Anisotropic Diffusivity of Creatine in Rat Hindleg Muscles Revealed by Diffusion Weighted Proton MRS

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INTRODUCTION: The cytosolic creatine pool are known to vary largely according to tissue and species differences (1). The apparent diffusion coefficient (ADC) of phosphocreatine (PCr) phosphorus was reported using a 31P diffusion weighted MRS (DW-MRS) in excised strips of fish and frog muscle and in the limbs of rats and rabbits and human muscle (2, 3). In this study, the ADCs of total creatine were measured with proton MRS. Measurements were performed on SD rat skeletal muscle. We hypothesized that diffusion characters of creatine or other metabolites could be used as a probe to reflect the intracellular structure.

MATERIALS AND METHODS: Animal Preparation: Normal adult male Sprague-Dawley (SD) rats (N=5; 2-mon old, 320-350g) were examined. During MR experiments, animals were anesthetized with a mixture of air and 1-1.5% isoflurane, positioned by an in-house hindleg fixation device, mechanically ventilated and muscle paralyzer (Pavulon 1mg/kg IP). **MRI Protocol:** All MRI measurements were acquired from a 7T Bruker animal scanner. For DW-MRS, a stimulated-echo (STEAM) based single-voxel MRS sequence was implemented by adding a pair of unipolar diffusion gradients during two TE/2 intervals. The hindleg was fixed along z axis, and diffusion gradient was applied with two directions either parallel or perpendicular to muscle fiber orientation, i.e. along x or z axis. DW spectra were acquired with diffusion duration δ=5ms, 6 b-values (0 to 5170 mm^2/s), 3 diffusion times Δ (26, 46, 86ms), TR/TE=1500/30ms, NEX=32, spectral width=4kHz, and voxel size=8×8×8mm^3. **Data Analysis:** Spectral analysis was performed using the JMRUI and TOPSPIN software package. Creatine signals were quantified by fitting the spectrum to a Lorentzian line shape using the AMARES algorithm. Creatine ADCs were computed by fitting the b-value dependent signals to a monoexponential model. All measurements were expressed as mean ± standard deviation. Two-tailed unpaired student's t-test was employed to examine ADC difference between two different diffusion directions. Results were considered significant when $p<0.05$ (*$p<0.05$, **$p<0.01$, ***$p<0.001$, ns: not significant).

RESULTS AND DISCUSSIONS: Figure 1 domenstrated the typical in vivo diffusion weighted spectra acquired from rat hindleg muscle. The creatine (Cr3) peaks at 3ppm can be clearly distinguished from proton DW-MRS spectra. As seen in Figure 2A, 2B and 2C, in three different diffusion times, the relative signal magnitudes of perpendicular and parallel direction showed consistent discrepancy during the signal decay. Shown in Figure 2D, creatine (Cr3) ADCs were significantly larger in the parallel direction, i.e., $(4.206\pm0.16)\times10^{-4}$ mm^2/s for ADC of parallel direction compare with $(2.856\pm0.18)\times10^{-4}$ mm^2/s for ADC of perpendicular direction. Statistically differences were found between two diffusion directions in all 3 diffusion times (Figure 2D). Measurement of total creatine ADC values in the rat muscle clearly showed that the creatine pools inside the muscle cells exhibit the anisotropic diffusivity. The creatine may be more hindered along the perpendicular direction according to the muscle fiber. This hindrance may be caused by the restriction of myolemma and/or the interaction with some subcellular structure like myofibrils and mitochondria.

CONCLUSION: Our experiment investigated the creatine diffusivity in the rat hindleg muscle by measuring the creatine ADC in two orthotropic diffusion directions. Creatine shows a highly anisotropic diffusion behavior within the myofiber, presenting a higher diffusivity in the fiber direction than in perpendicular direction.

REFERENCES: 1. Wyss M. and R. Kaddurah-Daouk Physiological reviews 2000;80:1107-213; **2.** Gabr R. E., American journal of physiology. Cell physiology 2011;301:C234-41. **3.** de Graaf R. A., Biophysical journal 2000;78:1657-64.

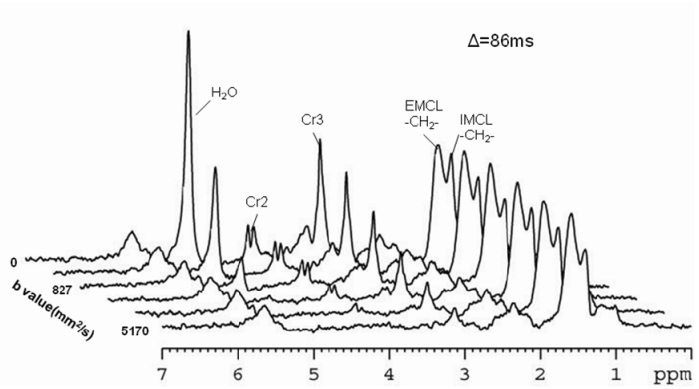

Figure 1. Typical diffusion weighted spectra observed from hindleg skeletal muscles of one adult SD rat. Major proton resonance are: H_2O, creatine $–CH_2–$ group (Cr2), creatine $–CH_3$ group (Cr3), extramyocellular lipid (EMCL) $–CH_2–$ group and intramyocellular lipid (IMCL) $–CH_2–$ group.

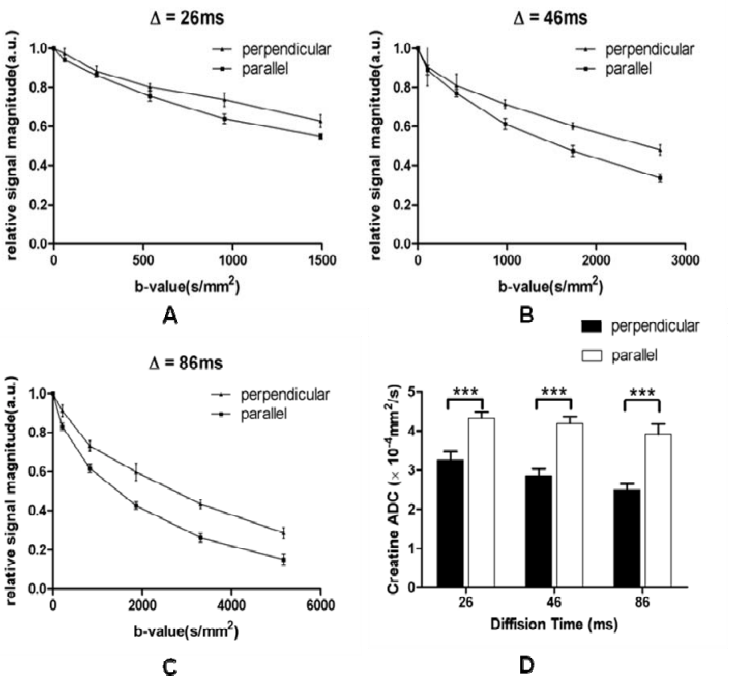

Figure 2. Anisotropic diffusivity of creatine in muscle. Perpendicular stand for the diffusion gradient perpendicular to the muscle fiber while parallel stand for the diffusion gradient parallel to the muscle fiber. A-C: Relative signal magnitude decay under 6 b-values in three diffusion time (i.e. A, Δ=26ms; B, Δ=46ms; C, Δ=86ms). D: Distinct Creatine ADCs in two diffusion gradient direction.