High Angular Resolution Diffusion Microscopy (HARDM) detects Retinal Disruption in mice with Diabetic Retinopathy

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Introduction: Diabetic retinopathy is the most common eye disease affecting diabetics and a leading cause of blindness. However, it cannot be diagnosed in its early stages. This study addresses this issue by using High Angular Resolution Diffusion Microscopy (HARDM) as a novel and non-invasive tool to detect this disease at an early stage in diabetic mice. Our key findings in this study are as follows: (1) High resolution anatomical images successfully display multiple retinal layers consistent with established anatomy. (2) HARDM of control eyes shows restricted water diffusion in the retina, reflecting a very organized, layered structure. (3) Compared to normal control eyes, the integrity of these layers appears to be compromised in certain areas in diabetic mice.

Methods: All animal protocols were in accordance to University of Florida Institutional Animal Care and Use Committee (IACUC) guidelines. C57 black mice were imaged *in vitro*, using a 17.6T, 750 MHz wide bore (89mm) Bruker NMR spectrometer. The eyes were fixed in 3% w/v formalin solution and suspended in Fluorinert™ for imaging. Diabetic eyes came from mice with Type I diabetes induced by a single dose of alloxan (62.5 mg/kg i.v.). The mice were not maintained on supplemental insulin (blood glucose >200mg/dl). DT images were obtained using a 21 direction high angular resolution diffusion imaging (HARDI) sequence. Typical parameters used were: TR=2500 ms, TE=15 ms, Δ=10 ms, δ=1.5 ms, NA=6, matrix size=128 x 128, FOV= 0.4 cm x 0.4 cm, spatial resolution = 31 µm. All DTI processing was done using fanDTasia software developed at UF [1]. Statistical comparisons were made using an unpaired Students t-test, with significance established at the $p < 0.05$ level.

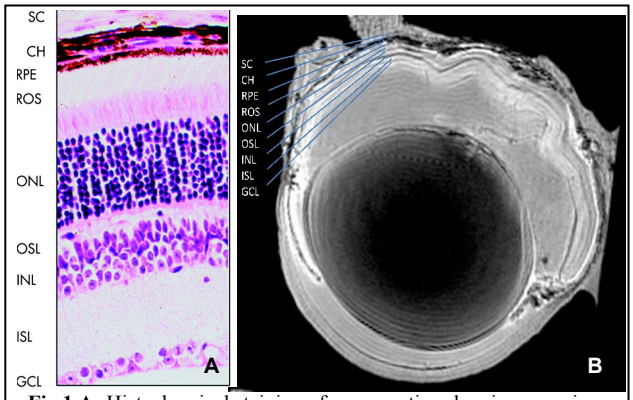

Fig 1.A: Histochemical staining of mouse retina showing organizational structure. **Fig 1.B:** 3D high resolution image of an *in vitro* eye with clearly differentiated retinal layers

Results: Histochemical studies of the mouse retina show an organized structure with several distinct layers as is seen in Fig.1.A. Our high resolution images (Fig. 1.B) were able to successfully identify multiple layers in the retina due to the high in-plane spatial resolution of 19 µm. Fig. 2.A shows the 3D reconstruction of fiber tracts of a control eye using a threshold of fractional anisotropy (FA) of 0.4. The retinal layers are marked by black arrows in Fig.2B. The direction of diffusion is observed to be across the retinal layers, that is, perpendicular to the layers rather than parallel to them. This layer is highly structured and appears to correspond to the photoreceptor segments of the rods and cones.

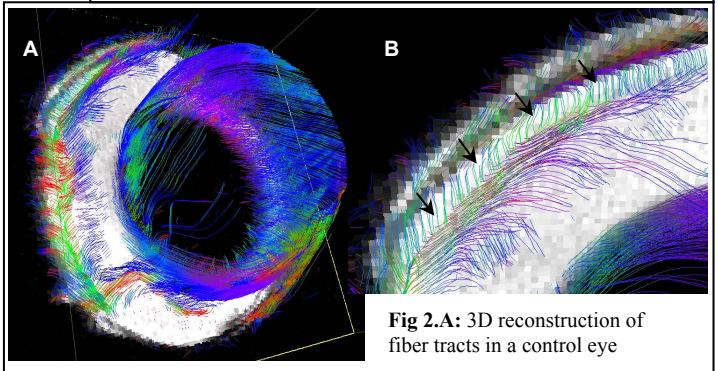

Fig 2.A: 3D reconstruction of fiber tracts in a control eye

Fig 2.B: Close up shows the photoreceptor layer has an organized structure.

Altered Retinal Diffusion in Diabetic samples: Fig. 3 shows a comparison between undamaged (A) and damaged (B) sections in the same diabetic eye. Fig. 3A is a representative of a control retina since it is undamaged and is highly structured. In Fig. 3B, alterations in retinal diffusion are clearly visible as the organization of the principal eigenvectors is no longer coherent following induction of diabetes, and the degree of fractional anisotropy is likewise decreased. In control eyes, the fractional anisotropy (FA) in the retina is observed to be significantly higher than that of the retinas from diabetic animals (Fig.4).

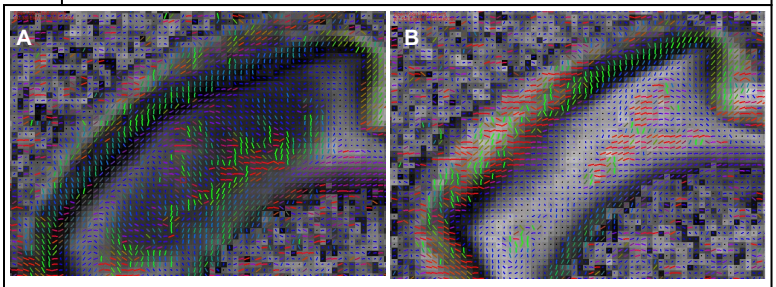

Fig.3: Illustration of focal nature of retinal disruption. Comparison between undamaged (A) and damaged (B) sections in the same diabetic eye.

Conclusions: Pathogenic retinas exhibit increased permeability, most likely due to the breakdown of the blood retinal barrier. Alterations in the eye following damage due to diabetic retinopathy can be detected by DTI. In control eyes, the organized structure of the retina is shown by the coherent nature of the principal eigenvector of diffusion. The difference in FA between retinas from diabetics and controls is also significant, and may reflect alterations in the extracellular volumes, perhaps the result of tissue edema, or changes in vascular permeability. These techniques may aid future studies by providing early detection of pathogenic conditions in the retina.

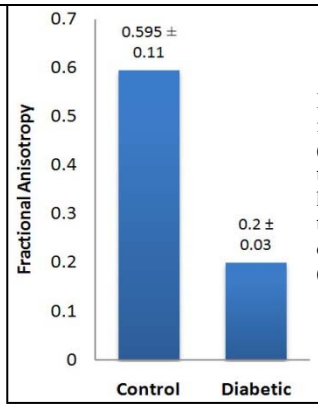

Fig 4: The mean FA in control retinas (n=4) was calculated to be significantly higher ($p<0.05$) than the mean FA of diabetic retinas (n=2).

References: [1] A. Barmpoutis and B. C. Vemuri, "A unified framework for estimating diffusion tensors of any order with symmetric positive-definite constraints", In Proceedings of ISBI10: IEEE International Symposium on Biomedical Imaging, 14-17 April 2010, Page(s): 1385-1388