Diffusion MRI measurement of muscle fiber size and sarcolemma permeability in normal skeletal muscle growth

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Purpose: Myofiber diameter and membrane permeability are important biophysical factors in muscle development, and are often associated with a wide range of myopathies. Crucially lacking, however, are non-invasive techniques that can reliably evaluate changes in muscle tissue microstructure. Recently, a random permeable barrier model[1] (RPBM) has been proposed to quantify[2,3] time-dependent diffusion tensor eigenvalues[4] in order to infer cell size and membrane permeability. The proposed DTI-RPBM framework[1-3] has been validated using numerical simulations[1], pilot human studies[2,3], and previously reported *ex vivo* DTI data[4]. The aim of this work has been to directly validate the DTI-RPBM framework using *in vivo* DTI experiments with mice in comparison to histopathological measurements of muscle fiber size and water channel distribution on the muscle membrane (sarcolemma).

Target Audience: Tissue microstructure with diffusion; MSK researchers and clinicians.

Methods: Two groups of two wild type (C57/blk males) mice were used in this study. One cohort of two mice was between 4-5 weeks old. The second cohort of two mice was between 10-12 weeks of age. These two groups of mice with different developmental stages of the muscle were used to compare the DTI-RPBM parameters with histological measures. All MRI studies were performed using a 7T Bruker Scanner with a Paravision 5 Console and a volume transmit and receive coil. Anatomical imaging was done with a T2-weighted rapid acquisition with relaxation enhancement (RARE) sequence (TR = 2s, TE = 35ms, RES = 0.18 X 0.18 X 1.5 mm^3, 10 slices) and a T1-weighted 2D FLASH sequence (TR = 5.8ms, TE = 2.2ms, flip angle = 30) in order to identify muscle groups of the lower leg. A diffusion weighted (DW) stimulated-echo (STEAM) pulse sequence with echo planar imaging (EPI) readout was also used to acquire images with diffusion gradients in six non-collinear directions and one reference image without diffusion weighting. The DW-STEAM-EPI scans were conducted with TR = 6 s, TE = 27 ms, FOV = 2.20 x 2.20 x 1.20 cm, and image matrix = 64 x 64 x 8. In order to measure muscle fiber size and aquaporin function, the DW-STEAM-EPI sequence was run repeatedly with eight diffusion times t ranging between 10ms – 1000ms. The trace of the diffusion weighting (the b matrix) was held constant at about 1000 s/mm^2 by varying diffusion weighting gradient strength depending on the increase of diffusion time. Total scan time was about 45 min per mouse. The animal body temperature was maintained at 32±2 °C during the scan. Data analysis was performed with region of interest manually drawn over the lower hind limb muscle. The average of second and third eigenvalues at each diffusion time t was assumed as the measure of diffusion $D(t)$ perpendicular to the muscle fibers, to which the DTI-RPBM model was fitted. The DTI-RPBM model fitting provided estimates of surface-to-volume ratio S/V, membrane permeability κ, and unrestricted diffusion coefficient D_0. After the imaging session mice were perfused with 4% paraformaldehyde and were allowed to fully fix overnight in preparation of immunohistochemistry staining of collagen IV and aquaporin-4 (AQP4). This study was approved by our Institutional Animal Care and Use Committee.

Results and Discussion: Figure 1 shows example images of the lower hindlimb muscle in T2-weighted and fractional anisotropy (FA) color map. The FA color map shows that most of lower limb muscles have a uniform directionality. Figure 1C shows the eigenvalues from the muscle in Figure 1A and 1B, demonstrating strong dependence of diffusion eigenvalues transverse to myofibers on diffusion time, as in Ref. 4. Figure 1D shows an asymptotically linear dependence on $t^{-1/2}$, which represents[1] a "fingerprint" of the membranes in the diffusion measurement. The DTI-RPBM measures from 4 mice are summarized in Table 1. In overall, young mice had on average lower unrestricted diffusivity, high S/V ratio, and lower membrane permeability κ than old mice. Figure 2 shows immunohistochemistry staining of collagen IV and AQP4 of one mouse from each group. In both staining methods, it can be easily noted that the old mouse has larger muscle fibers (consistent with smaller S/V ratio) than the young mouse. In addition, it appears that the muscle fibers of the young mouse are completely enclosed by thick collagen layers with high expression of AQP4, whereas the muscle fibers of the old mouse show only relatively thin layer of collagen and much lower level of AQP4 expression. This observation suggests that the higher membrane permeability in the older mouse group can be due to the reduced amount of collagen IV surrounding the muscle fibers. Collagen IV is known to support skeletal muscle cells and its impairment has been linked to muscular dystrophies[5]. The difference in the unrestricted diffusivity D_0 between two age groups may be attributed to sarcomere length difference with mouse age[6].

Conclusion: Our preliminary results demonstrate that the DTI-RPBM measures can be used to monitor muscle growth in wild type mice. It also substantiates the potential of using the DTI-RPBM measures for the study of muscular diseases, effects of atrophy and exercise, and, in general, may open a way to quantify cell size and membrane permeability in soft tissues with diffusion MRI. Further investigation is warranted with a larger cohort of mice together with quantitative analysis of immunohistochemistry staining images.

References: **1.** Novikov DS *et al.*, Nature Physics 2011, 7:508-514. **2.** Fieremans E *et al.*, ISMRM 2011, 1153. **3.** Fieremans E *et al.*, ISMRM 2013, 489. **4.** Kim S, Magn Reson Med 2005, 54:1387-1396. **5.** Sabetelli P, Matrix Bio 2012, 3:187-196. **6.** Goldspink, J Cell Sci 1968, 3:539-548.

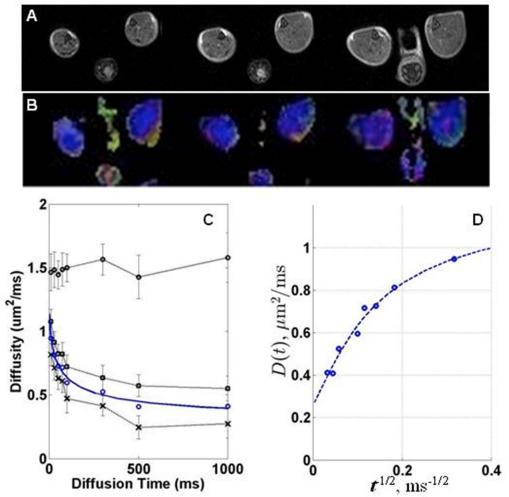

Figure 1: Anatomical T2-weighted RARE images to visualize the calf muscles of a mouse (A) and the corresponding FA colormap (B): red, left-right; blue, caudal-rostral; green, dorsal-ventral. (C) The plot of eigenvalues (black dots and lines) shows diffusion time dependence of the diffusion tensor in the hindlimb shown in (A) and (B). The blue line is a DTI-RPBM model fit to the transverse $D(t)=(\lambda_2+\lambda_3)/2$. (D) $D(t)$ replotted as a function of $t^{-1/2}$ becomes asymptotically linear at small $t^{-1/2}$, i.e. at large t.

Table 1: Estimated DTI-RPBM parameters

	Young mice		Old mice	
	1	2	3	4
D_0	1.205	1.279	1.411	1.435
S/V	0.340	0.228	0.184	0.316
κ	0.020	0.017	0.030	0.044

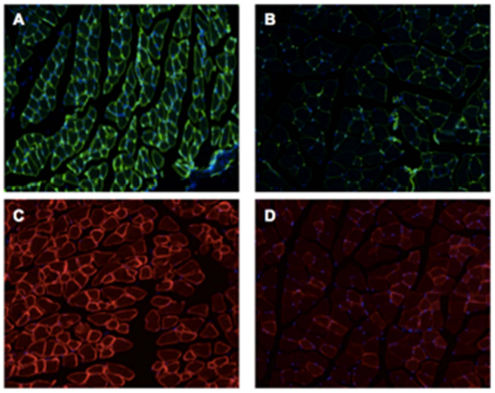

Figure 2: IHC for collagen IV **(A,B)** and AQP4 **(C,D)** of skeletal muscle across the fibers for a young mouse **(A,C)** and an old mouse **(B,D)**. DAPI stain is shown blue in all images.