

IMAGING REGENERATION IN DYSTROPHIC MUSCLE USING T2 AND DIFFUSION MRI

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Introduction: Dystrophic muscle is especially prone to injury during eccentric, or lengthening contractions. While the muscle repair mechanisms are intact, recently repaired dystrophic muscle fibers will be still be prone to repeated damage. During these cyclic bouts of damage and recovery, the muscle tissue undergoes various stages of remodeling. In order to visualize these stages, T₂ and diffusion weighted MRI were used to observe changes associated with damage and repair in muscle tissue. The goal of this study was to determine the sensitivity of diffusion and T₂ weighted MRI to study structural changes during pathogenesis and repair following acute damage in dystrophic skeletal muscle. Specifically, this study set out to image the time course of eccentric damage and recovery in dystrophic muscle caused by downhill (-14°) treadmill running for 15 - 20 minutes.

Methods: A total of 6 control C57BL/10SnJ and 6 *mdx* mice (age: 18-24 months) were run on a downhill treadmill at an angle of -14° for 15 to 30 min at a speed of 8 to 10 m/min. For the *mdx* mice, exercise was terminated when they could no longer run. The mice were first MR imaged before running and at 0 (immediately after), 1, 2, 5, and 10 days post exercise. Hindlimbs were imaged using a custom built 1 cm, single tuned, ¹H loop-gap coil (470MHz) and an 11.1T Magnex superconduction magnet coupled with a Bruker Avance (Rheinstetten, Germany) horizontal bore spectrometer (PV3). To determine transverse relaxation rates (T₂), multiple slice, single spin-echo, diffusion-controlled images were acquired with the following parameters: FOV=1x1cm², matrix=256x128, slices=12, slice thickness=1mm, slice gap=1mm, diffusion weighting=3 mm²/s, NEX=2 and TR=2 s. A Hahn spin-echo MR image sequence in which two separate acquisitions were acquired at echo times of 12 ms and 30 ms was implemented (1). Diffusion weighted image data were collected with the same parameters as above with the addition of diffusion gradients applied with low (b=100 s•mm⁻²) and high b-values (b=900 s•mm⁻²) in 6 directions (TE = 14 ms). The eigenvectors (e₁, e₂, e₃) and the corresponding eigenvalues (λ₁, λ₂, and λ₃) were calculated. The indices ADC and FA were determined, from which image maps were generated allowing measurement of specific regions of interest using the Paravision JIVE (Bruker Avance) software package.

Results: The *mdx* mice showed consistent damage in all muscle groups (with predictable regions in the medial compartment), while healthy C57 control mice did not at day 2 post exercise (Fig. 1). The mean T₂ in a lesion rapidly increased and remained so during most of the repair process (T₂: 18.9±0.5, 30.7±1.3**, 27.7±1.7**, 19.9±0.3, 17.64±0.2 (ms); for Days Pre, 0, 1, 5, and 10. **P<0.001 vs Pre, ANOVA).

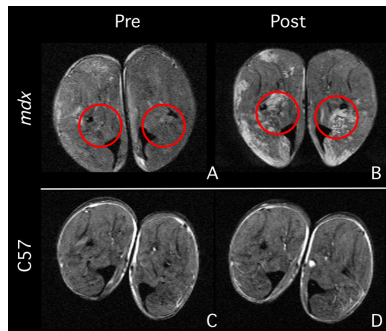


Figure 1. C57 and *mdx* hindlimb post downhill treadmill running. A) Pre-exercise B) 24hrs post running. C) and D): control C57 mice are not injured by the duration or intensity of the running protocol.

Similar to T₂, the mean diffusivity (ADC) also rapidly increases, but returned to the baseline at a faster rate during the time course of repair. The rapid decrease in FA inversely mirrors the changes of the ADC and reflects the loss of structure associated with the necrotic damage that soon follows the initial injury. The third eigenvalue, λ₃ exhibited a sustained significant increase for 24 hours (max = day 1).

Discussion: In order to reduce variation brought on by measuring dystrophic lesions of unknown temporal existence, in various stages of repair, we utilized a downhill treadmill running protocol to synchronize the incidence of injury. In the healthy C57 mice, immediately after injury an increase in ADC and a concomitant reduction in FA was seen in the exercised *mdx* muscle. This was consistent with an increase in tissue swelling and loss of restriction of water, presumably mostly in the perpendicular directions to fiber orientation (2). Interestingly, fluctuations were also seen in seemingly unaffected muscles as well, but they were not as pronounced as the changes in the lesions themselves (Fig. 2). The greatest difference between unaffected and affected (lesion) muscle was seen in λ₃ and FA. A combined analysis of T₂ and diffusion parameters appears to be a promising approach for monitoring recovery from damage in longitudinal studies of dystrophic skeletal muscle and will likely advance preclinical development of viable treatments for muscular dystrophy.

References:

1. Frimel T, et al. (2005) *Muscle Nerve* 32:605-612.
2. Heemskerk AM, et al. (2007) *Radiology* 243:413-421.

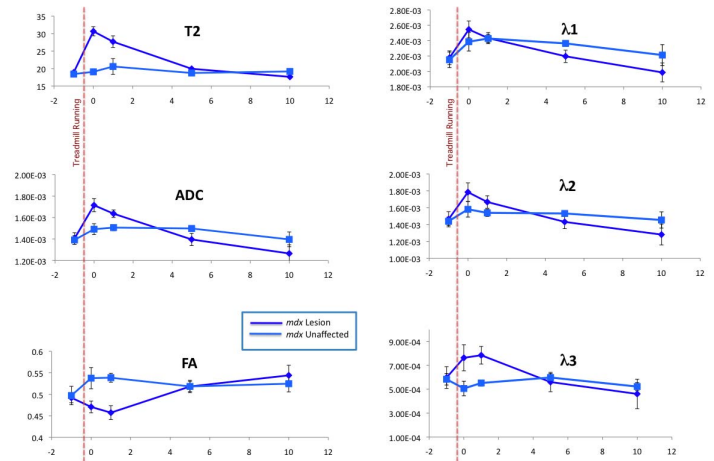


Figure 2. T₂ and diffusion indices during recovery from eccentric damage. Dark blue: dystrophy lesion and Light blue: a muscle that was not damaged.