

Visual deficits in DR patients have been reported before clinical vascular signs of DR, including deficits in contrast sensitivity²¹ and color sensitivity.⁵ Early changes in ERG have also been noted in DR patients and in STZ rats, specifically in the oscillatory potentials and scotopic threshold, which are believed to arise from inner retinal activity.^{40,49} Progressive decline of OKT visual parameters in Akita mice has recently been reported (Barber AJ, et al. *IOVS* 2010;51:ARVO E-Abstract 109).²⁸ Despite reduced ChBF in Akita mice at 2.5 months of age, OKT visual performance appeared normal at the same age, as did RBF, which might suggest that RBF is more important than ChBF for OKT performance. Unexpectedly, the contrast threshold was improved in the 7.5-month-old controls compared with 2.5-month controls. One reason for this was that the younger mice were more active in the OKT testing apparatus, tending to break visual attention and interrupt tracking with body movements, making it harder to subjectively detect subtle head movements close to threshold.²⁸ Decreased activity of older mice and relatively longer maintained periods of continuous tracking may thus have

made it easier to detect small and slow head-turning motions near threshold.

Although both spatial frequency and contrast thresholds were worse in the 7.5-month-old Akita mice, comparison of either RBF or ChBF measurements with visual performance parameters did not reveal consistent correlations in wild-type or Akita mice. Thus, vision loss in early DR may not necessarily be related to the BF magnitude but rather to other factors, such as duration of BF deficit or existence of a BF threshold under which vision is affected. Given that the OKT response is a behavioral test dependent on motor output, visual performance deficits could potentially arise from abnormalities in the motor system of Akita mice due to reasons unrelated to ocular BF, such as decreased muscle mass.⁵⁰ Furthermore, mice were imaged under anesthesia and in dim light conditions, whereas OKT responses were obtained in awake, behaving animals exposed to photopic light conditions. Differences in retinal function, metabolism, and BF may exist between light/dark and awake/anesthetized conditions. These differences could affect the correlation between BF and OKT responses. It is also possible that these differences in BF and vision between light/dark and awake/anesthetized conditions will not be the same in Akita mice compared with wild types.

Alternatively, although DR is traditionally considered a vascular disease, neuronal damage may occur in the retina independent of vascular abnormality, through factors such as hyperglycemia or hypoinsulinemia.²² If this is the case, then our data indicate that retinal vasculopathy precedes the neuropathy in Akita mice. Like most rodent models, Akita mice develop only the earlier signs of DR and do not develop the more severe vascular dysfunctions that occur later in human DR. Ultimately, severe vision loss in DR is caused by neovascularization (i.e., proliferative DR) and by diabetic macular edema resulting from increased vessel permeability.² In human diabetes, upregulation of angiogenic factors in the eye precipitates neovascularization and edema and is thought to occur in response to microvascular dysfunction in early DR, such as capillary nonperfusion. Thus, BF could be an indicator of the progression of DR. Human studies are likely needed to determine the association between BF changes in early DR and the progression to more severe and vision-threatening DR because rodent models have complications associated only with early human DR. MRI is well suited to perform these measurements because it allows imaging of both the retinal and choroidal vasculatures noninvasively.

CONCLUSIONS

This study demonstrates that ocular BF and vision are affected in the hyperglycemic Akita mouse model of diabetes. Reduced ChBF measured noninvasively with MRI was an early pathophysiological change found in 2.5-month-old Akita mice. Significantly reduced RBF and worse spatial frequency and contrast thresholds were also detected in Akita mice by 7.5 months of age but not at the earlier age. BF MRI could enable objective early detection, longitudinal disease staging, monitoring of therapeutic intervention, and improved understanding of pathophysiology by noninvasive measurements in animal models of disease.

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