Advanced pathology in aged mdx muscle characterized by quantitative multi-parametric MRI

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Target audience

A multi-parametric MRI approach was used to investigate advanced pathology in the leg muscles of *mdx* mice and compared the MRI findings to aged controls. T_2, DTI and qMT-MRI data were collected in a single imaging session. Preclinical and basic researchers will be able to incorporate these methods and findings into current and future studies. The results presented have improved our understanding the MR signal characteristics of advanced dystrophic/fibrotic muscle in small animal models of muscle pathology and are currently being translated to human patient studies[1].

Purpose

Degenerative neuromuscular diseases vary widely in their severity and impact on quality of life. These diseases share a common set of pathological features, including necrosis, variable fiber diameter, fat deposition, and fibrosis. In the early stages, areas of recently damaged muscle are easily detected with T_2-weighted MRI. But as the disease progresses, muscle fibers are increasingly replaced with fat and fibrosis in humans. This fat deposition is commonly measured using imaging methods such as Dixon imaging. However, the fibrotic nature of the muscle tissue is much harder to assess using routine methods. Small animal models of DMD, such as the *mdx* mouse, do not have the fat deposition seen in human patients but accumulate significant fibrosis in the leg muscles at late stages. This provides an opportunity to investigate the fibrotic aspect of myopathy without the complication of fat. In recent years, advanced MR methods with endogenous contrast have been employed to further characterize myopathy. Diffusion weighted imaging (DWI) and diffusion tensor imaging (DTI) are sensitive to alterations in tissue architecture. Magnetization transfer (MT) studies have shown a decrease in the magnetization transfer ratio (MTR) in dystrophic muscle. In this study, we used a multi-parametric approach to yield quantitative data and compare advanced pathology, including fibrosis, in two-year-old *mdx* mice to age-matched healthy muscle. In addition to relaxometery and DTI, we collected quantitative MT (qMT) data, yielding estimates of T_1 of the free liquid pool protons (T_{1f}), and pool-size-ratio (*PSR*; macromolecular protons vs. free water protons). The presence of fibrosis in the gastrocnemius (gastroc) muscles of the *mdx* mice was then confirmed by histological staining.

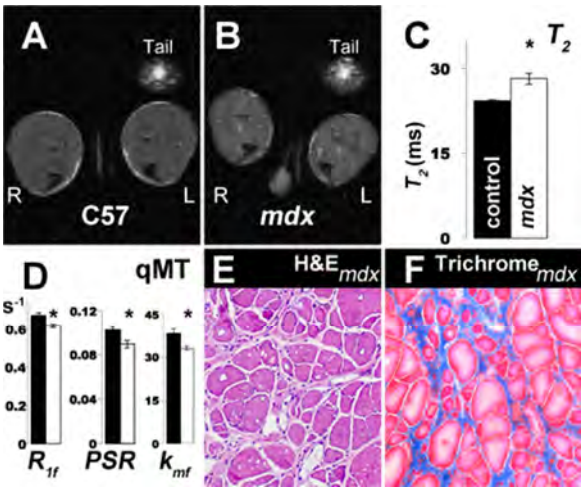

Figure 1. T_2-weighted (axial) MRI of the legs of A) healthy control and B) *mdx* mouse muscle. The *mdx* muscle appears unaffected. Yet, C) analysis of T_2 values reveal that *mdx* muscle has a significantly elevated T_2. Decreases were observed in the qMT D) parameters R_{1f}, *PSR*, and k_{mf} (Black: Control, White: *mdx*). E) H&E stained *mdx* gastroc displayed advanced pathology: increased intra-cellular space, variable fiber diameter, and central myonuclei. F) Trichrome staining confirmed that the *mdx* muscle also had extensive fibrosis.

Methods

Animals. Five healthy C57BL/j6 and five *mdx* mice, 19 - 24 mo. old were studied. **MRI Acquisition.** Mice were imaged *in vivo* at 4.7T Agilent/Varian Direct Drive scanner. For specific acquisition parameters, hardware and methods concerning high spatial resolution anatomical imaging, multiple-echo, single-slice imaging for T_2 data, DTI, calculation of diffusion tensors and derived metrics, qMT-MRI and fitting of *PSR*, R_{1f}, and k_{mf}, see Bryant *et al.* 2014[2]. ROI Analysis. Regions of interest (ROIs) were drawn in the gastroc muscles and parameters were fit from the mean signal intensities within the ROIs of the raw images. The right gastroc was chosen at random for ROI placement. Statistical significance was accepted at $p < 0.05$. Histology. Muscles were excised, frozen in melting isopentane, and stained with hemotoxylin and eosin (H&E) or Masson's trichrome to verify the presence of pathological feature in the aged *mdx* muscles.

Results

Age matched control muscle was consistently normal in appearance. T_2-weighted images had a homogenous signal intensity distribution in the healthy muscles. The aged *mdx* leg muscles were also consistently qualitatively normal in appearance (Fig 1A), but were determined to have a slightly, yet significantly elevated T_2 when compared to the healthy controls (Fig. 1B). At the age of two years, dystrophic lesions were rarely observed in these *mdx* mice. No significant changes were observed in any of the measured diffusion parameters (*ADC, FA,* or any of the tensor's eigenvalues). From the qMT data, the R_{1f}, *PSR*, and k_{mf} were all significantly decreased in the *mdx* muscle as compared to the healthy aged control muscle (Fig 1C)., H&E stained *mdx* gastroc muscle displayed an expanded extracellular space, variable fiber diameter, and central myonuclei. Investigation of tissue thin sections stained with Masson's trichrome stain consistently revealed regions extensive fibrosis in the aged *mdx* gastroc muscles.

Discussion

It was expected that the occurrence of active dystrophic lesions would be greatly reduced in the aged *mdx* mice, which appeared largely healthy in T_2-*w*-weighted images. From previous studies, it is also not surprising that the *mdx* T_2 values were slightly, but statistically significantly increased. Although fiber atrophy was expected to increase *FA* and λ_2 and/or λ_3 to decrease, no diffusion changes were observed. As for the qMT findings, it is interesting to see the *PSR* decrease, while there is an increase in collagen in the extra-cellular space and numerous small diameter myofibers. The decrease in *mdx* muscle R_{1f} suggests an increase in the T_1 of the liquid proton pool may be driving this drop in *PSR*. The histology data clearly confirm the presence of fibrosis and other advanced pathology in the aged *mdx* muscle.

Conclusion

The clinical benefit of new non-invasive biomarkers may aid the monitoring of patient condition and treatment response and aid pre-clinical treatment-development through providing a more accurate characterization of the tissue environment.

References

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2. Bryant N, Li K, Does M, et al. Multi-parametric MRI characterization of inflammation in murine skeletal muscle. NMR Biomed. 2014;(27): 716–725.