31P MRS at 7T shows a relation between the alkaline pH compartment content compared to phosphocreatine recovery kinetics at 1.5T

J. V. Oorschot[1], H. Kan[2], A. Webb[2], K. Nicolay[1], and J. Jeneson[1]
[1]Biomedical NMR, University of Technology Eindhoven, Eindhoven, Noord-Brabant, Netherlands, [2]C.J. Gorter Center for High Field MRI, Dept. of Radiology, Leiden University Medical Center, Leiden, Netherlands

Introduction. Non-invasive assessment of mitochondrial content in skeletal muscle is important in clinical research, for instance in diabetes, and sports medicine (1). ^{31}P MR spectroscopy has been widely used to determine muscle mitochondrial capacity non-invasively using the rate of phosphocreatine (PCr) recovery after exercise as index (2). In previous research in resting skeletal muscle, at a field strength of 7 tesla, a peak was observed 0.4 ppm downfield from the cytosolic Pi resonance (Pi_1), which was attributed to the Pi pool inside the mitochondrial matrix (Pi_2)(3). If correct, this signal could provide a good measure to determine mitochondrial capacity, assuming that the concentration of Pi is the same in both compartments. As the method can be applied in resting muscle, it provides significant advantages over approaches that require in-magnet exercise in terms of technical availability and patient cooperation. Endurance training leads to a significant increase of mitochondrial capacity in skeletal muscle (4), and a higher PCr recovery rate (5). In this study, we aimed to compare the PCr recovery rate, as the standard non-invasive measure for mitochondrial capacity, with the signal intensity of the Pi_2 peak, which is attributed to the mitochondrial Pi pool in endurance trained athletes compared to reasonably active subjects. If these indices are correlated, this will provide yet further evidence that the Pi_2 signal originates from the mitochondrial matrix.

Methods. The study was conducted in six healthy male volunteers (age range 21-25 years). Three subjects were highly trained active endurance athletes (exercise 6-9 times/week) (ATH). The other three subjects were reasonably physical active (1-2 times/week) (REG). Data from resting muscle were acquired on a 7 tesla Philips Achieva scanner using a custom-built transmit and receive double-tuned ^{1}H and ^{31}P coil setup , with square coils for ^{31}P (10 cm) and ^{1}H (12 cm). The MR data were acquired from the lateralis muscle in the right upper leg. ^{31}P spectra were obtained using 2D CSI (FOV 160x160 mm; matrix size 8x8; Hamming weighted acquisition and post processing; 32 averages ;TR 1680 ms, image based shimming (6)). Adiabatic half passage 90 degrees RF pulses (3.3 ms) were applied with the transmitter frequency set to 5.0 ppm downfield from the PCr peak.

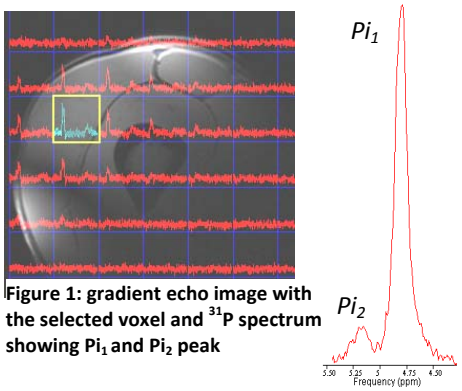

Figure 1: gradient echo image with the selected voxel and ^{31}P spectrum showing Pi_1 and Pi_2 peak

Within a week PCr recovery data were acquired on a 1.5 tesla whole-body Philips magnet using a custom-built transmit and receive double-tuned ^{1}H and ^{31}P coil setup with circular coils for ^{31}P (5 cm) and ^{1}H (6 cm), interfaced to a Bruker Biospin console. Exercise was performed using a MR-compatible bicycle ergometer for in-magnet exercise (7). ^{31}P spectra were obtained with surface coil localization on the right vastus lateralis (2 averages, TR 6 s). Adiabatic BIR60 pulses (4 ms) were applied. A light sensor setup was used to gate spectrometer data acquisition during cycling.

The CSI dataset was visualized using 3DiCSI software (8), and a voxel was selected in the lateralis muscle. The free induction decay was analyzed using the jMRUI software package. Peak areas for the two Pi signals from the 7T data and PCr signal from the 1.5T data were obtained by fitting Lorentzian line shapes and corrected for partial saturation effects. Using a least squares method the amplitudes of PCr were fit to a mono-exponential model, obtaining rate constant τ_{PCr} (9). Pi_2 signal intensities were correlated to PCr recovery rates using Pearsons r. Additionally, differences between the groups were compared with a t-test and considered significant at $p<0.05$.

Results. In the ^{31}P spectra obtained at 7T, a peak at 0.4 ppm downfield from the cytosolic Pi peak was reproducibly detected (Fig. 1), indicating an alkaline pH compartment. In the endurance trained athletes (ATH) the Pi_2 signal was 9.2 ± 2.7% of the Pi_1 resonance. In the reasonably active group (REG) the Pi_2 signal was significantly lower at 4.0 ± 0.7% of the Pi_1 signal. For the PCr recovery data a τ_{PCr} of 15 ± 5 s was found in endurance trained athletes, compared to significantly higher τ_{PCr} of 33 ± 4s in reasonably active subjects. The Pi_2/Pi_1 ratio was significantly correlated to the PCr recovery rate, with an r of -0.90.

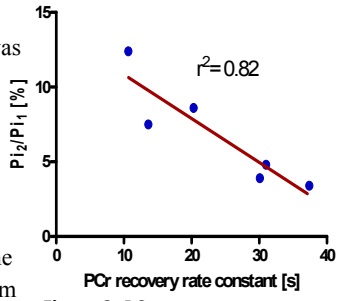

Figure 2: PCr recovery rate versus Pi_2/Pi_1 ratio.

Discussion. In this study, we observed a significant correlation between Pi_2/Pi_1 ratio and the PCr recovery rate, the standard measure for mitochondrial capacity (fig 2). This provides further evidence that the Pi_2 signal originates from the mitochondria and suggests that it can be used as a measure for the mitochondrial content. Previous studies reported both a lower (10) as well as a higher (11) cytosolic Pi content of leg muscle of endurance athletes compared to untrained subjects. Here we assume that cytosolic Pi is similar. Future studies employing broadband AHP pulses allowing additional quantification of the Pi_1/ATP ratio will therefore be necessary to objectify if the amplitude of the putative mitochondrial Pi resonance in resting spectra of human muscle indeed scales with mitochondrial content. A twofold increase was observed in Pi_2/Pi_1 ratio in athletes, the same magnitude of increase which was reported for mitochondrial volume fraction in other studies (12,13).

This method to determine muscle mitochondrial volume fraction would be a major improvement over current conventional methods in terms of technical availability and patient cooperation.

References. [1] **Chance B et al**. NMR Biomed 2006 [2] **Kent-Braun JA et al.** Radiol Clin 1994 [3] **Kan HE et al.** NMR Biomed 2010 [4] **Morgan TE et al.** New York Plenum 1971 [5] **McCully KK et al.** Physiol Pharmacol. 1992 [6] **Schar M et al.** Magn Reson Med 2004 [7] **Jeneson JA et al.** MR in Med. 2010 [8] **Zhao Q. et al.** 2009 [9] **Arnold D.L. et al.** MR in Med. 2005 [10] **Vandenborne K. et al.** Am. J. Physiol. 1995. [11] **Bernus G. et al.** Med. Sci. Sports Exerc. 1993 [12] **Fitts RH et al.** Am. Journ. Physio 1975 [13] **Johansen L et al.** Int Sports Med 2003