Layer-Specific Manganese-Enhanced MRI of the Diabetic Rat Retina in Light and Dark Adaptation at 11.7 Tesla

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PURPOSE. To employ high-resolution manganese-enhanced MRI (MEMRI) to study abnormal calcium activity in different cell layers in streptozotocin-induced diabetic rat retinas, and to determine whether MEMRI detects changes at earlier time points than previously reported.

METHODS. Sprague-Dawley rats were studied 14 days ($n = 8$) and 30 days ($n = 5$) after streptozotocin (STZ) or vehicle ($n = 7$) injection. Manganese-enhanced MRI at $20 \times 20 \times 700 \mu\text{m}$, in which contrast is based on manganese as a calcium analogue and an MRI contrast agent, was obtained in light and dark adaptation of the retina in the same animals in which one eye was covered and the fellow eye was not. The MEMRI activity encoding of the light and dark adaptation was achieved in awake conditions and imaged under anesthesia.

RESULTS. Manganese-enhanced MRI showed three layers, corresponding to the inner retina, outer retina, and the choroid. In normal animals, the outer retina showed higher MEMRI activity in dark compared to light; the inner retina displayed lower activity in dark compared to light; and the choroid showed no difference in activity. Manganese-enhanced MRI activity changed as early as 14 days after hyperglycemia and decreased with duration of hyperglycemia in the outer retina in dark relative to light adaptation. The choroid also had altered MEMRI activity at 14 days, which returned to normal by 30 days. No differences in MEMRI activity were detected in the inner retina.

CONCLUSIONS. Manganese-enhanced MRI detects progressive reduction in calcium activity with duration of hyperglycemia in the outer retina as early as 14 days after hyperglycemia, earlier than any other time point reported in the literature.

Keywords: ultra high-resolution MRI, high field, MRI contrast agents, light and dark adaptation, visual stimulation, manganese, MEMRI, photoreceptors, diabetic retinopathy

Diabetic retinopathy (DR), the leading cause of new blindness in working-age adults in the United States,¹ is presently diagnosed and treated based on changes in the retina caused by vascular abnormalities. However, recent evidence from electroretinography suggests that there are changes that occur in different retinal cell types before the abnormal manifestation of retinal vasculature.² Calcium plays an important role in cellular function. Calcium activity has been reported to be perturbed in experimental models of hyperglycemia.³ Inhibition of voltage-dependent calcium channels in the retinal microvasculature has been found in diabetes.⁴ Thus, imaging of calcium activity in vivo has the potential to provide valuable information about cellular dysfunction associated with DR.

Manganese-enhanced magnetic resonance imaging (MEMRI), in which contrast is based on manganese (Mn^{2+}) as a calcium analogue and an MRI contrast agent, has been used as a functional surrogate marker for calcium activity in vivo.^{5,6} In the retina, MEMRI has been used to study functional changes during light and dark adaptation^{7,8} and calcium dysregulation in DR⁹ and in glaucoma¹⁰ of rodents. The different neuronal cells in the inner and outer retina have different activity in varying lighting

conditions and likely have different vulnerabilities in various retinal diseases. However, reported MEMRI in the retina has shown one single layer,^{7,11} two layers⁹ assigned as the inner retina and the outer retina, or three layers⁸ assigned as the inner retina, outer retina, and the choroid. Consequently, this raises the question as to which of the MRI-detected retinal layers show calcium dysfunction in DR. The earliest reported MEMRI changes in the retina are at ~ 3 months in rats¹¹ and 1.5 months in mice⁹ after streptozotocin (STZ)-induced hyperglycemia.

The goals of this study were to use a high-resolution MEMRI protocol with validated layer assignments to study abnormal calcium activity in different cell layers in STZ-induced diabetic rats, and to determine whether MEMRI can detect changes at earlier time points than previously reported in STZ rats. Functional activity in different MEMRI-derived “layers” of the diabetic rat retina was measured during light versus dark adaptation. Manganese-enhanced MRI was carried out where one eye was covered (dark adaptation) and the fellow eye was not (light adaptation). Two identical radiofrequency transceiver coils, one for each eye, were used for MRI acquisitions.

MATERIALS AND METHODS

Animal Preparation

Animals used in this study were treated with Institutional Animal Care and Use Committee approval and in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Hyperglycemia was induced by intravenous injection of STZ (55 mg/kg) in citrate buffer (0.01 M, pH 4.5). Age-matched controls were injected with vehicle. Three groups of male Sprague-Dawley rats were studied: (I) 14 days post STZ injection ($n = 8$), (II) 30 days post STZ injection ($n = 5$), and (III) vehicle-treated age-matched controls ($n = 7$). Note that STZ injection in group II was given at a younger age than in group I such that MRI could be performed on ~60 day-old animals for all three groups. Hyperglycemia was verified by blood glucose levels (Alphatrak; Abbott Laboratories, Abbott Park, IL, USA) greater than 250 mg/dL, typically measured 3 days after STZ injection. No cataract was expected or detected in these animals in association with diabetes.

Animals were briefly anesthetized with 2% isoflurane for placement of an eye patch and an Elizabethan collar to prevent the animal from removing the eye patch.⁸ One eye, chosen at random, was patched for dark adaptation while the fellow eye was not patched for ambient light adaptation. The animal was allowed to recover from anesthesia for 2 hours to ensure dark adaptation before manganese injection. The animal was reanesthetized for intravenous manganese administration (88 mg $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ /kg body weight, via tail vein) over an hour. The animal was recovered from anesthesia and returned to its cage (with one eye still patched) under ambient room light for 5 hours for Mn^{2+} activity encoding under awake conditions. Tail vein infusion was chosen over intraperitoneal injection because a more consistent manganese dosage could be delivered via this route.

After 5 hours of Mn^{2+} activity encoding, animals were anesthetized with ~1.5% isoflurane and orally intubated for mechanical ventilation. Animals were then secured in an MRI-compatible rat stereotaxic headset; the eye patch was removed in dim red light, and atropine eye drops were applied topically to dilate pupils and to prevent ciliary muscle movements. Lubricating eye drops (Systane Ultra; Alcon, Fort Worth, TX, USA) were also placed on each eye. The MRI scanner room was kept dark during MRI.

Pancuronium bromide (0.5 mg/kg, intravenous) was administered after the animal was inside the scanner to eliminate eye movement.^{12–14} A second dose of pancuronium was given when the other eye was scanned sequentially. End-tidal CO_2 , rectal temperature, oximetry, and heart rate were continuously monitored and maintained within normal physiological ranges during MRI.

Magnetic Resonance Imaging

Magnetic resonance imaging experiments were performed on an 11.7 tesla/16 cm scanner (Biospec; Bruker, Billerica, MA, USA) with two identical custom-made surface coils with an inner diameter of 7 mm, one for each eye. Each eye was scanned separately. Scout images were acquired to plan a single midsagittal slice bisecting the center of the eye and optic nerve for subsequent imaging in order to minimize partial-volume effect due to retinal curvature.¹² Manganese-enhanced MRI was acquired using a gradient-echo sequence with repetition time = 150 ms, echo time = 4.63 ms, field of view = 7.5×7.5 mm, slice thickness = 0.6 mm, 18 repetitions acquired in time series, and acquisition matrix = 384×384 , yielding an in-plane resolution of 20×20 μm .

Image Data Analysis

Image analysis was performed using custom-written programs in Matlab (MathWorks, Natick, MA, USA) as described previously.¹⁵ Time-series data were corrected for drift and motion before offline averaging.¹⁶ The retinal images were linearized by radially projecting lines perpendicular to the retina. Intensity profiles were obtained over the posterior pole of the eye. Quantitative analysis was performed on a small region of the retina to minimize partial-volume effects due to the curvature of the retina and to avoid averaging heterogeneous retinal structure of variable laminar thicknesses across the length of the retina, as done previously by our group⁸ and Berkowitz et al.^{7,9,11}

In each animal, intensity profiles were normalized with respect to the vitreous of each eye to account for slight difference in radiofrequency coil sensitivity profiles. The vitreous region of interest was placed in homologous regions of the two eyes. The peak height was determined for the inner retina, outer retina, and choroid. Similar approaches have been employed previously using either peak height or region of interest analysis by our group⁸ and Berkowitz et al.^{7,9,11}

Statistical analysis comparing light versus dark layer intensities was performed by paired *t*-tests with Bonferroni-Holm adjustment to correct type I error in multiple comparisons. The significance level was set at $P < 0.05$. Preliminary statistical analysis comparing MEMRI intensity between control, 14 days, and 30 days post STZ with one-way ANOVA did not find any statistical differences in any layer or lighting condition. Light and dark peak percent differences were analyzed using one-way ANOVA with post hoc analysis using *t*-tests and Bonferroni-Holm correction with significance level set at 0.05.

RESULTS

The physiological parameters for all three groups are shown in the Table. In control animals, all physiological parameters were within normal physiological ranges. Diabetic animals weighed significantly less than age-matched controls, with weight significantly decreasing with duration of hyperglycemia ($P < 0.05$). Blood glucose of the 14- and 30-day post-STZ injection groups was significantly higher than in controls ($P < 0.05$). Blood glucose was also higher at 30 days post STZ than at 14 days post STZ. Respiration rate and end-tidal CO_2 were not statistically different among the three animal groups ($P > 0.05$). Heart rates of the 30-day STZ animals were statistically lower than those of control and 14-day STZ animals ($P < 0.05$). Oxygen saturation in 14-day STZ animals was significantly higher compared to controls and 30-day STZ animals ($P < 0.05$).

Manganese-enhanced MRI results from a typical control animal with light- and dark-adapted eyes are shown in Figure 1. The signal-to-noise ratios between the two eyes were similar overall. The MEMRI images showed layer-specific contrasts in the retinas, with alternating hyperintense, hypointense, and hyperintense bands. The sclera, posterior to the choroid, was hypointense because it has lower water content and short transverse relaxation time (T_2^*). The vitreous was hypointense because its signal was suppressed due to its long spin-lattice relaxation time (T_1).

Profiles were normalized with respect to each eye's vitreous as shown by the small rectangular box. A typical area of interest in the retina used for quantitative profile analysis is shown as the large rectangular box. We previously validated that peak 1 was the inner retina (ganglion cell and inner nuclear layer) with embedded retinal vessels, peak 2 was the avascular outer retina (outer nuclear layer and photoreceptor

FIGURE 1. (A) MEMRI of light- and dark-adapted eyes from the same control animal at $20 \times 20 \times 700 \mu\text{m}$ and (B) intensity profiles across the retinal thickness. Normalization was applied with respect to the vitreous. The region of interest in the vitreous shows the typical region used for normalization. ONH, optic nerve head. The region of interest on the retina shows the typical region where intensity profiles were obtained.

segments), and peak 3 was the choroid vascular layer.⁸ The choroidal peaks under dark and light adaptation had similar intensity. The outer retina peak showed higher intensity in dark relative to light. The inner retina in dark had slightly lower intensity relative to light.

Figure 2 shows the group-averaged peak intensity values under light and dark adaptation for controls, 14-days post-STZ animals, and 30-days post-STZ animals. In the control animals, the outer retina showed higher MEMRI intensity in dark relative to light ($P < 0.05$), the inner retina had lower intensity in dark relative to the light ($P < 0.01$), and the choroid intensity was not significantly different between dark and light adaptation ($P > 0.05$).

In the 14 days post-STZ group, MEMRI intensity in the outer, inner, and choroid layers was not significantly different between dark and light. In the 30-days post-STZ group, the outer retina MEMRI had lower intensity under dark compared to light ($P < 0.05$), opposite to what was seen in controls. The inner retina showed significantly lower intensity in the dark when compared to light ($P < 0.05$), similar to observations in control animals.

When compared among control and 14- and 30-days post-STZ groups, MEMRI intensity was not statistically different in any layer or lighting condition ($P > 0.05$, one-way ANOVA). Thus, we also analyzed the percent differences between light and dark adaptation (light minus dark) for the three different MEMRI layers at different stages of hyperglycemia (Fig. 3). In the inner retina, the percent signal differences were not statistically different among the different durations of hyperglycemia. In the outer retina, the percent signal differences were significantly different, changing from negative in controls to nearly 0, and then to positive with increasing hyperglycemic duration. In the choroid, the percent difference was positive in controls and at

30 days post STZ but was negative at 14 days post STZ ($P < 0.05$ compared to controls and 30 days post STZ).

DISCUSSION

We employed functional MEMRI to image calcium activity in the inner retina, outer retina, and choroid during light and dark adaptation of the normal and diabetic retina. The major findings are as follows. (1) In normal animals, the outer retina displayed higher MEMRI activity in dark compared to light, the inner retina displayed lower MEMRI activity in dark compared to light, and the choroid showed no differences in MEMRI activity. (2) The differences in MEMRI activity between light and dark changed significantly with the duration of hyperglycemia in the outer retina. (3) This change was detectable as early as 14 days after onset of hyperglycemia, to our knowledge earlier than times reported in the literature in rats. (4) Manganese-enhanced MRI activity in the inner retina did not change significantly with the progression of hyperglycemia.

The advantages of MEMRI^{5,6} are as follows: (1) Functional activity can be encoded outside the MRI scanner while the animal is awake, in contrast to conventional blood oxygen level-dependent (BOLD) functional MRI, which is done under anesthesia in the majority of animal studies. (2) Changes in calcium activity can be measured, which offers more direct mapping of neural activity compared to hemodynamic changes in BOLD functional MRI. (3) Conventional T_1 -weighted MRI can be used, which offers images free of susceptibility artifacts (image distortion, signal dropout, and so on), which are common in echo-planar imaging used for BOLD functional MRI. Functional MEMRI, however, has several limitations. Manganese-enhanced MRI generally requires groups with

FIGURE 2. Group-averaged peak intensity values (normalized to the vitreous) in the inner retina, outer retina, and choroid for (A) control ($n = 7$), (B) 14-days post-STZ ($n = 8$), and (C) 30-days post STZ ($n = 5$) animals. Error bars are standard error of the mean. * $P < 0.05$, ** $P < 0.01$.

stimulation and separate groups without stimulation for control because the intracellularly trapped Mn^{2+} has a long half-life. This also precludes the use of repeated or real-time paradigms often employed in conventional BOLD functional MRI studies. Normalization with respect to an external or internal standard is needed. Manganese-enhanced MRI application is also limited to studying animal models.

Layer Assignments

Consistent with our previous finding in normal animals,⁸ MEMRI of the rat retina showed three distinct bands. Layer assignments have been previously validated using an intravascular contrast agent, which demarcated the retinal and choroidal vascular layers but not the avascular outer retina in between. These three bands were the inner MEMRI band consisting of the vascularized inner retina (ganglion cell layer,

FIGURE 3. Percent changes between light and dark adaptation (light minus dark) for the three different layers at different durations of hyperglycemia. Positive values are indicative of relatively higher Mn^{2+} uptake in light, while negative values are indicative of relatively higher Mn^{2+} uptake in dark. Error bars are standard error of the mean. * $P < 0.05$.

inner plexiform layer, inner nuclear layer, and outer plexiform layer), the middle MEMRI band consisting of the avascular outer retina (outer nuclear layer and the inner and outer segments), and the outer MEMRI band consisting of the choroid. These layer assignments differed from those of Berkowitz et al.,^{7,9,11} which showed clearly in their images one single layer^{7,11} or two layers⁹ assigned as the inner retina and the outer retina. These authors did not mention the choroid signal in their studies. It is possible that the choroid, which was not distinguishable in their images, was partially included in their outer retinal layer assignment.

Light and Dark Adaptation in Normal Animals

In normal animals, the outer retina showed significantly higher MEMRI activity in dark compared to light adaptation, consistent with our previous results.⁸ This finding is also consistent with that of Berkowitz et al.,⁹ despite the differences in layer assignments of the outer MEMRI bands as discussed above. Photoreceptors in the outer retina hyperpolarize in the light and depolarize in the dark. The latter causes a net influx of positive ions (i.e., dark current),¹⁷ which triggers calcium (and thus Mn^{2+}) influx into the intracellular space, resulting in MEMRI signal enhancement in the dark relative to the light. This is consistent with the notion that the outer retina in the dark has higher oxygen consumption^{18–20} and higher glucose consumption¹⁹ compared to light adaptation.

The inner retina showed significantly lower MEMRI activity in dark compared to light adaptation, consistent with our previous finding.⁸ However, our results differed from Berkowitz's findings⁹ of no difference in MEMRI activity between light and dark adaptation in the inner retina. Some oxygen-electrode studies reported no difference in the inner retinal oxygen consumption between light and dark.^{18,20,21} Conversely, human retinal blood flow, which is tightly coupled to increased neural activity in the retinal vessels, has been reported to increase (~37%) from dark to light.²² A fluorescent microsphere study in rats also showed that retinal blood flow was higher in light than in dark²³ where the experimental conditions were essentially identical to those in the current MEMRI study except that the animals were under anesthesia. Our finding herein supports the notion that the inner retina has higher calcium activity in light relative to dark adaptation, suggesting that higher calcium and/or metabolic activity is

TABLE. Group-Average Physiological Parameters in Control ($n = 7$), 14-Day STZ ($n = 8$), and 30-Day STZ ($n = 5$) Rats (Mean $\pm$ SD)

	Weight, g	Blood Glucose, mg/dL	Respiration, Breaths/min	End-Tidal CO ₂ , %	Heart Rate, Beats/min	O ₂ Saturation, %
Control	335 $\pm$ 13	127 $\pm$ 1.4	61 $\pm$ 0.6	3.1 $\pm$ 0.05	395 $\pm$ 10	93.7 $\pm$ 0.4
14-day STZ	321 $\pm$ 4*	569 $\pm$ 43*	60 $\pm$ 0.3	3.1 $\pm$ 0.10	390 $\pm$ 9.4	96.1 $\pm$ 0.8*
30-day STZ	264 $\pm$ 13*†	618 $\pm$ 56*	61 $\pm$ 0.7	3.1 $\pm$ 0.05	307 $\pm$ 14*†	93.6 $\pm$ 0.8†

Note that the end-tidal CO₂ is underestimated for all groups due to the long sampling tube.

* $P < 0.05$ compared to controls.

† $P < 0.05$ between 14- and 30-day STZ animals.

needed in the inner retina to support conducting neuronal signals to the brain. The choroid showed no significant differences in MEMRI activity between light and dark adaption. We do not expect the choroidal vessels to differ between light and dark conditions.

Light and Dark Adaptation in Diabetic Animals

In the Outer Retina. Manganese-enhanced MRI activity decreased with hyperglycemia duration in dark adaptation relative to light. This finding likely indicates that the high calcium activity in dark decreased in the outer retina in chronic hyperglycemia. Ion homeostasis in the outer retina is necessary to maintain the dark current, synaptic activity, action potentials, and neurotransmitter release and recycling, among other normal cellular functions. Berkowitz et al.^{9,11} also reported the outer retina to have lower MEMRI activity in dark-adapted diabetic rats and mice, although their outer MEMRI layer assignments differed from ours as discussed above. These results suggest that dysfunction occurs only in high energy-demanding conditions under dark adaptation and not less energy-demanding conditions under light condition.

In the Inner Retina. The percent signal changes (light minus dark) were not statistically different with disease duration. However, there was not a significant difference between light and dark only in the 14 days post-STZ group. Inner retinal function assessed by ERG in animal models of DR indicates disruptions in the inner retina while the outer retina remains normal.^{24,25} The oscillatory potentials of the ERG, originating from the amacrine cells of the inner plexiform layer,²⁶ have been suggested to be sensitive to early DR changes, while the a and b waves remain normal.²⁵ Possible explanations for this apparent discrepancy between our MEMRI data and ERG data could be that the two methods measure different aspects of neuronal function or that MEMRI in the inner retina is less sensitive than ERG.

In the Choroid. The percent change (light minus dark) was positive in control, inverted to negative at 14 days post STZ, and reverted back to positive at 30 days post STZ. These data suggest that the choroid is also affected. There were large variations of percent differences in the choroid signals among the three animal groups, suggesting that hyperglycemia could alter choroid signals, similar to our previous blood flow findings.²⁷ This finding also suggests that the choroid signal should not be used for normalization for comparison with diabetic animals. The choroid is prone to vascular damage in DR and is reported to have decreased oxygen,²⁸ so it is possible that manganese accumulation in the retinal pigmented epithelium and endothelial cells outlining the choroidal vasculature is perturbed in chronic hyperglycemia. However, it is unclear why the choroid showed a biphasic pattern. One possibility is that STZ has direct toxic effects, in addition to those caused by hyperglycemia,^{29,30} which affect the choroid and are still present 14 days post STZ but recover by 30 days. Further experiments are needed to investigate this and other possibilities.

Another major finding is that MEMRI detects changes as early as 14 days after STZ injection, earlier than shown in previous reports. Previous MEMRI studies, which measured only the normalized intensity in dark adaptation, reported changes in the retina after 3 months of STZ-induced diabetes in rats.¹¹ Another study using mice found significant changes after 1.5 months but not at 0.9 months.⁹ Such discrepancy could be due to species differences, different severity of DR, or the use of only dark-adapted conditions. We focused on the effects of diabetes on the difference between light and dark in the same animal, which reduced the variability between subjects.

Other MRI Methods Applied to DR

Other MRI methods have also been used to study DR. Muir et al.²⁷ reported that both retinal and choroid blood flow decrease with progression of diabetes, and notably they detected choroidal blood flow to be reduced first. This is not unexpected because choroidal angiopathy and capillary dropout are associated with progression of hyperglycemia.^{31,32} It is unknown how or if choroidal dysfunction is related to outer retinal abnormalities, although there is some evidence that photoreceptor damage in severe DR is related to choroidopathy.³³ Berkowitz et al.³⁴ detected diffusion changes in the retina of STZ animals and found that retinal thickness increased after STZ injection compared to age-matched controls.

Limitations of the Study

The sample sizes were relatively small. However, this study is a demonstration of feasibility, and these sample sizes are generally sufficient to achieve statistical significance. A previous MEMRI study from our group,⁸ as well as many similar MEMRI studies by Berkowitz et al.,^{7,9,11} used similar sample sizes. In addition, we also have changes at two time points, supporting our main conclusion. Another concern is that changes in peak values might not reliably reflect the peak shapes. We use peak values as a representative measurement since we have previously validated that each peak corresponds approximately to the center of each region (inner retina, outer retina, and choroid). Other values, such as the average or area of each layer, could be explored, although previous studies found using the values from peaks or comparable regions to be reliable.^{7,8} We have previously explored the analysis of decomposing peak height and width using multiple model functions but found them to be unreliable because the baseline (signal outside the retina) was not flat and there are too many unknowns in the model to be reliably extracted from the limited number of data points across the retinal depth (usually ~ 12 or 13).

Another possible confounder of these results would be if hyperglycemia affected the vitreous MEMRI signal due to Mn²⁺ leakage into the vitreous or altered vitreous composition (and

thus T_1 value). If this were the case, both eyes in the same animals would likely be affected to the same extent. Thus, the paired percent MEMRI changes (Fig. 3) between light and dark eyes of the same animals should be minimally affected even if there are some disease-induced perturbations of the vitreous. Our data did not show dramatic changes in the vitreous MEMRI signals, supporting the notion that disease-induced perturbation of the vitreous MEMRI signal is small. There were also differences between groups in physiological parameters under anesthesia that could potentially affect the MEMRI results, although given that the animal was awake for most of the Mn^{2+} uptake time, this likely had minimal effects. Future studies could include larger sample sizes and additional time points to achieve sufficient power to regress these physiological parameters from MEMRI signal changes.

Conclusions

This study used high-resolution MEMRI to image layer-specific, calcium-dependent functional activity in the inner retina, outer retina, and choroid during light and dark adaptation in diabetic animals at multiple time points. The findings indicate that calcium activity is reduced in the outer retina in chronic hyperglycemia. Manganese-enhanced MRI detects changes as early as 14 days after onset of hyperglycemia, to our knowledge earlier than shown in previous reports. Manganese-enhanced MRI can be used to investigate other retinal diseases such as glaucoma and retinal degeneration in animal models.

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References

- Aiello LP, Gardner TW, King GL, et al. Diabetic retinopathy. *Diabetes Care*. 1998;21:143–156.
- Phipps JA, Fletcher EL, Vingrys AJ. Paired-flash identification of rod and cone dysfunction in the diabetic rat. *Invest Ophthalmol Vis Sci*. 2004;45:4592–4600.
- Ng YK, Zeng XX, Ling EA. Expression of glutamate receptors and calcium-binding proteins in the retina of streptozotocin-induced diabetic rats. *Brain Res*. 2004;1018:66–72.
- Matsushita K, Fukumoto M, Kobayashi T, et al. Diabetes-induced inhibition of voltage-dependent calcium channels in the retinal microvasculature: role of spermine. *Invest Ophthalmol Vis Sci*. 2010;51:5979–5990.
- Lin YJ, Koretsky AP. Manganese ion enhances T_1 -weighted MRI during brain activation: an approach to direct imaging of brain function. *Magn Reson Med*. 1997;38:378–398.
- Duong TQ, Silva AC, Lee SP, Kim SG. Functional MRI of calcium-dependent synaptic activity: cross correlation with CBF and BOLD measurements. *Magn Reson Med*. 2000;43:383–392.
- Berkowitz BA, Roberts R, Goebel DJ, Luan H. Noninvasive and simultaneous imaging of layer-specific retinal functional adaptation by manganese-enhanced MRI. *Invest Ophthalmol Vis Sci*. 2006;47:2668–2674.
- De La Garza BH, Li G, Shih YY, Duong TQ. Layer-specific manganese-enhanced MRI of the retina in light and dark adaptation. *Invest Ophthalmol Vis Sci*. 2012;53:4352–4358.
- Berkowitz BA, Gadianu M, Bissig D, Kern TS, Roberts R. Retinal ion regulation in a mouse model of diabetic retinopathy: natural history and the effect of Cu/Zn superoxide dismutase overexpression. *Invest Ophthalmol Vis Sci*. 2009;50:2351–2358.
- Calkins DJ, Horner PJ, Roberts R, Gadianu M, Berkowitz BA. Manganese-enhanced MRI of the DBA/2J mouse model of hereditary glaucoma. *Invest Ophthalmol Vis Sci*. 2008;49:5083–5088.
- Berkowitz BA, Roberts R, Stemmler A, Luan H, Gadianu M. Impaired apparent ion demand in experimental diabetic retinopathy: correction by lipoid acid. *Invest Ophthalmol Vis Sci*. 2007;48:4753–4758.
- Cheng H, Nair G, Walker TA, et al. Structural and functional MRI reveals multiple retinal layers. *Proc Natl Acad Sci U S A*. 2006;103:17525–17530.
- Li Y, Cheng H, Duong TQ. Blood-flow magnetic resonance imaging of the retina. *Neuroimage*. 2008;39:1744–1751.
- Li Y, Cheng H, Shen Q, et al. Blood-flow magnetic resonance imaging of retinal degeneration. *Invest Ophthalmol Vis Sci*. 2009;50:1824–1830.
- Muir ER, Duong TQ. MRI of retinal and choroidal blood flow with laminar resolution. *NMR Biomed*. 2011;24:216–223.
- Muir ER, Duong TQ. Layer-specific functional and anatomical MRI of the retina with passband balanced SSFP. *Magn Reson Med*. 2011;66:1416–1421.
- Hagins WA, Penn RD, Yoshikami S. Dark current and photocurrent in retinal rods. *Biophys J*. 1970;10:380–412.
- Linsenmeier RA. Effects of light and darkness on oxygen distribution and consumption in the cat retina. *J Gen Physiol*. 1986;88:521–542.
- Wang L, Tornquist P, Bill A. Glucose metabolism in pig outer retina in light and darkness. *Acta Physiol Scand*. 1997;160:75–81.
- Cringle SJ, Yu DY, Yu PK, Su EN. Intraretinal oxygen consumption in the rat in vivo. *Invest Ophthalmol Vis Sci*. 2002;43:1922–1927.
- Braun RD, Linsenmeier RA, Goldstick TK. Oxygen consumption in the inner and outer retina of the cat. *Invest Ophthalmol Vis Sci*. 1995;36:542–554.
- Riva CE, Logean E, Petrig BL, Falsini B. Effect of dark adaptation on retinal blood flow [in French]. *Klin Monbl Augenheilkd*. 2000;216:309–310.
- Shih YY, Wang L, De La Garza BH, et al. Quantitative retinal and choroidal blood flow during light, dark adaptation and flicker light stimulation in rats using fluorescent microspheres. *Curr Eye Res*. 2013;38:292–298.
- Sakai H, Tani Y, Shirasawa E, Shirao Y, Kawasaki K. Development of electoretinographic alteration in streptozotocin-induced diabetes in rats. *Ophthalmic Res*. 1995;27:57–63.
- Kohzaki K, Vingrys AJ, Bui BV. Early inner retinal dysfunction in streptozotocin-induced diabetic rats. *Invest Ophthalmol Vis Sci*. 2008;49:3595–3604.
- Shirao Y, Kawasaki K. Electrical responses from diabetic retina. *Prog Retin Eye Res*. 1999;17:59–67.
- 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.

28. Padnick-Silver L, Linsenmeier RA. Effect of acute hyperglycemia on oxygen and oxidative metabolism in the intact cat retina. *Invest Ophthalmol Vis Sci.* 2003;44:745-750.
29. Kume E, Fujimura H, Matsuki N, et al. Hepatic changes in the acute phase of streptozotocin (SZ)-induced diabetes in mice. *Exp Toxicol Pathol.* 2004;55:467-480.
30. Imaeda A, Kaneko T, Aoki T, Kondo Y, Nagase H. DNA damage and the effect of antioxidants in streptozotocin-treated mice. *Food Chem Toxic.* 2002;40:979-987.
31. Adhi M, Brewer E, Waheed NK, Duker JS. Analysis of morphological features and vascular layers of choroid in diabetic retinopathy using spectral-domain optical coherence tomography. *JAMA Ophthalmol.* 2013;131:1267-1274.
32. McLeod DS, Luttly GA. High-resolution histologic analysis of the human choroidal vasculature. *Invest Ophthalmol Vis Sci.* 1994;35:3799-3811.
33. Johnson MA, Luttly GA, McLeod DS, et al. Ocular structure and function in an aged monkey with spontaneous diabetes mellitus. *Exp Eye Res.* 2005;80:37-42.
34. Berkowitz BA, Bissig D, Ye Y, Valsadia P, Kern TS, Roberts R. Evidence for diffuse central retinal edema in vivo in diabetic male Sprague Dawley rats. *PLoS One.* 2012;7:e29619.