Lamina-specific anatomical and functional imaging of normal and diabetic rat retina

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Introduction Diabetic retinopathy (DR) is a leading cause of blindness. In its late stages DR is primarily a vascular disease, but neural dysfunction may contribute at earlier stages [1]. Current diagnosis of DR relies on confirmation of vascular extravasation, thrombosis, or hemorrhage, and no clinically significant method has been devised to identify DR patients before established vasculopathy.

Streptozotocin (STZ) induced diabetes is an established rat model of DR. While nine to twelve months of hyperglycemia are usually required to observe typical vasculopathic DR derangements, abnormal tissue oxygenation in the retina has been reported as early as 3.5 months after STZ injection using phosphorescence [2] and the ΔpO2 technique [3]. Histologic changes in retinal thickness occur within 7.5 months [4]. Although powerful, existing techniques have significant limitations. In addition to the lack of laminar specificity the phosphorescence technique cannot distinguish between oxygen arriving from retinal and choroidal vasculature and is applicable only for large vessels. The ΔpO2 technique measures vitreous humor oxygenation *near*, but not *in*, the retina and layer-specific attribution is impossible.

Non-invasive MRI has the potential to provide the unique advantages of anatomical, physiological (blood flow and tissue oxygenation) and functional information in a single setting without regard to tissue depth or limitation of light path. However, until recently, MRI has demonstrated generally inferior spatial and/or temporal resolution relative to many optical techniques. Our lab has recently developed MRI techniques to image the retina at very high spatial resolution with layer specificity. Using anatomical MRI we have resolved three retinal tissue "layers" in cat and rat retinas [3].The addition of Gd-DTPA contrast-enhanced MRI further resolved two vascular layers. BOLD fMRI of the retina associated with visual stimuli and physiological challenges in the cat [5] and rat retina [6] was also recently reported.

The goal of this study was to apply these lamina-specific MRI and fMRI techniques to evaluate early signs of DR in rat retina. We hypothesized that MRI can be used to resolve lamina-specific structural and functional changes associated with diabetic retinopathy. As a first step, we quantified the thickness of different retinal layers and investigated the BOLD fMRI responses to hyperoxia and hypercapnia in diabetic (DM) and non-diabetic control (Con) rats.

Methods Age-matched, gender-matched rats were injected i.v. with streptozotocin (STZ, 100 mg/kg., n = 6) or vehicle (n = 8). The diagnosis of diabetes mellitus was based upon two consecutive random blood glucose determinations greater than 250 mg/dl (14 mM) using tail vein blood and a hand-held blood glucose analyzer (Freestyle, Abott Laboratories). Rats developed hyperglycemia within 2-3 days of STZ injection, and were monitored 3 times per week for weight, blood glucose, glucosuria, , and urine ketones Imaging was performed at ~3.5 months after STZ injection.

MRI was performed on a 4.7T/40cm scanner using a single-loop coil (0.8 cm) for the left eye for high sensitivity and reduced FOV. Anatomical T_1-weighted imaging was acquired with FLASH, TR=100ms, TE=4ms, slice thickness=0.8mm, FOV=8x8mm, matrix=128x128 (62x62μm), and NT=16. BOLD fMRI was acquired using two-shot spin-echo EPI with diffusion weighting to suppress the vitreous signal, TR=1s, TE=20ms, slice thickness=1 mm, FOV=1.1x1.1mm, matrix=128x128. During baseline the animals breathed air for 100s followed by 100% O_2 or 5% CO_2 (21% O_2) gas for 220s. Thickness of multiple layers was quantified as described elsewhere [5]. Cross correlation analysis was used to derive BOLD % changes maps, BOLD percent changes and number of activated pixels for multiple layers.

Results and Discussion Figure 1 shows the anatomical MRI, hyperoxia- and hypercapnia-induced BOLD percent-change maps and time courses from a Con (top row) and a DM (bottom row) animal. The anatomical image bisects the optic nerve (green arrow). The inner strip closest to the vitreous (red arrow) and the outer strip appear bright (thicker red arrow) appeared bright, while the middle strip appeared dark. Anatomic assignments validated previously in normal animals [6] are: *i)* The inner strip was as the ganglion and bipolar cell layer and the embedded retinal vasculature. *ii)* The middle strip as the photoreceptor layer. *iii)* The outer strip as the choroidal vascular layer. **Table 1** summarizes the group-averaged laminar thickness of DM and Con retinas. The outer layer of DM was significantly thicker than Con, whereas the inner and middle layers were similar. These observations suggest that choroidal vascular layer may be damaged, consistent with diabetic retinopathy being primarily a vascular disease. Our results do not contradict a previous study reporting alterations in neural retina thickness by 7.5 months post STZ injection [4].

Table 1 Retinal thickness (μm)

	Control	Diabetic
Inner layer	160 ± 24	170 ± 23
Middle layer	84 ± 16	72 ± 12
Outer layer	93 ± 16 [a]	132 ± 34 [a]
TOTAL	293 ± 33 [b]	353 ± 30 [b]

[a,b] indicate statistical difference between control and diabetic

BOLD fMRI signals in response to hyperoxia and hypercapnia were detected (Fig. 1). In hyperoxia, BOLD change in the outer strip was significantly larger than that in the inner strip. This is because hyperoxia induced vasoconstriction of the *retinal* blood vessels [6]. Thus, despite increased O_2 saturation, BOLD increases remain small. In contrast, hyperoxia has little effect on choroidal blood flow, and induces large BOLD signal changes. In hypercapnia, BOLD change in the outer strip was significantly smaller than that in the inner strip. This is because hypercapnia has little effect on choroidal blood flow [2], but potently induces vasodilation of inner retinal vessels [6]. Thus CO_2 induces only minimal increases in BOLD signal in the outer strip, but induces large increases in BOLD signal in the inner retinal layer.

While the percent changes of *activated pixels* were not statistically different between DM and Con, the numbers of activated pixels in all layers were diminished in diabetic. The group-average histogram plots of number of pixels versus BOLD percent changes are shown in **Figure 2**. For O_2 challenge, the area under the curve for diabetes was 42% smaller than controls (P<0.01). For CO_2 challenge, the area under the curve for diabetes was 33% smaller than controls (P<0.01). These results suggest that neurovascular coupling is perturbed in an early phase of DR. BOLD fMRI thus has the potential to be used to stage the progression of diabetic retinopathy.

Conclusion This study demonstrates the potential of MRI to provide powerful insights into how *retinal* and *choroidal* blood flow and oxygenation are regulated. The MRI data show how diabetic retinopathy affects the two vasculatures and the neural tissues they subserve, providing a means to better understand the disease processes *in vivo*. MRI thus has the potential to be used for early detection, longitudinal monitoring of diabetic retinopathy and other retinal diseases. Future studies will involve imaging at earlier time points and correlating MRI findings with electroretinogram findings and histology.

References: [1] Aiello et al, Diab Care 1998. [2] Shonat et al, Appl Optic 1992. [3] Berkowitz et al, IOVS 1998. [4] Barber et al, J Clin Ophth 1998. [5] Shen et al, JMRI 2005 in press; Cheng et al ISMRM 2006, submitted. [6] Sharma et al, in Alder's Physiology of the Eye 1992. Funded by Whitaker Foundation & NEI/NIH.

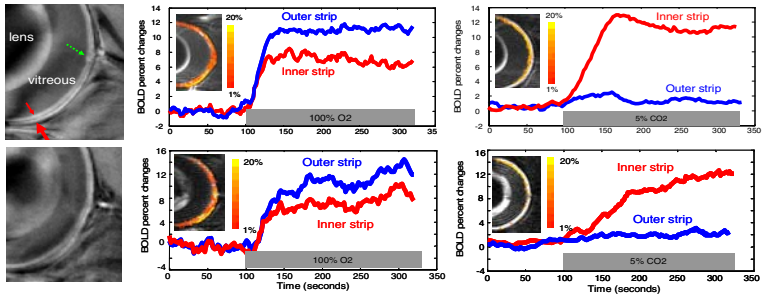

Fig 1. MRI of control (top row) and diabetic (bottom row) rats.

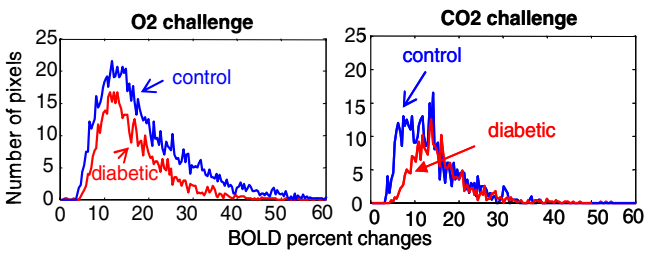

Fig 2. Histograms of BOLD % changes for control and diabetic rats.