# Monitoring skeletal muscle regeneration and dystrophy in mice using $T_2$ and diffusion tensor MRI and fiber tracking

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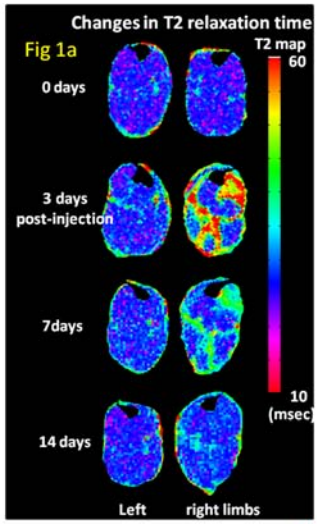
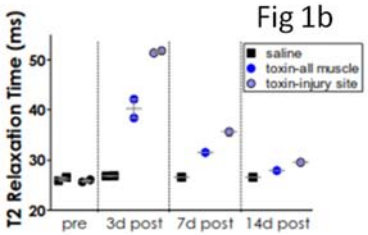
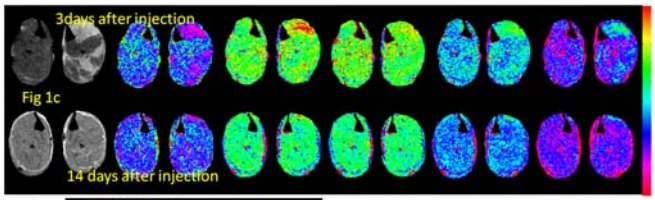
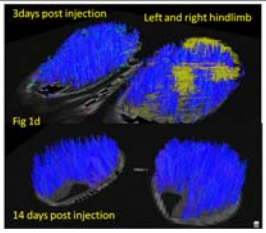
**Introduction:** Dystroglycanopthy is a type of muscular dystrophy caused by abnormal glycosylation of α-dystroglycan, which impairs its binding to the extracellular matrix. Loss of dystroglycan glycosylation causes muscle cell death, leading to cycles of degeneration and regeneration with progressive muscle wasting. In our dystroglycanopathy mouse model [1] and in patients, disease phenotype can vary from severe to milder muscular dystrophy. We previously used quantitative $T_2$ MRI to screen these dystroglycanopathy mice and showed hyper-intense regions in hindlimb muscle of 7.5 week old knockout mice. Surprisingly, the $T_2$ relaxation time was reduced in 13 week old knockout (KO) mice and not different from 13 week old littermates (LC). The cause of the reduced $T_2$ signal in older KO mice is unknown. We hypothesized that the dystroglycanopathy disease course may have very active degeneration/regeneration at younger ages (e.g. 7.5 wks), but less active regeneration at older ages (e.g. 13 wks), as a similar phenomenon has been described in a mouse model of Duchene muscular dystrophy [2]. We would anticipate that the molecular signature of regenerating muscle fibers by T2 MRI must be different and higher than mature muscle fibers. To test this hypothesis, we induced regeneration in one hind limb of wild-type mice using a muscle toxin and employed $T_2$ MRI. We also used fiber tracking to investigate water diffusion, orientation of fibers and muscle fiber damage in regenerating muscle of wild type mice and in KO and LC dystroglycanopathy mice.

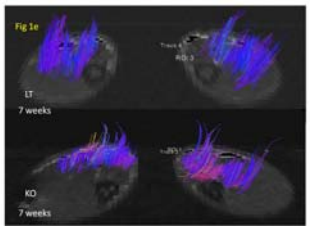
**Materials and Methods:** *Mice and MRI:* C57BL/6J mice (9 - 11 weeks old, n=4) were injected with saline (left) or cardiotoxin (right) using 4x25ul injections into the hindlimbs (1 to tibialis anterior, 3 to calf). T2 relaxation and diffusion tensor imaging was performed at 1 to 3 days pre-injection, then at 3 d, 7 d, and 14 d post-injection. Conditional dystroglycanopathy *Fktn* knockout (KO) and littermate (LC) mice underwent diffusion tensor imaging at 7.5 and 13 weeks old. For MRI, all mice were anaesthetized with isoflurane (1.0-1.5% in air), body temperature was maintained at 37°C, and the respiratory rate was monitored. MRI was performed on a 21 cm ID horizontal bore 7T Agilent magnet using a 38mm ID birdcage coil and 1.5 cm homebuilt RF coil. Quantitative $T_2$ for toxin injected mice were obtained using multislice spin echo (FOV=30 $mm^2$, TR=4 sec, TE=10 msec, number of echos #12, NSA=4, matrix size 128 x128)[2]. Diffusion tensor imaging was acquired with diffusion gradients applied along 12 non-colinear directions and one reference image was recorded without diffusion weighting. Scan parameters were: FOV=15 $mm^2$, matrix size=128x128, NSA=2, TE=30 ms, TR=2 sec, Δ=20 ms, δ=10 ms and b-value = 342 sec/$mm^2$. From the DTI data, the eigen values (λ1, λ2 and λ3), trace diffusion (TD) and fractional anisotropy (FA) were calculated using TrackVis. Mean values were obtained from an entire ROI within each slice from DTI processed data. Tractography was performed using TrackVis (http://www.trackvis.org). Statistical measurements were performed using ANOVA.




**Results and Discussion:** Muscle toxin causes widespread necrosis and edema followed by muscle regeneration. At 3 d post-toxin, necrosis is complete and regeneration has begun, but residual edema is still expected as is evident by the hyper-intense regions in toxin-injected (right) muscle by T2 map (Fig. 1a) and average T2 in the injury ROI of >50 ms (Fig 1b). There was no sign of edema after saline (left) injection (Fig. 1a,b). At 7 d post toxin, fiber regeneration is known to be well developed with no remaining edema, however elevated T2 persisted, suggesting that the newly regenerated fibers have a distinct T2 signature. Fiber maturation is fully complete by 14 d post-toxin where T2 relaxation time normalized. In DTI processing, 3 d post-toxin muscle (top) was significantly different from 14 d post-toxin muscle (bottom) in eigen values, TD an TR of the injury regions (Fig 1c, values not shown). Fiber tracking results obtained from toxin treated mice supports a complete loss of tracts at 3 days post-injection as expected for the pre-myoblast fusion phase of regeneration, and full fiber tract maturation in muscle at 14 days (Fig. 1d). Notably, DTI (performed on ROI with enhanced T2) in 7.5 wk KO muscle, where degeneration/ regeneration is not synchronized, also showed abnormal fiber tracts with short fibers, curved indices, and several disruptions of vertical orientation compared to littermates (Fig. 1e). Alternately, in eigen value analysis, only FA was different in dystrophic mice (LC vs. KO; 0.711±0.05 vs. 0.604±0.05, p=0.011*; TD 1.442±0.032 vs. 1.397± 0.0104).

**Conclusions:** The present study demonstrates that regenerating muscle has a distinct T2 profile. Furthermore, by DTI, there is a unique fiber tractography in regenerating and dystrophic muscle. Therefore, our data suggest that the DTI method is useful for monitoring dynamic changes in muscle fiber tracts during muscle degeneration/regeneration. Combining DTI, T2 and tractography will facilitate studies of muscle damage and repair over time.

**References:** 1. Beedle et al. J Clin Invest. 2012, 4; 122 (9): 3330.
2. Gardan-Salmon D et al., 2011 Eur J Appl Physiol. 111 :2763-73.
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