In Vivo quantification of intramyocellular lipid in Mouse hindleg muscle

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Purpose:

In the last years, in vivo proton NMR spectroscopy (^{1}H-MRS) has been applied successfully in human, dog and rat muscle to investigate the muscle lipids metabolism for some metabolic disorders [1,2] and drug studies [3]. Thank to the magnetic susceptibility difference experienced by intramyocellular (IMCL) and extramyocellular lipids (EMCL), the ^{1}H-MRS is currently the only technique that enables an *in vivo* non-invasive monitoring of IMCL levels.

Nevertheless, no *in vivo* quantification of IMCL pools from mice has been published. The lack of mice muscle lipids investigations is mainly due to the small size of muscle and the strong EMCL signal contamination. Therefore, the aim of the present study is to set up ^{1}H-MRS for muscle IMCL quantification in the mouse.

Methods:

C57/bl6 mice of 13.6±1 months (n=9, 27±4g) were used for this study. MR experiments were performed using a 7T Bruker Biospec system with a volumic birdcage coil and a surface coil for RF transmission and MR signal reception respectively.

Mouse hindleg was positioned parallel to B_0 and scoot images were obtained of muscle in three perpendicular directions [Fig1a] for an optimal selection of VOI. First and second order shims were adjusted using the FASTMAP method. Localized proton spectra were acquired from 5.7±0.5 µl of tibilais anterior muscle (TA) using PRESS sequence. (TR/TE=2000/18ms, 1024 acquisitions). VAPOR and OVS modules were used for water suppression and outer volume saturation respectively.

Data were processed in time domain using the j-MRUI software. Suppression of residual water components was achieved using the HLSVD algorithm. Spectra were corrected for B_0 shift by referencing total creatine (tCr) resonance at 3.04 ppm. Quantification of components was performed using AMARES with inclusion of some prior knowledge (frequencies and linewidths constraint). The IMCL level was expressed relatively to the methyl tCr signal intensity.

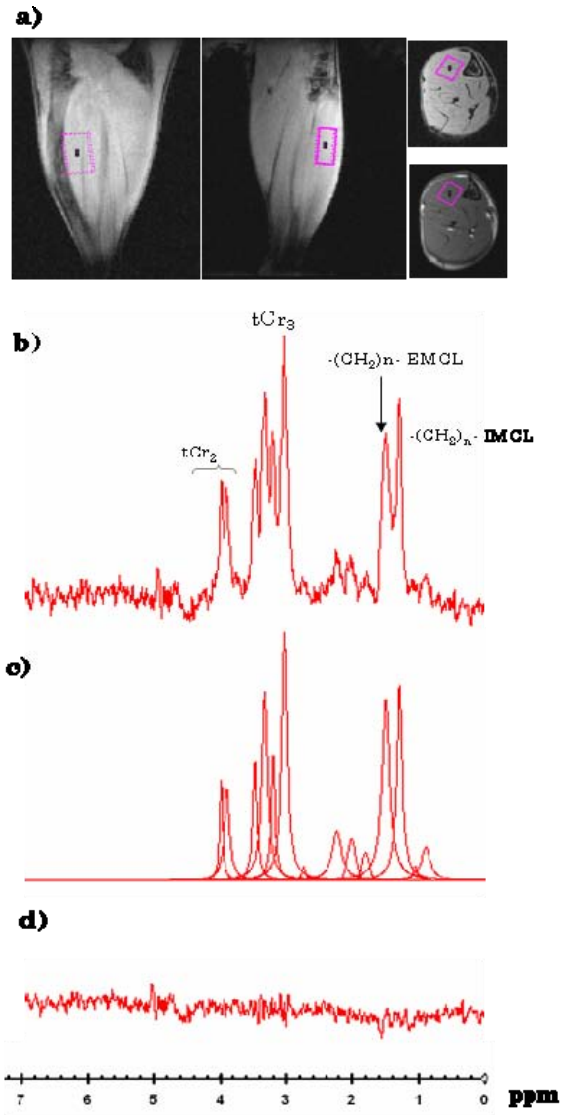

Fig 1: Scoot images of muscle in three perpendicular directions with the VOI (a) and a typical proton spectrum of mouse tibialis anterior muscle (b), the individual component (c) and the residue (d).

Results:

Figure 1a shows the MR images with the selected voxel for localized MR spectroscopy and a typical proton spectrum of mouse tibialis anterior muscle (Figure 1 (b)). The individual components and the residue of achieved quantification are presented in the Figure 1c and 1d.

The most relevant spectroscopic signals of muscle tissue stem from methyl EMCL (1.1 ppm), IMCL (0.9 ppm), methylene IMCL (1.3 ppm) and EMCL (1.5 ppm); other lipid protons resonances appear between 1.6 and 2.5 ppm. The resonance present at 3.04 ppm and the doublet structure at 3.94 ppm are assigned to total creatine methyl and methylene respectively.

An optimal local shimming was obtained in all mice resulted with a water line width of 22±2.5 Hz. The mean Signal to Noise Ratio (SNR), assessed for the methyl creatine signal, was 7.1±3.3.

The IMCL quantification was expressed as IMCL to tCr ratio. The mean value of the nine mice was 0.62±0.12.

Discussion:

A good selection of the VOI, out of bone and adipose tissue, and high shim quality are necessary to obtain a good differentiation between IMCL and EMCL peaks in all of obtained spectra. SNRs of spectra, acquired with an acquisition duration of 34 min, were sufficient to successfully achieve the IMCL/tCr quantification using j-MRUI software.

This study demonstrates the feasibility of the non-invasive *in vivo* ^{1}H-MRS of mouse muscular lipids. That could be interesting for metabolic investigations of obesity, diabetes and insulin resistance mouse models.

References:

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