Journal of Magnetic Resonance 204 (2010) 91–98

Contents lists available at ScienceDirect

Journal of Magnetic Resonance

journal homepage: www.elsevier.com/locate/jmr

In vivo 1D and 2D correlation MR spectroscopy of the soleus muscle at 7T

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ARTICLE INFO

Article history:
Received 26 November 2009
Revised 7 January 2010
Available online 12 February 2010

Keywords:
MR spectroscopy
L-COSY
7 Tesla
Soleus muscle
In vivo
Human
Intra-myocellular and extramyocellular lipids

ABSTRACT

Aim: This study aims to (1) undertake and analyse 1D and 2D MR correlation spectroscopy from human soleus muscle *in vivo* at 7T, and (2) determine T1 and T2 relaxation time constants at 7T field strength due to their importance in sequence design and spectral quantitation.
Method: Six healthy, male volunteers were consented and scanned on a 7T whole-body scanner (Siemens AG, Erlangen, Germany). Experiments were undertaken using a 28 cm diameter detunable birdcage coil for signal excitation and an 8.5 cm diameter surface coil for signal reception. The relaxation time constants, T1 and T2 were recorded using a STEAM sequence, using the 'progressive saturation' method for the T1 and multiple echo times for T2. The 2D L-Correlated SpectroscopY (L-COSY) method was employed with 64 increments (0.4 ms increment size) and eight averages per scan, with a total time of 17 min.
Results: T1 and T2 values for the metabolites of interest were determined. The L-COSY spectra obtained from the soleus muscle provided information on lipid content and chemical structure not available, *in vivo*, at lower field strengths. All molecular fragments within multiple lipid compartments were chemically shifted by 0.20–0.26 ppm at this field strength. 1D and 2D L-COSY spectra were assigned and proton connectivities were confirmed with the 2D method.
Conclusion: In vivo 1D and 2D spectroscopic examination of muscle can be successfully recorded at 7T and is now available to assess lipid alterations as well as other metabolites present with disease. T1 and T2 values were also determined in soleus muscle of male healthy volunteers.

1. Introduction

Two-Dimensional MR spectroscopy, first reported by Jeener [1,2] as a simple two-pulse sequence and known as the Correlated SpectroscopY (COSY) experiment, has proven invaluable in many disciplines. Unlike stable chemical compounds and relatively stable proteins, cultured cells and tissue biopsy specimens have a limited experimental time due to metabolic degradation. This changed the perspective of data acquisition and analysis for 2D MR data sets. The 2D COSY method was first applied to cells in 1984 [3] and then to tissues in 1988 [4].

It was demonstrated that both viable cells and tissues give rise to a plethora of resonances, up to 60 different chemical species all of which can alter simultaneously with the onset and development of disease [5]. It was, however, realized that the study of cells and tissues involved a detailed and complex inter-relationship of biochemical pathways that with appropriate clinical and pathological correlation provided, biochemical, diagnostic and prognostic information [6–14]. The wide range of molecules with equally diverse T1 and T2 relaxation values meant that any one 2D data set needed to be post processed using different mathematical criteria in order to inspect all molecules active on the MR timescale [15].

The literature on 2D MRS of biopsies paved the way for *in vivo* 2D studies on humans. The first COSY studies were undertaken on the brain [16] at a 2T field strength with a voxel size of 240 cm^3 and total experimental time of 102 min. The resultant 2D spectra were broad and most cross-peaks were weak and overlapped. Whilst these early results proved that 2D data could be accrued *in vivo*, it also became clear that the spectral dispersion obtained at higher fields is important for diagnostic purposes.

With the introduction of higher field magnets for clinical usage i.e. 3T and above, the utilization of *in vivo* 2D spectroscopy became an option for the interrogation of biochemistry of disease and could assist with unambiguous resonance assignment. The higher the field strength, the greater the potential for improved SNR, spectral resolution and reduced spectral acquisition time [17].

Our objective here was to firstly evaluate the chemical information available from the 2D L-COSY [18] recorded from human soleus muscle, *in vivo* at 7T, and to ascertain if this technology could provide information on the pools of lipids and their contents. Localized MRS has been applied successfully, by others, to human

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doi:10.1016/j.jmr.2010.02.008

skeletal muscle *in vivo* where two lipid pools, referred to as intramyocellular lipids (IMCL) and extramyocellular lipids (EMCL) [19–22], were reported. It was proposed that the resonances from the lipids in the muscle spectra were seen as two signals due to the geometrical arrangement and anisotropic susceptibility of these lipid compartments [19].

Secondly we aimed to measure the relaxation characteristics of human soleus muscle at 7T. Relaxation measurements can be challenging at high fields as the evolution of J-coupled species is TE dependent and can complicate spectral line shapes. Such measurements are necessary for the design of pulse sequences. Despite the reports of relaxation properties for soleus and tibialis anterior muscles (3T) [23], soleus muscle (1.5T) [20], skeletal muscle (1 and 2.4T) [24], soleus, tibialis posterior and tibialis anterior (4T) [25], tibialis anterior muscle (7T) [26], the literature to date has failed to provide a comprehensive report of all T1 and T2 values for the soleus muscle.

We report here the complete relaxation properties (T1 and T2 values) for the 1D spectra from the soleus muscle of healthy volunteers at 7T, and the chemistry recorded *in vivo* using the 2D L-COSY method which provides better spectral dispersion and more certainty in resonance assignment.

2. Experimental

2.1. Volunteers

Apparently healthy male volunteers ($n = 6$, mean age = 33 ± 9.3 years), taking no medication, participated in this study. An all male cohort was chosen to avoid any sources of error due to the prior claim that the degree of unsaturation within IMCL and EMCL was lower in female subjects than male subjects, and that total lipid content in females was higher than in males [27]. To ensure reproducibility of measurements, the spectroscopic voxel was located in superior third of the soleus muscle. Informed consent was obtained from all participants. All subjects were supinely positioned (feet first) with their right leg inside a transmit coil. To improve separation between IMCL and EMCL and retain residual dipolar couplings, care was taken to position the leg parallel to the Bo field [19,28]. This study was approved by the local institutional review board.

2.2. MR imaging and spectroscopy

Experiments were performed on a 7T whole-body scanner (Siemens AG, Erlangen, Germany, software version VB15A), operating at a proton resonance frequency of 297.18 MHz, using and a 28 cm diameter detunable birdcage coil for signal excitation. Magnetic resonance imaging (MRI) and spectroscopy (MRS) in muscle were performed using an 8.5 cm diameter surface coil for signal reception.

2.2.1. Muscle MRI

Prior to the spectroscopy sequences being undertaken, FLASH 2D gradient echo images were acquired in all three dimension using the following parameters: TE/TR = 5/30 ms, FOV = 200 × 200 mm^2, matrix = 512 × 512, slice thickness = 5 mm, inter-slice spacing = 6 mm, and a flip angle = 10°.

2.2.2. T1 spectroscopic measurements

T1 was measured using the "progressive saturation" method [29–32]. A voxel size of 15 × 15 × 20 mm^3, and a TE of a stimulated echo acquisition mode pulse sequence (STEAM) [33,34] was set to 20 ms, mixing time to 10 ms, while TR was set to 430, 530, 650, 750, 950, 1500, 2000, 3000, 4000, 6000, 7000 ms, with a constant number of 16 averages, acquisition duration of 170 ms, four dummy preparation scans, 512 points per echo were acquired, a bandwidth of 3 kHz, RF offset frequency was set to 1.7 ppm lower than water frequency (i.e. on creatine methyl group), and water suppression routine 'WET' was activated [35]. 'WET' method is made out of three shaped frequency selective RF pulses, each followed by a spoiler gradient pulse aligned along orthogonal orientations. 'WET' water suppression technique was used for all spectroscopic experiments were water suppression is required. Raw data was zero-filled to 1.5 k before FT and was then manually phased. We made sure that at all times TE was much smaller TR so that progressive saturation method could be reliably implemented [32]. T1 was calculated as the slope of a straight line obtained by plotting – TR versus $\mathrm{Ln}\left(1-\frac{S}{S_o}\right)$, where S and S_o are resonance integrals at individual TR values and maximum TR values, respectively. To measure T1 value of water, the number of averages was dropped to four, water suppression was disabled, and all other parameters were kept the same. Spectral raw data processing and fitting were done with JMRUI [36], whereas T1 and error propagation were done with Mathematica [37].

2.2.3. T2 spectroscopic measurements

A series of STEAM 1D experiments, using the same voxel size and positioning as described above, were acquired at TE values of 50, 100, 150 and 200 ms and TR set to 7s, spectral width 3 kHz, 512 acquired points, 170 ms acquisition duration, four dummy preparation scans, 16 averages per TE value, RF offset frequency was set to 1.7 ppm lower than water frequency (i.e. on creatine methyl), and water suppression was activated ('WET' technique) [35]. In some subjects, data points TE = 250 and 300 ms were also acquired, but T2 results were not affected by these additional points, and thus were omitted for remaining subjects. Raw data was zero-filled up to 1.5 k before FT and was then manually phased. T2 was calculated as the slope of a straight line obtained by plotting TE versus Ln(S), where S is resonance integral at different TE values. Multi-exponential behavior of T2, i.e. presence of multiple compartments, was tested by multi-exponential fitting of data acquired at above TE values as well as TE values of 250 and 300 ms. To measure T2 value of water, the number of averages was dropped to four, water suppression was disabled, and all other parameters were kept the same. Spectral raw data processing and fitting were undertaken using JMRUI [36], whereas T1 and error propagation calculations were undertaken using Mathematica (Wolfram, version 6) [37].

2.2.4. L-COSY measurements

The same voxel as was used for T1 and T2 measurements was also used for L-COSY. The L-COSY sequence was applied with a TE (initial) of 30 ms, TR of 2s, eight averages per increment, spectral width in F2 was 4000 Hz, t1 increment size of 0.4 ms, indirect spectral width used was 2500 Hz and the number of increments was 64. The "WET" water suppression method [35] was applied before the acquisition sequence. The L-COSY acquisition time was 17 min. Data processing was done using Felix software package [38], using the following processing parameters used were: F2 domain (skewed sine-squared window, 2048 points, magnitude), F1 domain (sine-squared window, linear prediction to 128 points, zero-filling to 512 points, magnitude). The total creatine methyl resonance at 3.02 ppm was used as an internal chemical shift reference in F1 and F2 [20]. Specific absorption rate (SAR) was within acceptable limits at all times.

2.2.5. Statistical analysis

T1, T2 and L-COSY data were acquired from a single subject two times to ensure intra-subject reproducibility. Inter-subject vari-