In vivo ^{1}H MRS monitoring of intra-myocellular lipids after acute muscle injury in healthy and dystrophic mouse muscles

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Introduction

Duchenne muscular dystrophy (DMD) and *mdx* mice (an animal model for human DMD) are considered homologous based on the lack of dystrophin. The parallels in damage between injured and dystrophic skeletal muscles are striking; both are characterized by inflammation, enzyme efflux, fiber splitting, cytoskeletal changes, membrane damage, fibrosis, and myogenesis [1]. Quantification of the EMCL is difficult due to its sensitivity to the orientation of the molecules with respect to the main magnetic field. However, IMCL is not sensitive to the orientation of the main magnetic field. [2,3]. In this study, we assess the potential of *in vivo* ^{1}H MRS to detect and monitor the IMCL in acute skeletal muscle injury in both healthy and *mdx* mice.

Materials and Methods

Unilateral injury to the tibialis anterior muscle (TA) was induced by 15 maximal lengthening contractions as previously described [4] in *mdx* mice (C57BL/10*mdx,* n=4, 2 left TA & 2 right TA) and C57 mice (C57BL/10, n=4, 2 left TA & 2 right TA). The animal was anesthetized with a gas mixture of O_2 (0.5 L/min) and 2% isoflurane. With the animal supine, one of the hind limbs was stabilized and the foot was secured onto a plate, the axis of which was attached to a stepper motor (model T8904, NMB Technologies). A custom program based on commercial software (Labview version 8.5, National Instruments) was used to synchronize contractile activation and the onset of ankle rotation. Injury results from forced lengthening (plantarflexion) contractions through a 70^0 arc of motion, superimposed onto a maximal contracting TA.

All MRI/MRS experiments were performed on a Bruker Biospec 7.0 Tesla 30 cm horizontal bore scanner using Paravision 5.0 software. A Bruker ^{1}H surface coil array was used as the receiver and a Bruker 72 mm linear-volume coil as the transmitter. Mouse were anesthetized in an animal chamber with a gas mixture of O_2 (1 L/min) and 3% isoflurane. The animal was then placed supine on a custom made body holder to align the two legs parallel to the static magnetic field to maximize the chemical shift difference between the two lipid components [2, 3]. The RF coil was positioned and fixed with surgical tape in the region of interest on the animal legs. After the animal was moved into the center of the magnet, the isoflurane level was maintained 1.0 to 1.5% for the remainder of the experiment. An MR-compatible small-animal monitoring and gating system (SA Instruments, Inc.) was used to monitor respiration rate and body temperature. Mouse body temperature was maintained at 36-37^{o}C using a warm water circulation. The experimental protocol was approved by the institutional animal use and care committee at the University of Maryland.

Three-slice (axial, mid-sagittal, and coronal) scout rapid acquisition with fast low angle shot MR imaging (FLASH) was used to localize the volume of interest. High resolution proton density-weighted (PD) anatomic images in both longitudinal and cross-sectional views were acquired using rapid acquisition with relaxation enhancement (RARE) sequence with TR/TE = 1800/9.25 ms, RARE factor = 4, in-plane resolution = 0.1 x 0.1 mm^2, slice thickness = 0.75 mm, averages = 8. Total acquisition time was 7.5 minutes in each view. A point-resolved spectroscopy (PRESS) pulse sequence (TR/TE=2000/18 ms) was used to acquire the spectral information on a 1 x 1 x 4 mm^3 voxel in TA (outlined by red lines in Fig. 1a, b) for 34 minutes on each voxel. ^{1}H MRS data was fitted using the LCModel package [5]. The *in vivo* mean IMCL peak area relative to total creatine was measured. Statistical analysis was performed using the Student t-test.

TAs were harvested after the *in vivo* MRI/MRS experiments, snap frozen in liquid nitrogen, then stored at -80^{0}C. Oil red O (ORO) staining was used to identify lipid depositions in skeletal muscle.

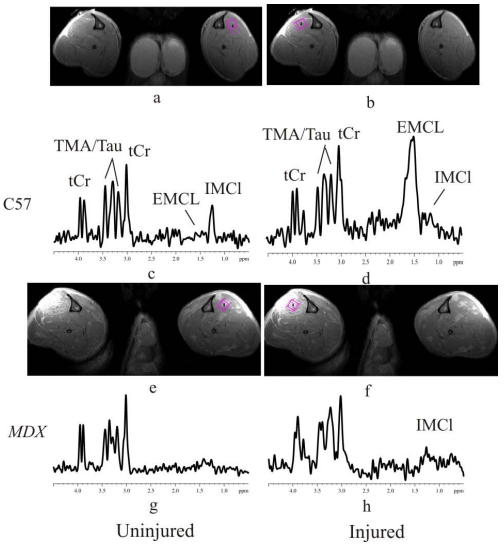

Fig.1. *In vivo* MRI (a, b, e, f) and ^{1}H MRS (c. d. g, h) from TA muscles from C57 and *mdx* mice before and after acute injury.

Results

Fig.1 a, b, e, f illustrate the PD MR images from the cross-sectional view of hind limbs and localized region in TA for the ^{1}H MRS acquisition in a C57 (Fig. 1a, b) and an *mdx* (Fig. 1c, b) mice. Images of C57 uninjured muscles were homogeneously dark with no focal hyperintense regions. By contrast, the injured areas showed hyperintensities in both groups likely due to edema at the injury site soon after the injury. In general, the cross-sectional view of the legs was significantly larger in *mdx* mice compare to the C57 ones. The MR images of *mdx* mice were heterogeneous with multiple unevenly distributed focal hyperintensities throughout the bulk of the *mdx* muscles. Fig. 1c & d show ^{1}H spectra acquired from TAs in the C57 mouse. The high quality spectra clearly discriminated various resonances with non-excessive EMCL contamination. In general, the *mdx* mice demonstrated very low and sometimes undetected IMCL levels before the injury (Fig. 1g). The uninjured C57 group demonstrated significant high levels of IMCL to total creatine (IMCL/tCr, 0.7092 ± 0.1622 vs 0.0634 ± 0.0634, $p < 0.01$) compared to the *mdx* group. In addition, the injury caused a significant increase in IMCL/tCr (1.5123 ± 0.5657 vs. 0.0634 ± 0.0634, $p<0.02$) in the *mdx* group but not the C57 group.

ORO staining confirmed the changes quantified by the ^{1}H MRS findings and showed increased lipid deposition after injury in *mdx* TAs.

Discussion

This study shows that *in vivo* ^{1}H MRS can serve as a sensitive surrogate outcome measure of muscle injury, especially for the IMCL profiles. Clinically, it may allow one to monitor synchronous muscle repair after injury. Previous studies have shown that the area of the histological lesions in *mdx* muscles exactly localized within the hyperintense regions on MRI, but with little or no adipose tissue [1]. Our data support that finding, or at least show that it is unlikely that the hyperintense regions in the *mdx* are related to the intra-myocellular lipids. In addition, the increase of the IMCL in the *mdx* mice after injury may indicate a mitochondrial dysfunction or reduced fatty acid oxidation.

References

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