Changes in foot orientation alters residual dipolar couplings of Creatine and Phosphocreatine in the skeletal muscle of rats

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Introduction: Approximately 95% of the human body's total creatine is located in skeletal muscle. When muscles are put to work, creatine gets converted to creatine phosphate (phosphocreatine), which in turn releases ATP, which is the source of energy for living cells [1]. Thus a constant supply of creatine and phosphocreatine are essential in maintaining a healthy state in muscles. In proton MRS Creatine (Cr) and phosphocreatine (PCr) gives rise to singlets at 3.03 and 3.93 ppm due to the methyl and methylene groups respectively. The chemical shift separation between the protons of these two molecules is much smaller than in vivo linewidths, causing them to be indistinguishable under normal conditions [2]. However skeletal muscle spectroscopy strongly depends on the muscle fiber orientation with reference to the magnetic field and is influenced by [3,4]:

Bulk Susceptibility effects:

- These depend on the orientation of the leg in the scanner and affect the separation between IMCL and EMCL

J coupling causes spectral line splitting due to interaction of nuclei separated by bonds

- Residual Dipolar Coupling (direct dipole-dipole coupling between nuclei) can alter chemical shifts by few Hz and also introduce additional relaxation mechanisms.

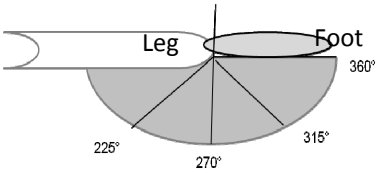

Figure1. Different foot orientations with respect to the leg and magnetic field

Earlier studies have looked at the skeletal muscle spectra by rotation of entire leg with respect to the magnetic field [5,6]. In this work we have assessed the residual dipolar couplings of Creatine (Cr) and Phosphocreatine (PCr) in the skeletal muscle spectra of the Tibialis Anterior muscle compartment in rats as a function of changing the foot orientation and hence rotating the fibers of the leg with respect to the magnetic field.

Methods: All experiments were performed on a 7 Tesla Bruker Cliniscan MRI/MRS scanner using a 72 mm volume transmit in conjunction and a 20mm surface receive coil. Localized PRESS MRS spectra were acquired on a 7 week old Wistar rat over a volume of 3mm3 with TR/TE of 4s/13ms, 256 averages and 2048 complex points from the tibialis anterior (TA) muscle. A device was developed to fix the leg and rotate the foot of the rat. The knee was placed at 90° and the leg was kept in line with the knee. The ankle position was fixed leaving only the foot mobile. The foot was flexed at 360 °, 315 °, 270 ° and 225 ° as illustrated in Figure1.

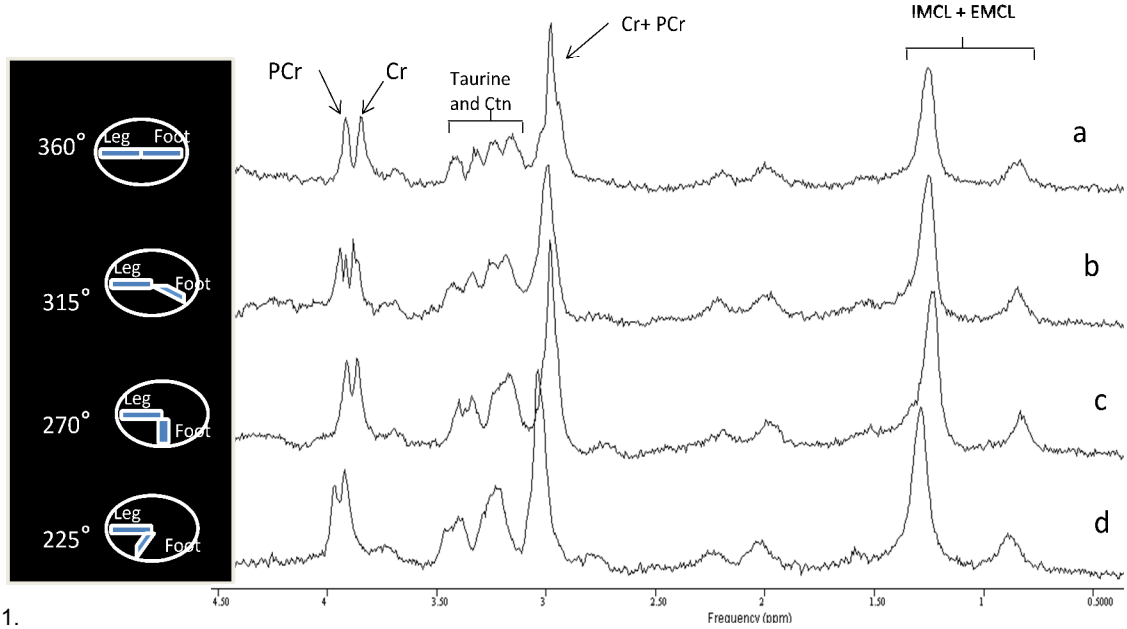

Figure2. MRS PRESS spectra of tibialis anterior muscle at Different foot orientations with respect to the leg and magnetic field

Results: The separation of Cr and PCr at 3.9 ppm depends on the foot angle with respect to the leg. In Figure 2, maximum separation of 22Hz (Fig 2a) was observed between Cr and PCr at 360°, 18 Hz (Fig 2c) at 270° and 17 Hz (Fig 2d) at 225° respectively. The effect of residual dipolar splitting was also observed for both Cr and PCr (Fig 2b) and was dependent on the foot angle. The maximum residual dipolar splitting of 6.8Hz was observed for both Cr and PCr and chemical shift separation of 18.5 Hz at 315°. In addition the resonances of Taurine and carnitine also demonstrate the the changes in chemical shifts and residual dipolar couplings due to foot orientation. We observed very minimal extramyocellular fat in young rats and was in agreement with the earlier work [7].

Conclusion: We have demonstrated the residual dipolar couplings for both Cr and PCr as a function of foot angle with reference to leg in Tibialis Anterior compartment of young rats. The chemical shift separation of Cr, PCr, Taurine and carnitine is dependent on the foot angle which alters the orientation of the muscle fibers with respect to the main magnetic field. In addition the strength of the residual dipolar couplings for both Cr and PCr is also dependent on the leg position.

References: 1. Creatine, MedLine Plus Supplements, U.S. National Library of Medicine. (2010) 2. Lei H, Poitry-Yamate C, Preitner F, Thorens B, Gruetter R, NMR Biomed, 23: 578 (2010) 3. Boesch C, Slotboom J, Hoppeler H, Kreis R, Magn Reson Med, 37: 484 (1997) 4. Hanstock CC, Thompson RB, Trump ME, et al, Magn Reson Med, 42: 421(1999) 5. Ntziachristos V, Kreis R, Boesch C, Quistorff B, Magn Reson Med, 38:33 (1997) 6. Boesch C, Kreis R, NMR Biomed,14:140 (2001) 7. Neumann-Haefelin C, Kuhlmann J, Belz U, Kalisch J, Quint M, Gerl M, Juretschke HP, Herling AW, Magn Reson Med, 50:242 (2003)