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Communication

Proton magnetic resonance spectroscopy of skeletal muscle: A comparison of two quantitation techniques

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ABSTRACT

Rationale and objectives: The aim of this study was to develop and compare two methods for quantification of metabolite concentrations in human skeletal muscle using phased-array receiver coils at 3 T.
Materials and methods: Water suppressed and un-suppressed spectra were recorded from the quadriceps muscle (vastus medialis) in 8 healthy adult volunteers, and from a calibration phantom containing 69 mM/L N-acetyl aspartate. Using the phantom replacement technique, trimethylamine specifically [TMA] and creatine [Cr] concentrations were estimated, and compared to those values obtained by using the water reference method.
Results: Quadriceps [TMA] concentrations were 9.5 ± 2.4 and 9.6 ± 4.1 mmol/kg wet weight using the phantom replacement and water referencing methods respectively, while [Cr] concentrations were 26.8 ± 12.2 and 24.1 ± 5.3 mmol/kg wet weight respectively.
Conclusions: Reasonable agreement between water referencing and phantom replacement methods was found, although for [Cr] variation was significantly higher for the phantom replacement technique. The relative advantages and disadvantages of each approach are discussed.

1. Introduction

There is increasing interest in the use of proton magnetic resonance spectroscopy (MRS) in the musculoskeletal (MSK) system both for research and clinical investigation [1,2]. Traditionally, results from *in vivo* MRS have been expressed as ratios of metabolite levels, but this approach may be misleading if all metabolite levels in the spectrum are changed relative to normal tissue. Therefore, it is generally preferable to estimate individual metabolite concentrations using spectral quantitation techniques. For MRS in the brain, spectral quantitation techniques using a variety of principles are now well-established [3]. However, there have been few quantitative MRS studies in the MSK system [4–7], and the design of quantitation techniques for MSK MRS presents additional challenges, in that the presence of lipid compartments within the region-of-interest (ROI) needs to be carefully considered. In addition, phased-array receiver coils are increasingly being used for MSK MRS, and quantitation methods designed for use with single-channel transmit-receive coils (e.g. [8]) require further modifications for use with phased-array coils [9].

The most commonly used approach to quantifying metabolite signals uses a reference MRS signal as a standard [3], although other approaches have been explored, such as the 'ERETIC' method which uses an electronically-generated reference signal [10,11]. The reference signal may be 'internal', i.e. from the same region of interest as the metabolites to be determined [12,13], or it may be external to the region of interest, most commonly a standard sample placed adjacent to the subject [14]. A third option is the 'phantom replacement' method, for which the reference sample is scanned separately from the *in vivo* study [8,15]. Each method has its own advantages and disadvantages. The internal reference method assumes that a signal is present in the spectrum (from the same ROI as the compounds to be quantified) that originates from a molecule of stable, known concentration. While the internal referencing method is simple in its implementation, relatively insensitive to inhomogeneities of the B_0 and B_1 fields, and requires no or little additional scan time, its most obvious limitation is that the concentration of the reference compound may not be accurately known. For example, *in vivo* water is often used as the concentration reference, and this may not be constant between

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subjects or regions within the MSK system. The external reference method requires the collection of a spectrum from an external calibration sample placed next to the subject during the same scanning session; while the concentration of the reference compound is precisely known with the external referencing method, the disadvantages of this method include its sensitivity to inhomogeneities of the B_0 and B_1 fields, the additional scanner time required while the patient is in the magnet, and the possible deleterious effects on the *in vivo* B_0 field homogeneity due to the magnetic susceptibility effects of the external sample.

The phantom replacement method combines some of the advantages of internal and external referencing, by utilizing a phantom reference of known concentration; however, the phantom is scanned in a separate session. The advantages of this method include the lack of need for additional patient scan time, the known reference concentration, and the absence of potentially deleterious magnetic susceptibility effects [8]. Nevertheless, despite its advantages, the method remains sensitive to B_1 inhomogeneity and variable radiofrequency coil loading [9]. For transmit-receive coils, the coil loading can be estimated, and corrected for, using the reciprocity theorem [8], and this may be extended for use with receive-only phased array coils by comparing the relative sensitivities of each element of the receive array to that of the transmit coil [9,16,17].

In this manuscript, two approaches for quantitative MRS in the MSK system are compared, namely the internal reference method using the tissue water signal, and the phantom replacement method. Spectra from the quadriceps muscle (vastus medialis) of eight healthy volunteer subjects were quantified using both approaches and compared. The ERETIC method was not used in the current study, because it involves special hardware not available on standard clinical MRI scanners, and also may be sensitive to variable receiver coil loading, which is also difficult to measure in clinical phased-array coils.

2. Methods

Eight healthy normal subjects were recruited for MRS (4 female, age 32 ± 4 years, mean ± standard deviation, range 25–37 years). Written informed consent was obtained from all subjects after institutional review board approval had been obtained. MR images and spectra were acquired on a 3 T scanner (Magnetom Trio, Siemens Medical Solutions, Inc., Malvern, PA) using a four-element 'body matrix' receiver coil and a circularly polarized (CP) body transmit coil. Axial T_2 weighted anatomic images (TR/TE 2886 ms/100 ms, FOV 20 cm, slice thickness 6 mm, acquisition time 4 min) were collected to provide a guide for spectroscopy voxel localization within the right quadriceps muscle. Specifically, the voxel was carefully positioned in the vastus medialis muscle with attention to avoid blood vessels, subcutaneous and other fat, and the femur bone (Fig. 1). For each voxel, a single voxel Point-RESolved Spectroscopy Sequence (PRESS), TR 2 s; TE 135 ms, voxel size $2 \times 2 \times 4$ cm (16 cm^3), 128 averages, acquisition time 4 min 20 s) spectrum was acquired with a 4-pulse CHESS water-suppression scheme [18], followed by two acquisitions without water suppression (16 averages, scan time 40 s), one collected with 'body matrix' receive and the other with the CP-transmit coil used as receive. Prior to data collection, field homogeneity was optimized using linear, manual shimming. For each experiment, the transmitter voltage (V) required for a 90° pulse was recorded.

In vivo water and metabolite T_1 and T_2 relaxation times were also determined in six human subjects, as well as in the phantom used for quantitation (see below). T_1 and T_2 values were estimated by fitting signal intensities recorded as a function of TR and TE using standard equations ($S(\mathrm{TR}) = S_0(1 - \exp(-\mathrm{TR}/T_1)$, $S(\mathrm{TE}) = S_0\exp(-\mathrm{TE}/T_2)$). TR was varied from 530 ms to 20 s, and TE from 30 ms to 500 ms.

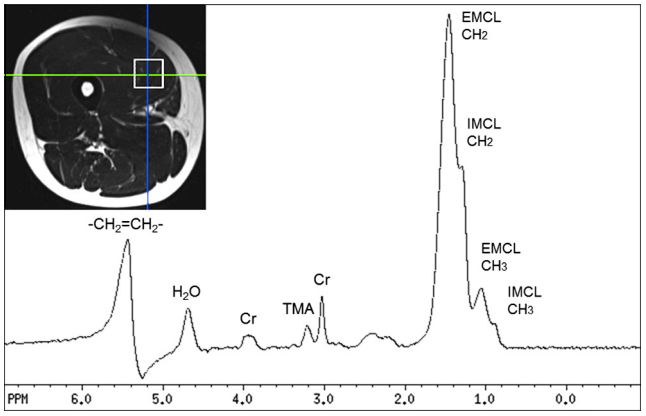

Fig. 1. T_2-weighted MRI showing voxel location used for MRS in one subject, and the corresponding water suppressed spectrum from that region. Signals assigned to unsaturated fats (—CH_2=CH_2—), water (H_2O), trimethylamines (TMA), creatine (Cr – both CH_3 and CH_2 groups at 3.0 and 3.9 ppm, respectively), extra- and intramyocellular lipids respectively (EMCL and IMCL).

Spectral peak areas were determined using AMARES [19] method in the jMRUI [20] software package. Spectra were manually zero and first order phase corrected, eddy-current corrected [21] and 5 Hz exponential line broadening applied. The spectra were fitted to Lorentzian line shape by AMARES. Full details of the AMARES method can be found in reference [19].

2.1. Phantom replacement method

In addition to the scans described above, an additional measurement was performed in a reference phantom consisted of a 3.8 l cylindrical bottle containing a solution of 69 mM/L N-Acetyl Aspartate (NAA). The same voxel size was used both *in vivo* and in the reference phantom. Molar metabolite concentrations [M], where M is either creatine (Cr) or trimethylamines (TMA, primarily carnitine (Ctn) [22]), were calculated from

$$[\mathrm{M}] = [\mathrm{P}] \times \frac{S_i}{S_r} \times \frac{n_r}{n_i} \times \frac{k_r}{k_i} \times \frac{\mathrm{TA}_i}{\mathrm{TA}_r} \times \frac{1}{\mathrm{CF}_{vol}} \quad (1)$$

where the subscripts refer to the scans performed *in vivo* (i) or in the reference phantom (r). [P] is the molar concentration of the reference phantom (69 mM/L NAA), S is the signal intensity (e.g. spectral peak area as determined by AMARES), n is the number of protons contributing to the peak, k is a term to account for T_1 and T_2 relaxation effects, TA is a measure of the phased-array coil loading, and CF_{vol} is a correction term to account for the lipid composition of the voxel. Since metabolites are believed to only be present in the aqueous fraction of the voxel volume, CF_{vol} was calculated from the water/lipid ratio observed in the un-suppressed spectrum, corrected for relaxation time effects, and the proton molar concentration of water and lipid respectively [7]. Molar concentrations were converted to mmol/kg wet weight by dividing by muscle tissue density, which was assumed to be 1.05 gm/mL [23]. As mentioned above, while coil-loading may be directly estimated from the transmitter amplitude required for a 90° pulse in the case of transmit-receive coils, this cannot be done for receive-only phased-arrays. Instead, a 'virtual' transmitter amplitude (TA) for the phased-array is estimated from a knowledge of the CP-transmit coil voltage (V) required for a 90° pulses and a measure of the relative signal intensities (e.g. the tissue or phantom water signal) of the CP-coil and the matrix phased array [17]. The relative sensitivity