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

J. V. Oorschot¹, H. Kan², A. Webb³, K. Nicolay⁴, and J. Jeneson¹

¹Biomedical NMR, University of Technology Eindhoven, Eindhoven, Noord-Brabant, Netherlands. ²C.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). 31P 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 (Pi₁), which was attributed to the Pi pool inside the mitochondrial matrix (Pi₂) (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 Pi₂ 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 Pi₂ 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 physically 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 ¹H and ³¹P coil setup, with square coils for ³¹P (10 cm) and ¹H (12 cm). The MR data were acquired from the lateralis muscle in the right upper leg. ³¹P 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. 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 ¹H and ³¹P coil setup with circular coils for ³¹P (5 cm) and ¹H (6 cm), interfaced to a Bruker Biospin console. Exercise was performed using a MR-compatible bicycle ergometer for in-magnet exercise (7). ³¹P 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 τPCr (9). Pi₂ 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 ³¹P 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 Pi₂ signal was 9.2 ± 2.7% of the Pi₁ resonance. In the reasonably active group (REG) the Pi₂ signal was significantly lower at 4.0 ± 0.7% of the Pi₁ signal. For the PCr recovery data a τPCr of 15 ± 5 s was found in endurance trained athletes, compared to significantly higher τPCr of 33 ± 4s in reasonably active subjects. The Pi₂/Pi₁ ratio was significantly correlated to the PCr recovery rate, with an r of -0.90.

Discussion. In this study, we observed a significant correlation between Pi₂/Pi₁ ratio and the PCr recovery rate, the standard measure for mitochondrial capacity (fig 2). This provides further evidence that the Pi₂ 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 Pi₁/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 Pi₂/Pi₁ 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.
