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

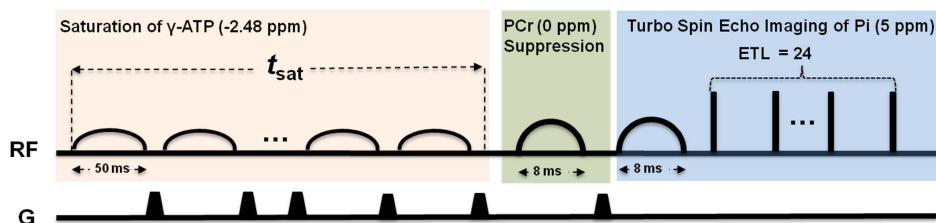Prodromos Parasoglou[1], Ding Xia[1], and Ravinder R Regatte[1]
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TARGET AUDIENCE: Those interested in muscle physiology, muscle bioenergetics, metabolism, and technical developments in high-field multinuclear MRI. **PURPOSE**: Phosphorus (^{31}P) saturation 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[1] and have therefore generated interest in the further development of ^{31}P-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)[2], which results in low MR signal, the ATP synthesis rates and fluxes have been studied mostly with unlocalized spectroscopy[3]. 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_f) can be measured through the progressive ST experiment[4], 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]: $M(t_{sat})/M_0 = 1-k_f T_1'[1-exp(-t_{sat} / T_1')]$ where $M(t_{sat})$, the magnitude of the Pi signal as a function of t_{sat}, M_0, the reference Pi signal (without saturation), and T_1' the longitudinal relaxation of Pi when γ-ATP is saturated. By measuring Pi for a sequence of t_{sat} we obtain a curve of $M(t_{sat})/M_0$. A two-parameter fitting of the data to the above equation can determine k_f and T_1'. The product of k_f and Pi concentration estimates the unidirectional flux of Pi to form ATP, V_f. In the skeletal muscle, PCr concentration is significantly higher than that of Pi[2]. Therefore, imaging of Pi may be contaminated by PCr signals that can affect quantification both of k_f and V_f. To address this issue we developed a ^{31}P-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 ^{31}P/^{1}H quadrature transmit-receive knee coil (Rapid MRI, Ohio) (18 cm inner diameter). We measured mean k_f and V_f 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 k_f and V_f 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 k_f was 0.07 ± 0.03 s^{-1} and V_f was 0.26 ± 0.08 mM s^{-1}. In the soleus k_f was 0.10 ± 0.04 s^{-1} and V_f 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 ^{31}P-MRS[3]. We are only aware of one study with localized ^{31}P-MRS measurements in the gastrocnemius and the soleus[6], 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 (^{31}P-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 echo and echo spacing 26 ms, echo train length (ETL): 24, FOV: 300 x 300 x 200 mm^3, 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).
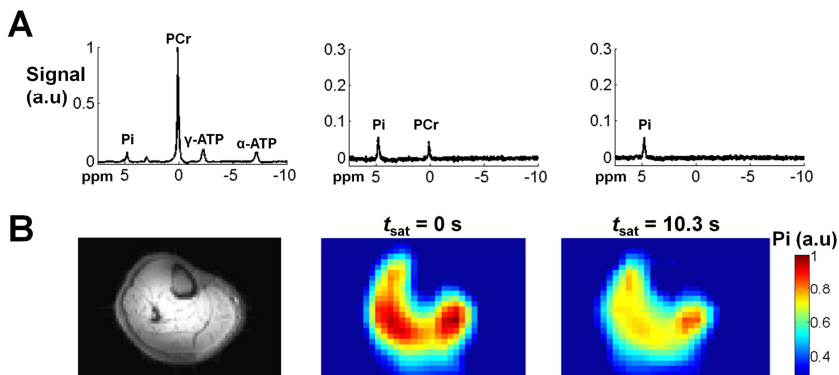
Fig.2: A) Validation of efficient saturation of γ-ATP and suppression of PCr with unlocalized ^{31}P-MRS. Unlocalized spectra of the lower leg muscles without any preparation pulses (left). Spectrum acquired with γ-ATP saturated and no PCr suppression shows off-resonance excitation of PCr (middle). Spectrum with γ-ATP saturation and PCr suppression results in excitation of only the Pi resonance (right). **B)** Anatomical ^{1}H 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).

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