Skeletal Muscle ^1H_2O T_2 Analyzed for Multiple Components in *mdx* Mice after Downhill Running

Forbes, S.C.; Batra, A. (UF, Physical Therapy); Vohra, R. (UF, Physiology and Functional Genomics); Vandenborne, K. (UF, Physical Therapy); Walter, G.A (UF, Physiology and Functional Genomics)

Introduction

Duchenne muscular dystrophy (DMD) is an X-linked recessive disorder that is due to a mutation in the dystrophin gene[1] and is characterized by muscle damage, inflammation, early loss of functional abilities, and reduced life expectancy.[2] We have previously observed that ^1H_2O T_2 of skeletal muscles is greater in boys with DMD than controls using single exponential T_2 analysis[3] and that there is an increased amplitude of a long component in DMD than controls when analyzed for multiple components using non-negative least squares analyses (NNLS)[4]. In this study we evaluate multi-exponential T_2 in dystrophic (*mdx*) mice before and after downhill running to exasperate skeletal muscle damage. We hypothesized that a long T_2 component would be more predominant in dystrophic muscle after downhill running than before running and in controls due to greater damage and inflammation.

Experimental

The medial compartment of the lower hindlimb of dystrophic (*mdx*, n=5) and wild-type (n=5) mice were examined using ^{1}H-MRS single voxel STEAM (128 TE's, 5-300 ms, TR 9 s) before and 24 hours after downhill running (30-60 min, 14° grade, 6-12 m/min) with a 4.7T Varian/Agilent MR operating system of the Advanced Magnetic Resonance Imaging and Spectroscopy (AMRIS) facility. The amplitude of the water peak was determined using complex principal component analysis followed by T_2-NNLS analysis (500 time constant bins linearly spaced from 1 to 251.5 ms, plus an energy constraint). The signal decays were resolved into three components (short<20 ms; medium 21-40 ms; long>40 ms). The relative amplitude of each component was determined by integrating its peak and dividing by the sum of all peak integrals.

Results and Discussion

Using T2-NNLS, a long component was not resolved in any of the wild-type mice before or after downhill running; however, a long component was resolved in some of the *mdx* mice before treadmill running (average amplitude: 1±2%) and in all of the *mdx* mice after downhill running (amplitude: 6±2%). The increase in prevalence and contribution of the long component after downhill running is consistent with muscle damage and inflammation contributing to this component.

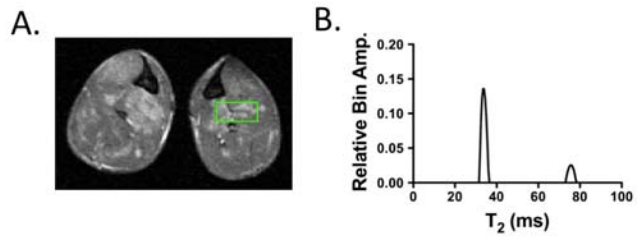

Figure 1. Example *mdx* lower leg axial SE image (TE 40 ms) showing voxel placement in the medial compartment (A). ^{1}H-MRS relaxometry spectra were acquired and analyzed with NNLS, in which a medium (20-40 ms) and long component (>40 ms) was observed (B).

Conclusions

Single voxel ^{1}H-MRS STEAM measures of ^1H_2O T_2 in skeletal muscle revealed differences between dystrophic and control skeletal muscle using T_2-NNLS analysis. DMD had a more predominant long component (>40 ms) than controls, which is consistent with muscle damage/inflammation contributing to this component. Furthermore, this long component was affected by downhill running in dystrophic muscle. Overall, T_2-NNLS analysis may provide valuable insight into disease progression and potential treatment interventions when interpreting ^1H_2O T_2 changes in dystrophic muscle.

Acknowledgements

This research was supported by the Muscular Dystrophy Association (175552) and Senator Paul D. Wellstone Muscular Dystrophy Cooperative Research Centers (U54AR052646). A portion of this work was performed at the National High Magnetic Field Laboratory, which is supported by National Science Foundation Cooperative Agreement No. DMR-1157490, the State of Florida, and the U.S. Department of Energy.

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