Age Dependent Changes in Metabolite Profile and Lipid Saturation in Dystrophic Mice

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Introduction - Duchenne Muscular Dystrophy (DMD) is a fatal X-linked genetic disorder. In DMD, the absence of the dystrophin protein causes decreased sarcolemmal integrity resulting in progressive replacement of muscle with fibrofatty tissue. The effects of lacking dystrophin on muscle and systemic metabolism are still unclear. Therefore, to determine the impact of the absence of dystrophin on metabolism, we investigated the metabolic and lipid profile at two different, well-defined stages of muscle damage and stabilization in a mouse model of DMD (*mdx*). We measured NMR-detectable metabolite and lipid profiles in the serum and muscles of *mdx* mice at 6 weeks and 24 weeks of age. Metabolites were determined in muscle *in vivo* using ^{1}H MRI/MRS, in isolated muscles using ^{1}H- high-resolution magic-angle spinning (HR-MAS), and in serum using high sensitivity ^{1}H/^{13}C NMR.

Experimental - C57BL/10ScSn-DMDmdx mice (*mdx*) and age matched C57BL/10ScSn (controls) were purchased from Jackson Laboratories. *In vivo* MRI/MRS were obtained on AMRIS' horizontal 11.1T MR system. *In vivo* ^{1}H spectra were acquired from the gastrocnemius (1x2x2mm^{3}) muscle using LASER (TR/TE=2s/14ms; np=1024, SW=15ppm). 1D ^{1}H and ^{13}C mouse serum spectra were on an Agilent VNMRS-600 spectrometer using a custom 1.5 mm ^{13}C high temperature superconducting (HTS) probe [1]. ^{1}H spectra were acquired from the isolated mouse gastrocnemius using HR-MAS NMR with a 4mm HR-MAS probe on a Bruker 600 MHz spectrometer (AVIII;Topspin 3.2).

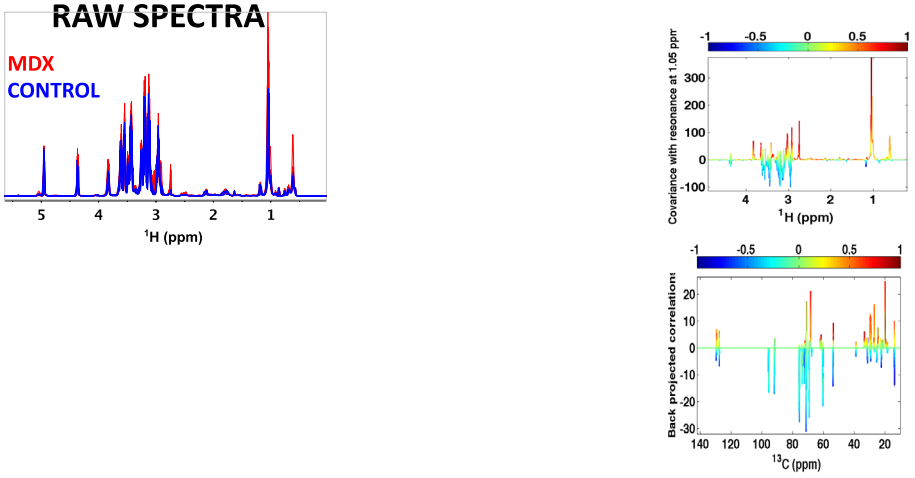

Fig.1. ^{1}H and ^{13}C spectra obtained from dystrophic and control mouse serum. ^{1}H and ^{13}C raw spectra of 6-month *mdx* (red) and 6 month control (blue) mice (**left**). Partial least squares discriminant analysis (PLS-DA) (**middle**). The insets are the scores plot showing the separation of the two groups from the supervised predicative modeling algorithm, PLS-DA. The spectra in the middle panel are representations of the peaks(features) with the largest contribution to the separation. In the ^{1}H PLS-DA spectrum there is a single peak around 1 ppm whereas in the ^{13}C PLS-DA there are many peaks indicative of glucose that are responsible for the separation. Statistical total correlation spectroscopy (STOCSY) plots, which find peaks that correlate to a peak of choice (**right**). The ^{1}H STOCSY plot represents peaks positively correlating to the peak at 1 ppm indicative of lactate, creatine, and taurine in the positive and negatively correlating to peaks indicative of glucose. The ^{13}C STOCSY peaks shows peaks that correlate to the 1 ppm peak in the ^{1}H plot.

Results and Discussion - Dystrophic mice were found to have a unique lipid saturation profile compared to control mice, revealing an age-related metabolic change. In the 6 week old *mdx* mice, serum lipids were increased and the degree of lipid saturation changed between 6 and 24 weeks. PLS-DA indicated that *mdx* and control could be separated according to a ^{1}H resonance at 1ppm and glucose in the ^{13}C spectra. STOCSY analysis indicated correlations between the 1ppm ^{1}H peak with lactate, creatine, glucose and taurine. The serum Tau:Cr ratio increased over the life span of *mdx*, but not in control mice. Furthermore, the saturation index of lipids increased in the serum but decreased in the tissue over time. Finally, we demonstrated associations between MRI-T2, a strong indicator of inflammation/edema, with tissue and serum lipid profiles.

Conclusions - These results indicate the complex temporal changes of metabolites in the tissue and serum during repetitive bouts of muscle damage and regeneration that occur in dystrophic muscle.

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References - [1] Ramaswamy V et al. *Journal of Magnetic Resonance*. 2013; 235:58-65