# Multi parametric MRI evaluation of muscle development

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**Target Audience:** Clinicians/Researchers interested in observing microstructural changes in various myopathies

**Purpose:** The current non-invasive gold standard for investigating myopathies is the three-point Dixon MRI, which quantifies individual contributions of fat and water in each voxel of tissue to then calculate fat fractions[1]. Though the technique has successfully determined an increase in fat fractions with age, the fatty infiltration process may not necessarily be the best marker to study the wide range of myopathies, particularly at their early stages[2]. Myofiber diameter and membrane permeability are ideal markers for tracking the wide range of myopathies, considering these biophysical factors are crucial for muscle development and are often associated with the pathologies. In our previous simulation and *in vivo* studies, we utilized the random permeable model[3] (RPBM) of diffusion MRI data to assess muscle fiber size and sarcolemma permeability in normal muscle development[3,4,5,6,7]. In this study, we have furthered our work by comparing our DTI-RPBM technique with the conventional IDEAL-Dixon imaging and T2 mapping to better understand microstructural changes in the muscle.

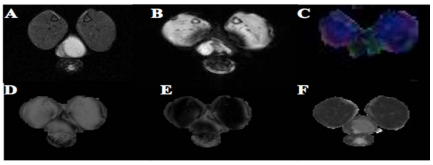


**Figure 1:** Anatomical T2-w RARE and T1-w images to visualize the calf muscles of a mouse **(A, B)** and the corresponding FA color map **(B)**: red, left-right; blue, caudal-rostral; green, dorsal-ventral. **(D)** and **(E)** are the water and fat images, respectively, with **(F)** T2 map

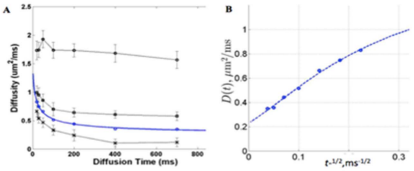


**Figure 2:** The plot of eigenvalues shows diffusion time dependence of the diffusion tensor in the hindlimb. The blue line is a DTI-RPBM model fit to the transverse $D(t) = (\lambda_2+\lambda_3)/2$. (D) $D(t)$ replotted as a function of $t^{-1/2}$ becomes asymptotically linear at small $t^{-1/2}$, i.e. at large $t$.

**Methods:** This study was done using wild-type mice (C57/blk males, n=5) at different ages. The age difference provided a means of evaluating muscle development at various stages through the use of DTI-RPBM metrics, fat fraction, and T2. All images were acquired using a 7T Bruker Scanner with a Paravision 5 Console and a volume transmit and receive coil. Anatomical image was acquired with a T2-weighted rapid acquisition with relaxation enhancement (RARE) sequence (TR = 2s, TE = 35.4ms, RES = 0.156 x 1.56 mm$^3$, 10 slices) and a T1-weighted 3D FLASH sequence (TR = 40ms, TE = 3.6ms, flip angle = 10) 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 twenty non-collinear directions and one reference image without diffusion weighting. The DW-STEAM-EPI scans were conducted with TR = 3 s, TE = 27.4 ms, FOV = 2.0 x 2.0 x 1.0 cm, and image matrix = 64 x 48 x 10. The DW-STEAM-EPI sequence was run repeatedly with seven diffusion times $t$ ranging between 20ms – 700ms. 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. For IDEAL-Dixon imaging, gradient echo sequence was ran to acquire 6 echoes with TR = 15ms, TE = 7.1ms, FOV = 2.12 x 2.0 x 1.0 and an image matrix of 128 x 96 x 10. T2 relaxation times were measured through a sequence of 32 echoes with TR = 2.4ms, TE = 7.1ms and a flip angle = 180. Total scan time was about 2 hours per mouse. Data analysis was performed with region of interest (ROI) manually drawn over the lower hindlimb 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 $D_0$. Water and fat fractions were determined by the IDEAL data analysis method and T2 was calculated using a monoexponential fit to the multi-spin echo data above noise level. Both fat fraction and T2 values were compared with the DTI-RPBM parameters, S/V and κ, by drawing similar ROIs in each images.

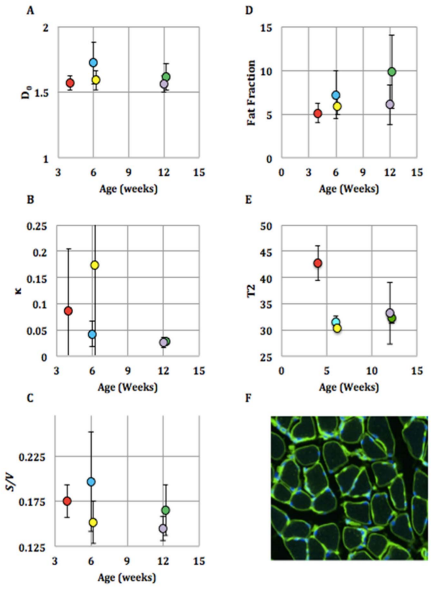


**Figure 3:** DTI-RPBM was used to measure $D_0$ **(A)**, κ **(B)** and $S/V$ **(C)** with mice at different ages. As the mice matured κ and $S/V$ were shown to decrease slightly. Fat fractions were also measured **(D)**, and increased with age. The recorded T2 relaxation times **(E)** with the corresponding developmental stages. IHC of skeletal muscle with WGA that will validate all MRI data **(F)**

**Results and Discussion:** Figure 1 serves as an example of the lower leg muscle images acquired during an MRI session: in T2-weighted, fractional anisotropy (FA), water/fat images and T2 map. Figure 2A shows the eigenvalues from the mouse hindlimb in Figure 1B and illustrate the relationship between diffusion eigenvalues perpendicular to the myofibers and diffusion time similar to the ex vivo DTI study[9]. Figure 2B shows an asymptotically linear dependence on $1/t^{1/2}$, which represents a "fingerprint" of the permeable membranes in the diffusion measurement. The DTI-RPBM data of these mice helps establish trends between mouse ages and the unrestricted diffusivity $D_0$, κ, and $S/V$. With age, both the myofiber $S/V$ and permeability decrease[5], while $D_0$ remains unchanged. Our previous work showed correlations between muscle fiber size and sarcolemma permeability[4]. IDEAL-Dixon imaging results show that the older mice had higher fat fractions[2]. This trend is more evident in an aging population in which individuals are known to have fat deposits within the skeletal muscle due to metabolic changes and decreased activity. T2 measurements were also recorded at these distinctive developmental stages and were shown to decrease with age.

**Conclusions:** In this study, we report a successful development of multi-parametric imaging methods for comprehensive assessment of the myofiber development in the mouse hindlimb, combining conventional MRI measures with DTI-RPBM for the first time. Our preliminary results demonstrate that the microstructural changes measured using our imaging parameters during muscle development are in line with the literature. This tool can be useful in assessment of disease progress or treatment response in various myopathies, such as Duchenne muscular dystrophy where pathology in the sarcolemma is followed by fat infiltration. Further study is warranted to validate these methods with immunohistochemistry staining of WGA and AQP4.

**Acknowledgments:** The authors acknowledge funding from the NIH (R21 NS081230) **References: 1.** Wren T *et al.,* Musculoskeletal Imaging Original Research **2**. Marcus R *et al.,* J Nutr Health Aging. 2010 May; 14(5): 362–366. **3.** Novikov DS *et al.*, Nature Physics 2011, 7:508-514. **4.** Winters K *et al.,* ISMRM 2014 2695 **5.** Fieremans et al, ISMRM 2011 1153; **6.** Fieremans et al, ISMRM 2013 0489; **7.** Lemberskiy G *et al.* ISMRM 2014 4423 **8.** Novikov DS *et al.*, PNAS 2014, 111:5088. **9.** Kim S, Magn Reson Med 2005, 54: 1387-1396