

## Retinal and Choroidal Blood-Flow MRI and Visual Function in Diabetic Retinopathy in Mice

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**INTRODUCTION:** Diabetic retinopathy (DR) is the leading cause of new blindness in working age adults between 20 and 74 years of age (1). Vascular dysfunction in the retina is the most prominent feature of DR. Changes include thickening of vascular layers, capillary non-perfusion, and vascular leakage. The retina is nourished by two separate vasculatures - the retinal vessels in the inner retina next to the vitreous and the choroidal vessels outside the neural retina - which are separated by an avascular layer (2). Optical imaging techniques, widely used to study the retina, have difficulty resolving retinal blood flow (RBF) and choroidal BF (ChBF). In contrast, MRI was recently used to quantify RBF and ChBF in mice at 42x42x400  $\mu\text{m}$  (3). In this study, BF MRI was used to measure layer-specific RBF and ChBF in a mouse model of DR. Visual function in the same mice was assessed using the optokinetic head-tracking response to rotating gratings (4).

**METHODS:** Experiments used male mice heterozygous for the *Ins2Akita* (Akita) mutation, which leads to hyperglycemia by 1 month of age, on a C57BL/6J background; wild type, normoglycemic littermates were used for controls (n=9 wild type, n=10 Akita). Akita mice were confirmed to be hyperglycemic (blood glucose >250 mg/dl) at 4.5-6 weeks of age. Optokinetic responses of awake animals were determined, as in (4), at 7-8 months of age. Visual acuity was tested by varying the spatial frequency (cycles/degree (cpd)) of the rotating gratings. Contrast sensitivity was tested by varying the contrast (%) of the gratings and was assessed at two spatial frequencies, 0.064 and 0.103 cpd (4).

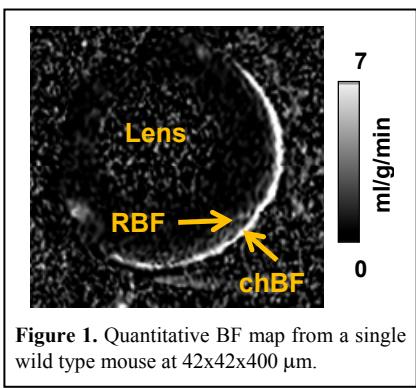
MRI was done on the same mice within 2-7 weeks of the optokinetic tests using a 7T/30cm Bruker scanner with a 150 G/cm gradient. Mice were anesthetized with 1.1-1.4% isoflurane and kept under 30% O<sub>2</sub>. Respiration rate, heart rate, O<sub>2</sub> saturation, and temperature were maintained within normal ranges. A small surface eye coil with active decoupling (diameter=6 mm) was used for imaging. BF was measured using continuous arterial spin labeling, with a circular coil (diameter=8 mm) for labeling placed at the heart (3). Images were acquired in coronal orientation with gradient-echo EPI with FOV=6x6 mm, matrix=144x144 zero-filled to 256x256, 2 segments, a single 0.4 mm slice, TR=3.0s per segment, TE=12.6ms, labeling duration=2.6s, and post labeling delay=350ms. BF images in ml/g/min were calculated as in (3). Profile analysis was used to average BF along the retinal length (2). Statistical analysis used unpaired t-tests and Pearson's correlation.

**RESULTS:** Physiological parameters are summarized in **Table 1**. Akita mice had increased blood glucose and decreased weight. Lower isoflurane levels were used on Akita mice (~1.1% compared to ~1.4% in wild type) to maintain physiology close to wild type mice, but respiration rate was still lower in Akita mice. A representative BF image from a wild type mouse is shown in **Figure 1**. RBF was significantly lower in Akita mice (p=0.018), and ChBF tended to be lower (p=0.054) (**Figure 2**). Visual acuity of Akita mice was significantly worse, indicated by a lower spatial frequency threshold (p=0.002). Contrast sensitivity at 0.064 and 0.103 cpd were significantly worse in Akita mice, indicated by larger contrast thresholds (p=0.023 and 0.009, respectively).

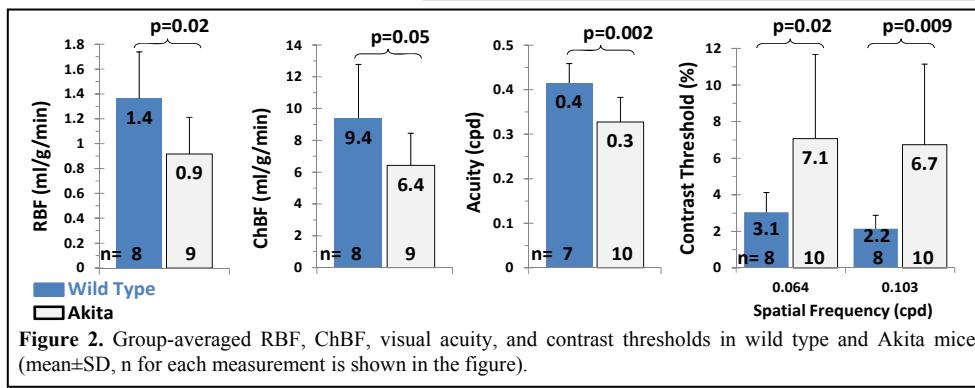
**DISCUSSION:** BF MRI offers quantitative measures of retinal physiology with a large field of view. In human and rodent models of DR, RBF and ChBF are reported to increase (5,6), decrease (7), or be unchanged (8). Measured BF changes likely depend on many factors such as stage of disease, measured hemodynamic parameters, and differences among methods and animal models. Higher RBF and ChBF correlated with better vision in wild type mice. The Akita mice had both BF and vision deficits which were only weakly correlated to each other. DR is often considered a vascular disease, but neuronal damage may occur in the retina independent of vascular abnormality (9), so it could be that the vision deficit in Akita mice is mainly due to direct neuronal damage, independent of vascular and BF deficits.

BF (5) and visual (9) deficits can precede clinical vascular signs of DR and may be potential early markers of disease. This novel BF MRI approach could enable early detection, longitudinal disease staging, and monitoring of therapeutic intervention in the retina. Future studies will involve measurements at earlier time points of DR as well as functional MRI of evoked responses.

**Reference:** 1) Aiello et al, Diabetes Care 1998, 21:143. 2) Cheng et al, PNAS 2006, 103:17525. 3) Muir et al, NMR Biomed 2010, in press. 4) Prusky et al, IOVS 2004, 45:461. 5) Grunwald et al, Br J Ophthalmol 1996, 80:327. 6) Tilton et al, Diabetes 1989, 38:1258. 7) Wright et al, Exp Eye Res 2008, 86:528. 8) Lorenzi et al, Diabetologia 2008, 51:361. 9) Antonetti et al, Diabetes 2006, 55:2401. Supported in part by R01 EY014211, R01 EY018855, CTSA-009A, UL1RR025767, VA MERIT.



**Figure 1.** Quantitative BF map from a single wild type mouse at 42x42x400  $\mu\text{m}$ .



**Figure 2.** Group-averaged RBF, ChBF, visual acuity, and contrast thresholds in wild type and Akita mice (mean $\pm$ SD, n for each measurement is shown in the figure).