Issues with Creatine/Phosphocreatine Quantification: Lipids Contamination and Water-suppression

J. Ren[1,2], I. Dimitrov[1,3], J. Murdoch[3], A. D. Sherry[1,4], and C. Malloy[1,5]

[1]Advanced Imaging Research Center, University of Texas Southwestern Medical Center, Dallas, Texas, United States, [2]Department of Radiology, University of Texas Southwestern Medical Center, Dallas, Texas, United States, [3]Philips Medical Systems, Cleveland, Ohio, United States, [4]Department of Chemistry, University of Texas at Dallas, Richardson, Texas, United States, [5]VA North Texas Health Care System, Dallas, Texas, United States

Background

A number of MRS studies have shown great interest in quantifying creatine/phosphocreatine (Cr/PCr), energy metabolites widely distributed in brain, heart, and skeletal muscles. This is partially because of the importance of their role in energy metabolism along with ATP/ADP, and partially due to their role in standardizing other *in vivo* metabolites. The methyl (CH_3) resonances of Cr and PCr, give rise to a narrow and intense singlet at ~3.05 ppm, easily identifiable in a typical *in vivo* ^{1}H MRS. However, as diallylic methylene protons of fatty acids (-CH=CH-C**H$_2$**-CH=CH-, *dial*-CH_2-FA) also resonate at ~3.0 ppm, the presence of FA in tissue may cause problems with CH_3-Cr/PCr quantification, especially in the skeletal muscles where plenty of fat tissues are embedded between muscle fiber bundles as extramyocellular lipids (EMCL). FA molecules also exist within muscle cells in the form of droplets of intramyocellular lipids (IMCL). The impact of FA on Cr/PCr measurement has not been adequately addressed in literature, and we therefore aim to bring an awareness to this issue. Furthermore, several recent publication on muscle ^{1}H MRS at low fields have reported the observation of signal attenuation on CH_3-, but not CH_2- (resonance at ~ 4.0 ppm) of Cr/PCr due to magnetization exchange involving surrounding immobile protons pools or creatine in immobile state . We also investigate the reduction of *dial*-CH_2-FA resonance upon water suppression (WS).

Methods

Forty two healthy volunteers participated the study with written informed consent. The protocol was approved by the institutional IRB. Single-voxel STEAM and/or 2D chemical shift imaging (CSI) spectra were acquired from selected calf muscle regions, with and without WS, on a 7T human scanner (Philips Medical System, Best, The Netherlands), using a quadrature transmit/receive coil. WS optimization was done automatically by the scanner with a sinc-shaped excitation pulse. Typical imaging parameters: TR = 2s, TE = 20 and 40 ms. For single voxel scans, volume ~1 ml and NSA = 128 or 256. For 2DCSI scans, NSA = 2, FOV = 150x150 mm^2, matrix: 12x12 to 20x20, slice thickness = 5 or 10 mm. Spectral fitting was done with ACD software (Advanced Chemistry Development, Inc).

Results and Discussion

As shown in **Fig. 1a,** a typical ^{1}H MRS from muscle tissue is usually dominated by fat signals from two pools, EMCL and IMCL(give reference here). The fat resonances are identified by reference to a spectrum from a known near-pure fat content close to muscle such as tibialis bone marrow (**Fig.1b** and **1c**; **Fig.1c** here is a duplicate of **1b but shifted as in the IMCL case,** for helping resonance assignment). The *dial*-CH_2-FA signal at 3 ppm overlaps with that of CH_3-Cr/PCr. This makes the 3-ppm resonance 3.5-fold larger than the CH_2-Cr/PCr peak at 4ppm, which is significantly different from the theoretical 3:2 ratio for pure Cr/PCr signal.

We further examined the effect of water suppression on signal intensity on both FA and Cr/PCr. **Figure 2** shows that water suppression has no attenuation effect on FA proton signalsfrom marrow tissue. **Figure 3**, however, indicates that water suppression has ~40% attenuation effect on FA protons signal. In this particular case, the orientation of muscle and fat tissue allows a partial separation between *dial*-CH_2-FA (marked as **E** in **Fig.3** inset) and CH_3-Cr/PCr (marked as **D**) Cr/PCr resonances at 7T. In addition, both CH_2-Cr/PCr (marked as **A**) and CH_3-Cr/PCr showed no attenuation effect. Such observation was found in all 42 human cases, though the extent of the FA resonance attenuation varies from case to case and from one muscle location to another. The results suggest that there may be a third source, beside EMCL and IMCL, contributing to FA signals acquired without water suppression, and myocellular membrane lipids (MCML) may be such a candidate since water permeating membranes permits close interaction between lipids and water. Alternatively, in the past this phenomenon has been attributed, to MT effects due to unspecific coupling interaction between CH_3-Cr/PCr and immobile protons pools from macromolecules in muscle tissues.

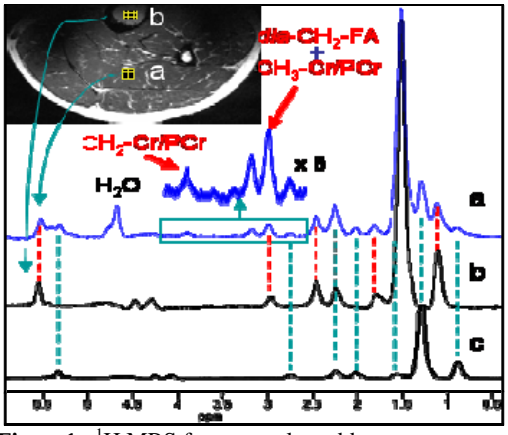

Figure1. ^{1}H MRS from muscle and bone marrow. Signal at ~3ppm is from both Cr/PCr and FA.

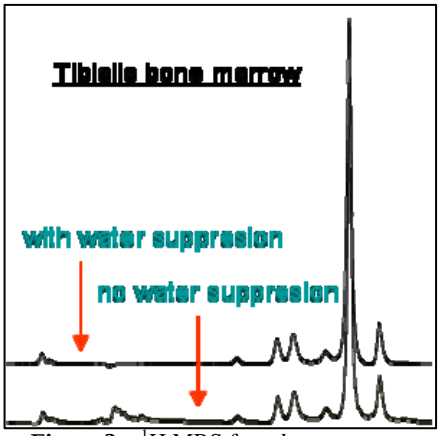

Figure 2. ^{1}H MRS from bone marrow. No attenuation of FA signals by WS is seen.

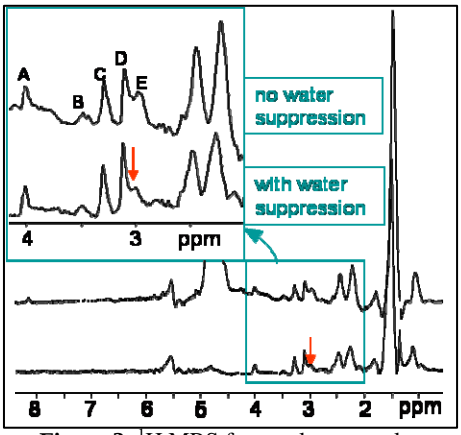

Figure 3. ^{1}H MRS from soleus muscle. FA signal attenuation by WS is seen.

Conclusion

We demonstrated that muscle CH_3-Cr/PCr signal may be contaminated with fat resonances, which opens the issue of metabolite quantification using the CH_3 Cr/PCr resonance. We also show that WS attenuates muscle FA proton resonances but not those of Cr/PCr.

References: Boesch C et al *MR Biomed*. 19:968(2006); Keis R et al JMR 137, 350(1999); Schneider J et al MRM 43:497(2000); Kruiskamp MJ at al JMR 149,8(2001); Machann J, et al *Ann. Rep. NMR Spectrosc*. 50:1(2003)