Cross validation of retinal and choroidal blood flow using arterial spin labeling MRI and fluorescent microsphere

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INTRODUCTION The retina is nourished by both the central retinal artery and the choroidal circulation in most mammals to maintain proper function and the metabolic needs of the retina. Quantitative measurement of retinal and choroidal blood flow (BF) provides a means to early detect retinal diseases [1]. MRI provides non-invasive, quantitative measurement of the BF and its application to the retina to resolve retinal and choroid BF was recently reported in mice [2]. Unfortunately, there are no comparable imaging methods to cross validate this novel quantitative BF in the retina and choroid *in vivo*. Conventional optical imaging methods are non-quantitative, depth ambiguous (which limited its investigation to either the optic nerve head or the fovea), and is constrained by media opacity. The non-invasive arterial spin labeling (ASL) BF MRI technique to study the retina necessitates vigorous validation. The goal of this study was to use the established microsphere technique to quantitatively validate MRI measurement of retinal and choroidal BF in rats. We used a modified microsphere technique [3] to account for different vessel sizes of the retinal and choroidal circulation to improve BF measurement accuracy and ASL MRI at 11.7 T to measure ocular BF with laminar specificity in albino rats for the first time.

METHODS Adult male Sprague Dawley rats (n = 24, 250–300 g) were anesthetized with 1-1.2% isoflurane, ventilated, and paralyzed. The respiratory rate was set between 57-60 stroke/min. End-tidal CO_2 and arterial pCO_2 were measured and maintained within normal physiological ranges by adjusting the tidal volume. A regulated heated pad was used to maintain body temperature at 37^0 C. MRI BF measurement was performed on an 11.7 T/16 cm magnet and a 74 G/cm gradient insert (Bruker, Billerica, MA). A custom-made small circular surface coil (ID~7 mm) was placed on the left eye. Magnetic field homogeneity was optimized using FASTMAP shimming with first order shims on an isotropic voxel of 7x7x7 mm, encompassing the entire eye. Quantitative BF MRI was acquired using continuous ASL technique in the eye with 4-shot, gradient echo EPI acquisition, spectral width = 208 kHz, TR = 3000 ms, TE = 12.7 ms, labeling duration = 2.96 s, FOV = 9x9 mm, slice thickness = 0.6 mm, acquisition matrix = 228x228, yielding an in-plane resolution = 40×40 µm. Microsphere BF measurement employed a mixture of 2.5 million 8 µm yellow-green (absorption/emission: 505/515 nm) and 0.5 million 10 µm red (absorption/emission: 580/605 nm) fluorescent microspheres (FluoSpheres, Molecular Probes, Eugene, OR). The microspheres were suspended within approximately 350 to 550 µl solution of 0.15 M NaCl and 0.05% Tween 20. The suspension was stored in a 37˚C water bath and sonicated for 10-20 seconds immediately before injection. A 2-cm vertical incision was made along the midline of the abdomen below the xiphoid. The incision was kept open using an ocular speculum to allow the apex of the heart to be visualized through the diaphragm. Heparin (0.5-1 mg/kg, i.v.) was administered to prevent blood clotting. A 27-gauge needle, connected to 15-cm PE50 tubing, was inserted into the left ventricle through the diaphragm. Slight pressure toward the heart was applied to hold the needle position. A mixture of dual-size microspheres was then injected into the left ventricle. The injection duration was 35-40 sec. Arterial blood sample was collected for one minute starting at the onset of microsphere injection. The animals were then euthanized by euthansol. Both eyes were enucleated. The anterior portion of the eye, ~2 mm behind the limbus, was removed. The entire retina was dissected from the remaining eyecup and flat-mounted on a glass slide. The choroid together with the underlying sclera was flat-mounted with four tension-relief cuts. The tissue was sealed with fluorescence mounting medium (Vectashield, Vector Laboratories, Burlingame, CA) and cover slips. Microspheres were counted for the retina and the choroid under a fluorescent microscope. Microspheres in the arterial reference blood samples were also counted after the blood was hypo-osmotic haemolyzed and applied to a hemocytometer counting chamber. The BF per tissue was calculated using the following equation,

$$Blood\ flow\ per\ tissue\ (\mu l/min) = \frac{number\ of\ microspheres\ per\ tissue}{number\ of\ microspheres\ in\ the\ reference\ blood\ sample} \times reference\ sample\ flow\ (\mu l/min)$$

Statistical analysis was performed by paired t-tests. A probability value of $P < 0.05$ was used.

RESULT & DISCUSSION This study measured retinal and choroidal BF in quantitative units using MRI and fluorescent microsphere. Arterial pCO_2, pH, and MABP values were 38.5±3.53 mmHg (mean±SD), 7.46±0.04, and 101.4±12.4 mmHg, respectively (n = 18). **Fig. 1** shows basal BF measurement by noninvasive high resolution MRI technique (40x40 µm in plane resolution). The averaged BF values in the retina and the choroid from the mid-sagittal section were 1.2±0.6 and 8.1±1.8 ml/g/min, respectively (n = 6), with the retinal:choroid BF ratio of 1:6.8. The BF values in the retina and the choroid were significantly different (P = 0.00008). **Fig. 2** shows representative fluorescent images of the retina and choroid. The green (8 µm) microspheres were counted in the retina and the red (10 µm) microspheres were counted in the choroid. The retinal and choroidal BF values were 9.1±2.8 and 73.4±45.3 µl/min per tissue, respectively (n = 18), with the retinal:choroid BF ratio of 1:8.1. The BF values in the retina and the choroid were significantly different (P = 0.0008). Taking the wet weight of the choroid-retinal pigment epithelium (20 – 25 mg) measured by Kadam et al. [4], the averaged choroidal BF values by fluorescent microsphere was 2.95 ml/g/min. This value was underestimated because the weight of retinal pigment epithelium was included and cannot be separated from choroid (choroid is a vascular structure and its wet weight is almost impossible to measure alone). Using the wet weight of the rat retina reported by Kadam et al., (25 – 35 mg), [4] Tilton et al. (5 – 10 mg) [5,6], Citirik et al. (15 – 23 mg) [7], and Winkler et al. (10 mg) [8], the retinal BF measured by microsphere in the present study ranged 0.23 – 1.64 ml/g/min. Despite the quantitative choroid BF warrants further elucidation, the quantitative MRI retinal BF values were in comparable range of that measured by microsphere technique under the same condition.

In conclusion, this study demonstrated that the dual size and dual-dose microspheres mixture can be used to measure the retinal and choroidal BF. The basal BF values measured by MRI were in good accordance with the microsphere techniques. Non-invasive MRI approach offers opportunities to investigate ocular BF regulation in normal and diseased subjects. The same approach can be used to validate MRI measurement of cerebral blood flow.

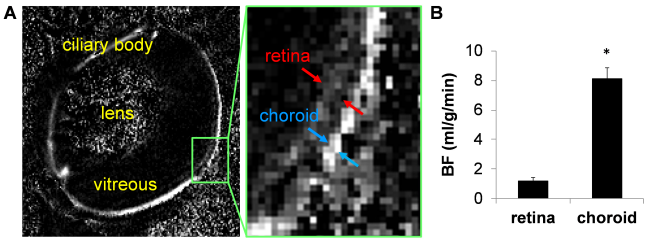

Fig 1. Quantitative basal retinal and choroidal BF measurement by high resolution CASL MRI. **(A)** A representative basal BF map. **(B)** Group-averaged BF values (n=6). *P < 0.05, error bars are S.E.M.

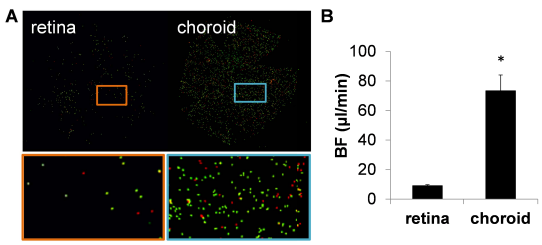

Fig 2. Quantitative basal retinal and choroidal BF measurement by fluorescent microsphere. **(A)** A representative fluorescent images of the retina and choroid . **(B)** Group-averaged BF values (n=18).

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