Optimization of Acetyl-carnitine Detection in Human Skeletal Muscle by 7T 1H MRS

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Introduction

In skeletal muscle the level of acetyl-CoA regulates the activity of multiple metabolic pathways including TCA cycle, lipid oxidation and glycolysis (1). Unfortunately, the small size of CoA pool (~ 10 μM) is currently beyond conventional MRS detection limit (~ 0.5 - 1 mM). However, indirect measurement is possible through the detection of 100-fold larger carnitine pool, owing to the fact that the level of acetyl-carnitine in carnitine pool mirrors that of acetyl-CoA in CoA pool (2). In ^{1}H MRS, acetyl-carnitine is marked by a sharp singlet at 2.12 ppm and can become detectable in certain circumstances, for example after high-intensity exercise (3). Detection of low level of acetyl-carnitine in resting skeletal muscle is limited by the contamination of dominant lipid signals in the chemical shift range of 2.0-2.5 ppm, contributed from –OOC-CH_2- and -CH_2-CH=CH- of both IMCL and EMCL. Spectral fitting of acetyl signal for quantification of acetyl-carnitine is often difficult due to the irregular lineshape of these overlapping lipid signals and the asymmetric characteristics of EMCL components. In the current study, we utilize inversion-recovery technique to optimize the detection of acetyl signal by eliminating the lipid contamination. The method is based on the difference in T_1 relaxation time between acetyl signal (long T_1) and the lipid signals (short T_1) to null the lipid signals in the early phase of recovery process post-inversion, and contamination-free acetyl signal can be obtained with a few minutes using a STEAM sequence.

Methods The protocol was approved by the Institutional Review Board. Informed consent was obtained from all participants (n = 4). The left calf of each subject was placed on a 2-channel partial-volume T/R surface coil with the subjects leg parallel to B_o. Localized single voxel ^{1}H MR spectra were obtained from the medial soleus muscle (typical voxel size: 5 mL) using a 7 Tesla Achieva scanner (Philips Medical Systems) and a STEAM sequence with TR = 8000 ms, TM = 17 ms and TE = 140. A hyperbolic secant pulse with an inversion bandwidth of 3 kHz was used for inversion of the whole spectrum with delay time Td = 250 ms, which was optimized by varying Td in the range from 35 to 6500 ms. The chemical shift was referenced to creatine methyl signal Cr3 at 3.02 ppm.

Results and Discussion

With an inversion delay time of 250 ms, the contaminating lipid signals in the chemical shift region of 2.0-2.5 ppm were completely eliminated by the inversion pulse while the acetyl-carnitine acetyl signal at 2.12 ppm was largely preserved (Figure 1c). The lipid signals in the chemical shift region of 0.7-1.6 ppm region was also reduced 8-fold. In contrast, only partial reduction was observed for the Cr3 signal (17%) and carnitine TMA signal (39%). Thus the inverted contamination-free spectrum (Figure 1d) can be used for quantification of acertyl-carnitine without complication of difficult lineshape fitting.

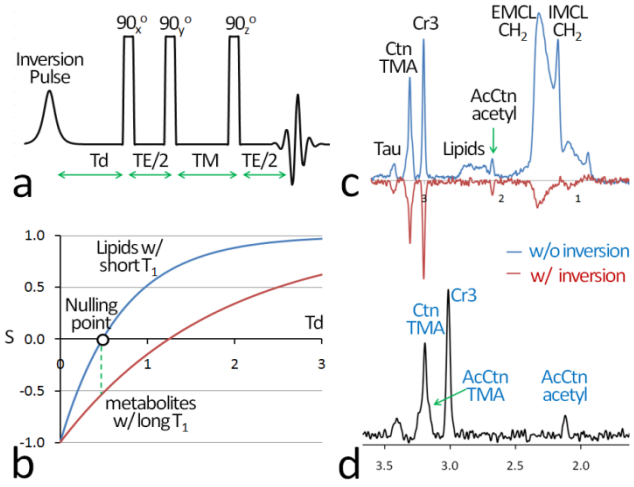

Figure 1. a) ^{1}H MRS STEAM sequence with a inversion preparation pulse; b) Schematic inversion recovery curves of short-T_1 lipids and long T_1 acetyl group showing the null point Td for elimination of lipid signals; c) 7T ^{1}H MR spectra acquired from soleus muscle of female subject without (blue trace) and with (red trace, Td = 250 ms) inversion pulse, NSA 16, scan time 2 min; d) The lipid-contamination-free spectrum obtained by inverting the inversion spectrum in c) (red trace). Ctn: free carnitine; AcCtn: acetyl-carnitine; TMA: trimethylamine group; Cr3: total creatine methyl group; Tau: Taurine; IMCL: intramyocellular lipid; EMCL extramyocellular lipid.

Conclusion: The inversion-recovery strategy, which enables the elimination of lipid contamination, greatly simplified acetyl-carnitine detection and analysis.

References: 1) Brass, E. P. Am J Clin Nutr 2000, 72(supp):618S-23S. 2) Ramsay et al Mol Aspects Med 2004, 25(5-6):475-93. 3) Kris R. et al NMR Biomed 1999, 12(7):471-6