MAGNETIZATION TRANSFER CONTRAST IN MRI AS A DIAGNOSTIC AND MONITORING TOOL FOR MUSCULAR DYSTROPHY

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Introduction: Throughout the progression of dystrophic myopathies, the affected muscle tissues undergo numerous cycles of degeneration and regeneration due to their high susceptibility to injury. Upon exhaustion of the regenerative capacity of these muscles, damaged fibers are progressively replaced by fat and connective tissue. Underlying this elevated vulnerability to damage is a deficiency in dystrophin or dystrophin associated glycoproteins (DAGs). Since the pathological effects are often due to the lack of a single protein, the muscular dystrophies are well suited for gene therapy, stem cell therapy, as well as pharmaceutical treatment. Current methods of monitoring efficacy of therapeutic intervention and diagnosis rely heavily on tissue biopsy and histology. The development of non-invasive imaging modalities is greatly needed to evaluate novel therapeutic approaches in their ability to restore the dystrophin/DAG complex. MRI has been shown to be a valuable tool in the study of these neuromuscular diseases, but limitations on current methodologies have become apparent. One such limitation is the inability to detect fibrosis within dystrophic muscles as the disease progresses. Previous studies have indicated that magnetization transfer (MT) is sensitive to tissue fibrosis in the liver[2]. Therefore the goal of this study was to monitor the progression of tissue fibrosis with aging in murine models of Duchenne's muscular dystrophy (DMD) and limb-girdle muscular dystrophy (LGMD) using MT contrast. In addition, we determined whether MT could be used to detect gene correction in dystrophic mice.

Methods: A total of 25 *mdx*, 11 C57BL10, 13 *sgcg*$^{-/-}$, and 4 *sgca*$^{-/-}$ mice were studied. One leg of the *sgca*$^{-/-}$ mice was treated by IM injecting neonates with a muscle specific recombinant adeno-associated virus (1x10^{11}vg of rAAV2/1-tMCK-*sgca*) which expresses the human form of the missing α-sarcoglycan (sgca). **MRI/MRS.** During all *in vivo* MR experiments animals were anesthetized using gaseous isoflurane in oxygen. MRI were acquired with a FOV=1cm, matrix=256x128, slice thickness=1mm, nex=2 and a TR= 2s. To determine transverse relaxation rates (T_2) a spin-echo diffusion weighted sequence in which the diffusion weighting (***b***) is kept constant, but is minimized to just the imaging terms (***b***=4.2 s/mm^2), was performed at two echo times (TE=14,40ms)[1]. Magnetization Transfer (MT) was measured using the same spin echo sequence as used for T_2, except that a single transaxial image slice location was acquired using a MT preparation pulse consisting of 25 msec square pulses. Z spectra were acquired with frequency offsets of ±20, ±15, ±10, ±5 KHz using a 1s presaturation 6 μT pulse. MT contrast was determined at each offset frequency. Finally, unsuppressed and suppressed STEAM spectra (NP=2048 complex points, TR=2s, TM=15ms, sw=3kHz) were acquired with a *maximal* size of 2x2x3mm^3 in the tibialis anterior, soleus, and gastrocnemius muscles. **Quantification of Fibrosis.** Collagen content was evaluated by collecting digital micrographs of muscle cross sections stained with Masson's Trichrome Stain. Images were analyzed using the ImageJ program. ROIs, containing the entire muscle cross section, were manually selected and the percentage of positive collagen staining tissue was calculated using a Hue-Saturation-Intensity color model for pre- an post-thresholding measures (H:146-206, S:0-255, I:0-255; Pass Filter). All values are reported as mean±SEM and differences between groups were determined using ANOVA and paired t- tests with a level of significance set at $p<0.05$.

Results and Discussion: Masson's trichrome staining showed significantly larger amounts of fibrotic tissue in the gastrocnemius (gastroc) muscle of 24month old *mdx* mice compared to 28month C57/Bl10 mice (Fig 1). Based on quantitative histochemistry, the gastrocs of old *mdx* mice contained 20.71±5.52% fibrotic tissue by area and old control gastroc muscles contained only 3.84±1.04% fibrotic tissue. In the diaphragm extensive fibrosis was already observed at 1 year of age. Unlike, young dystrophic mice, old dystrophic mice also showed small deposits of lipids in the gastroc muscles which were detectable *in vivo* by ^{1}H-MRS and confirmed by Oil red O staining on serial sections. Similar results have previously been reported in old (>88 wks) *mdx* muscles.[3] Overall the Z spectra obtained from the tibialis anterior (TA) and gastroc muscles of old (>72wk) mdx mice were upwardly shifted compared to that of age matched controls (Fig1B). We further compared MT in old (18-28 month) and young (1-5month) dystrophic and control mice at a single offset frequency (Fib 1C). Specifically, we compared regions that appeared hyperintense (HI) on T_2 weighted images with normal, unaffected regions. HI regions showed significant MT contrast when compared to control muscles or unaffected regions (based on T_2) in *mdx* animals. Interestingly, large differences in MT were also detected in TA regions of old *mdx* mice in which there were no detectable difference in T_2. Finally, we found that expression of the missing protein alpha sarcoglycan in the young LGMD IID mice (*sgca*$^{-/-}$), resulted in a 27% (range 11-26%; $p<0.05$) decrease in T_2 and a 5.4% (range 1.3- 7.2%; $p<0.05$) change in MTR in the treated vs. the untreated contralateral muscles, with a maximal difference of 48% in T_2 and 17% in MTR in the TA. In conclusion, we found significant differences in MT characteristics of healthy and fibrotic dystrophic muscles indicating that MT may provide a noninvasive measure of disease progression and therapeutic intervention for the dystrophies.

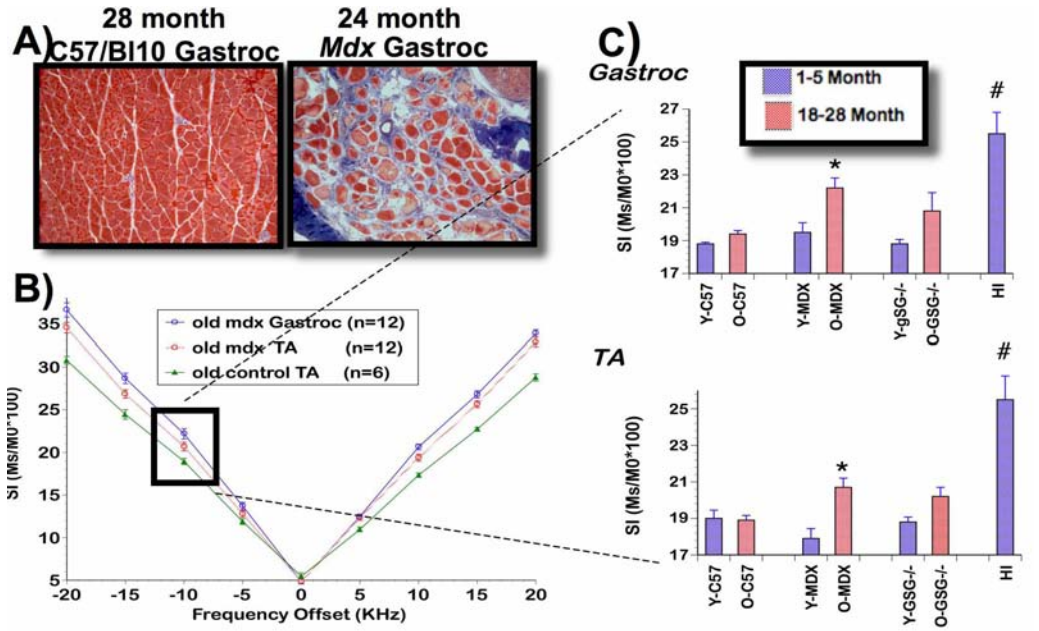

Fig 1. A) Masson's trichrome stain of 28 month old control and 24 month old *mdx* gastrocnemius (Gastroc) muscle. **B)** Z spectra from the gastroc and tibialis anterior (TA) muscles of old *mdx* mice and the TA of control mice. **C)** Magnetization transfer at the -10KHz offset from young (Y=1-5 month) and old (O=18-28month) control, *mdx*, and *sgcg*$^{-/-}$ mice. HI=hyperintense T_2 regions; M_s=image intensity with the MT pulse on; M_0=image intensity without the MT pulse; *p<0.05 between old and young *mdx* and between *mdx* and controls; **p<0.05 only between *mdx*; #p<0.05 between HI and controls.

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