Dystrophic Skeletal Muscle ^1H_2O T_2 Analyzed for Multiple Components

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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 shown that ^1H_2O T_2 of skeletal muscles is greater in DMD than controls using single exponential T_2 analysis, and that ^1H_2O T_2 is greater in younger subjects with DMD than older DMD subjects[3]. Furthermore, it has previously been shown that skeletal muscle T_2 can be resolved into multiple components using non-negative least squares analyses (NNLS)[4], and therefore resolving into multiple components in DMD may provide further insight into T_2 changes. In this study, we evaluated ^1H_2O T_2 in a large cohort of DMD and healthy unaffected control subjects in a range of age groups using NNLS. In a parallel study, we evaluated 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 than controls due to events associated with muscle damage and inflammation.

Methods: MR data were acquired from 118 boys with DMD (ages 5-14 years; mean 8.5±2.3 years; 5-6.9 years, n=41; 7-8.9 years, n=30; 9-10.9 years, n=24; 11-14 years, n=23) and 40 healthy controls (age 9.5±2.3 years; 5-6.9 years, n=6; 7-8.9 years, n=12; 9-10.9 years, n=14; 11-14 years, n=8) using 3T MR systems at three institutions (University of Florida, Oregon Health & Science University, and Children's Hospital of Philadelphia) as part of the ImagingDMD study. The subjects included both those prescribed corticosteroids (n=91) and those not on corticosteroids (n=27). ^1H spectroscopic relaxometry was performed using STEAM in the soleus muscle (16 TE's non-linearly spaced from 11-288 ms; TR 9 s; NA4; Figure 1). In a parallel study, the medial compartment of the lower hindlimb of dystrophic (*mdx, n=5*) and wild-type (n=5) mice were examined using ^1H-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. *Analysis*: The amplitude of the water peak was determined using complex principal component analysis followed by T_2-NNLS analyses (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.

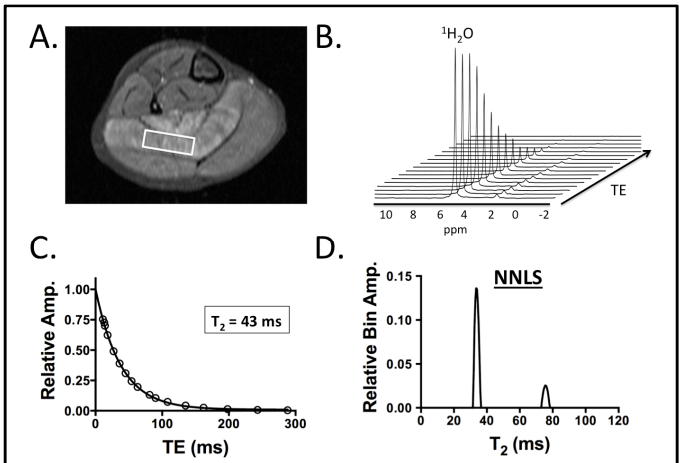

Figure 1. Example DMD lower leg fat suppressed axial SE image (TE 60 ms) showing voxel placement in the soleus (A),^1H-MRS relaxometry spectra (B), and subsequent T_2 decay curve (C) with NNLS analysis with a medium (20-40 ms) and long component (>40 ms; D).

Results and Discussion: Using NNLS analysis, we observed multi-exponential responses in the majority of subjects, with significant differences observed between DMD and controls in the medium and long components (Table 1). The relative contribution of the long component was greater ($p<0.05$) in subjects with DMD compared to controls, while the medium component was reduced ($p<0.05$) in DMD than controls. The amplitude of the long component tended to decrease with age groups in DMD (5-7 yrs: 8±3%; 7-9 yrs: 8±3%; 9-11 yrs: 4±1%; 11-14 yrs: 3±1%) and was greater ($p<0.05$) in DMD steroid naïve subjects (13±2%) compared to age-matched DMD subjects treated with corticosteroids (4±2%). In the mice, the 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.

Table 1. NNLS analysis of T_2 in controls and boys with DMD

	Short	*Medium*	*Long*
Controls	*Component*	*Component*	*Component*
T_2 (ms)	4.0±0.5	28.3±0.3	139.3±10.8
% of Total Amplitude	8+2	91±3	1±0.2
#of subjects with component (n=40)	14	40	29
DMD			
T_2 (ms)	5.4±0.4	31.0±0.3*	110.8±5.7
% of Total Amplitude	16±2	78±2*	6±1*
# of subjects with component (n=118)	67	118	87

* denotes significantly different ($p<0.05$) than controls.

Conclusion: Single voxel ^1H-MRS STEAM measures of ^1H_2O T_2 in skeletal muscle revealed differences between DMD and controls using NNLS analyses. 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 appeared to be affected by corticosteroid treatment and downhill running in dystrophic muscle. Overall, NNLS analyses may provide valuable insight into disease progression and potential treatment interventions when interpreting ^1H_2O T_2 changes in dystrophic muscle.

References:

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