Mapping the Unidirectional Pi-to-ATP Fluxes in Muscles of the Lower Leg by Using Progressive Saturation 31P-MRI with PCr Suppression at 7.0 T

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TARGET AUDIENCE: Those interested in muscle physiology, muscle bioenergetics, metabolism, and technical developments in high-field multinuclear MRI. PURPOSE: Phosphorus (31P) saturation transfer (ST) methods can assess the turnover rates of important metabolic reactions such as the adenosine triphosphate (ATP) synthesis/hydrolysis cycle, during which high-energy phosphate is reversibly transferred between ATP and inorganic phosphate (Pi). Recent studies have shown that certain diseases, including insulin resistance and diabetes, alter the Pi-to-ATP flux in skeletal muscle and have therefore generated interest in the further development of 31P-ST methods that could potentially be used for the clinical assessment of patients. Due to the relatively low concentration of Pi in skeletal muscle (~2-6 mM), which results in low MR signal, the ATP synthesis rates and fluxes have been studied mostly with unlocalized spectroscopy. However, localized measurements could potentially provide additional information on the effect of normal aging or disease on different muscle groups with different fiber type composition. In this study we focused on the development and implementation of an imaging method for simultaneously measuring the kinetics of ATP synthesis and the Pi-to-ATP fluxes in muscles of the lower leg within acquisition times that can be tolerated by patients (~ 50 min).

METHODS: The pseudo first order forward rate constant of the ATP synthesis reaction (k) can be measured through the progressive ST experiment, in which γ-ATP is saturated for different durations (t sat), resulting in Pi signal decrease. Under fully-relaxed conditions, assuming complete saturation of γ-ATP, the magnetization of Pi as a function of t sat, is described by the following equation 5: M(t sat)/M0 = 1 - k1T1' [1 - exp(-t sat/ T1')], where M(t sat) is the magnitude of the Pi signal as a function of t sat, M0, the reference Pi signal (without saturation), and T1' the longitudinal relaxation of Pi when γ-ATP is saturated. By measuring Pi for a sequence of t sat, we obtain a curve of M(t sat)/M0. A two-parameter fitting of the data to the above equation can determine k1 and T1'. The product of k1 and Pi concentration estimates the unidirectional flux of Pi to form ATP, Vγ. In the skeletal muscle, PCr concentration is significantly higher than that of Pi. Therefore, imaging of Pi may be contaminated by PCr signals that can affect quantification both of k1 and Vγ. To address this issue we developed a 31P-ST imaging sequence with PCr suppression (Fig.1). We tested the sequence on four healthy non-smoking volunteers (three men and one woman, 33.5 ± 6.8 years of age, with BMI 24.2 ± 2.8) without any medical history of disease affecting muscle function or blood flow. All subjects were examined on a 7 T MRI system (Siemens Medical Solutions, Erlangen, Germany) using a dual-tuned 31P/H quadrature transmit-receive knee coil (Rapid MRI, Ohio) (18 cm inner diameter). We measured mean k1 and Vγ in the tibialis anterior, the gastrocnemius and the soleus muscles of the lower leg. RESULTS: Figure 2A shows the complete saturation of γ-ATP and excitation of Pi using our sequence. The estimated k1 and Vγ in the tibialis anterior, were 0.08 ± 0.02 s⁻¹ (mean ± SD) and 0.21 ± 0.06 mM s⁻¹ respectively. In the gastrocnemius k1 was 0.07 ± 0.03 s⁻¹ and Vγ was 0.26 ± 0.08 mM s⁻¹. In the soleus k1 was 0.10 ± 0.04 s⁻¹ and Vγ was 0.30 ± 0.09 mM s⁻¹. DISCUSSION: Our preliminary results suggest that localized measurement of the ATP synthesis rate and the Pi-to-ATP fluxes can be obtained in several muscles of the leg using our imaging sequence. The exchange rates in our study are in close agreement with those reported previously using unlocalized 31P-MRS. We are only aware of one study with localized 31P-MRS measurements in the gastrocnemius and the soleus, where statistically significant differences were observed between the two muscles on five subjects. CONCLUSION: Mapping the kinetics of the ATP synthesis reaction and the Pi-to-ATP fluxes in several muscles of the lower leg using our imaging method is feasible at ultra-high field and may provide useful insights in the study of diseases such as insulin resistance and diabetes.


Fig.1: Saturation Transfer Imaging (31P-ST-MRI) pulse sequence: The ST module consists of a train of Gaussian pulses (50 ms each), which saturates γ-ATP. Spoiler gradients destroy any residual transverse magnetization between two consecutive pulses. The number of Gaussian pulses defines t sat in each experiment. A spectrally selective Gaussian pulse (duration: 8 ms, bandwidth: 250 Hz) suppresses PCr. Imaging is performed using a centric ordered three-dimensional turbo spin echo sequence (3D-TSE) with a frequency selective 90° excitation pulse (8 ms duration, 250 Hz bandwidth) that excites only the Pi resonance. Reference data are acquired with the ST module turned off. Parameters of the 3D-TSE are: TR: 20 s, Effective bandwidth: 250 Hz, FOV: 300 x 300 x 200 mm³, matrix size: 24 x 24 x 4, voxel size: 7.8 mL. Eight images were acquired with different t sat (range: 0.6 – 10.3 s).

Fig.2: A) Validation of efficient saturation of γ-ATP and suppression of PCr with unlocalized 31P-MRS. Unlocalized spectra of the lower leg muscles without any preparation pulses (left). Spectrum acquired with γ-ATP saturated and no PCr suppression shows off-resonance excitation of PCr (middle). Spectrum with γ-ATP saturation and PCr suppression results in excitation of only the Pi resonance (right). B) Anatomical 1H cross-section of the lower leg (left). Reference Pi image (t sat = 0 s) (middle), and Pi image with t sat = 10.3 s (right).