# In Vivo Quantification of ATP Synthesis Rates in Rat Skeletal Muscle by $^{31}$P Spectroscopic Magnetic Resonance Fingerprinting

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**Target audience**: Researchers and clinicians interested in high-energy phosphate metabolism and magnetization transfer.

**Background/Purpose:** $^{31}$P Magnetization Transfer spectroscopy (MT-MRS) has been proposed as a method of measuring ATP synthesis rates via both mitochondrial ATP synthase and creatine kinase (CK) in vivo. However, current $^{31}$P MT-MRS methods require prohibitively long imaging time to accurately measure ATP synthesis rates for many applications, particularly for ATP synthase[1]. Recent development of Magnetic Resonance Fingerprinting (MRF) provides a completely new framework for data acquisition that allows simultaneous measurement of several tissue properties with increased efficiency[2]. This study aimed to develop and validate a novel $^{31}$P Magnetization Transfer Fingerprinting (MT-MRF) method designed to increase measurement efficiency of ATP synthesis through ATP synthase while maintaining CK sensitivity.

**Methods:** The MRF acquisition consisted of 400 acquisitions organized into 3 sections (Fig. 1). The first section was preceded by a selective $P_i$ inversion pulse with the goal of measuring $P_i$ apparent recovery under minimal γATP attenuation. The second section was preceded by a selective γATP and PCr inversion with the goal of maximizing sensitivity of MT through ATP synthase[3]. The final section used selective γATP saturation to encode MT sensitivity via both ATP synthase and CK. All 3 sections were composed of repeating blocks of 4 acquisitions. A 1.1-ms Gaussian pulse with the carrier frequency set at the resonance of $P_i$ was used for excitation. The flip angle of the excitation pulses followed a pattern of 17.5°, -35°, 17.5°, 0°. The four acquisitions within each block used a constant TR of 7.7 ms. Following each block was a 240 ms inter-block delay. In the 3rd section, two selective sinc pulses were applied during the inter-block delay for γATP saturation. A 6 second interscan delay was used to reestablish longitudinal magnetization before starting the next repetition, resulting in a total repetition time of 34 s.

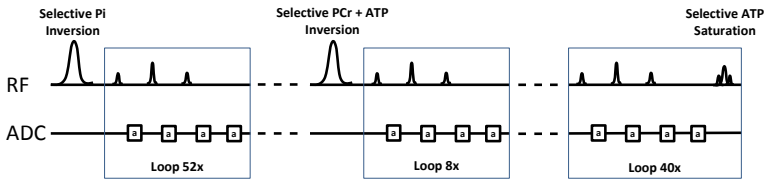


**Figure 1,** Pulse Sequence Diagram for MT-MRF Acquisition

FID signals were acquired in a 5.1 ms window with 10 μs dwell time. After Fourier transform, metabolite signals resolved to a single spectral bin (194 Hz spectral resolution). The signal evolutions of $P_i$, PCr, and γATP were extracted from their corresponding spectral bins. A Matlab-based Bloch-McConnell simulator was developed to simulate the signal evolution. The forward rate constant of ATP synthase ($k_f^{ATP}$) and creatine kinase ($k_f^{CK}$), metabolite concentration ratios, $T_1$ of $P_i$ and PCr, and the chemical shift of all three metabolites were determined by maximizing the inner product between the simulated evolutions and the acquired signal using Nelder-Mead simplex optimization algorithm.

Animal studies were performed on rat hind-limb at 9.4T scanner (Bruker) using a custom-built $^{31}$P saddle coil. For comparison, conventional saturation transfer inversion recovery (ST-IR) acquisitions were acquired using 6 inversion times ranging from 0.3 to 14 s, and a 14 s inter-scan delay. A control spectrum was also acquired with 14 s inversion time and the saturation pulse applied at the opposite side of the $P_i$ resonance peak. MRF and ST-IR acquisition used 18 and 4 averages, respectively, giving rise to same acquisition time of 10 min each. MRF and ST-IR were performed in an interleaved fashion with 10 acquisitions for each method.

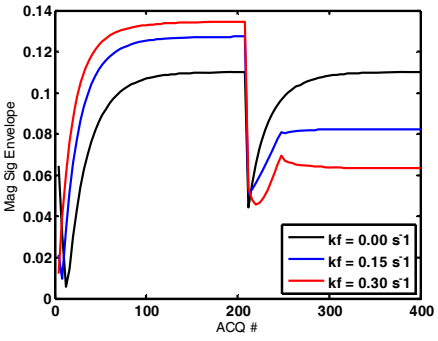


**Figure 2**, Simulated l2-normalized magnitude signal intensity envelopes for $P_i$ signal at different MT rate constants illustrating MT encoding.

**Results**: Fig. 2 shows simulated $P_i$ fingerprint envelopes that demonstrate the unique signal evolution pattern at three different rates of ATP synthase. Fig. 3 shows measured rate constants for both ATP synthase and creatine kinase using MRF and ST-IR. MT-MRF Measurement using 100 min signal averages showed good agreement with ST-IR method for both ATP synthase (0.19 vs 0.18 $s^{-1}$) and CK (0.42 vs 0.42 $s^{-1}$). The mean of the rate constants determined from the 10-min acquisitions were also similar between MRF and ST-IR for both ATP synthase and creatine kinase. However, the measurement of ATP synthase showed improved precision with a standard deviation half of that measured by ST-IR (0.07 vs 0.15 $s^{-1}$), suggesting higher measurement efficiency.

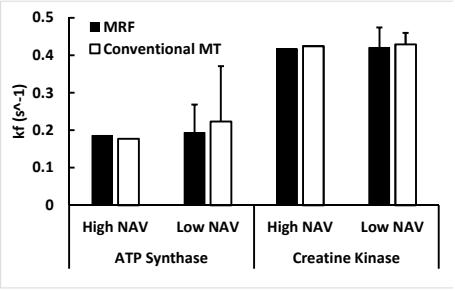


**Figure 3,** MT rate constant measurement comparisons between MRF and conventional MT method in rat hind limb.

**Discussion/Conclusion**: The flexibility of MRF sequence design allows a specific MRF sequence to be tailored to increase its sensitivity to specific parameters of interest. In this work, we present the use of an unconventional spectroscopic MRF method to increase the sensitivity to ATP synthase rate. The current iteration of our sequence shows a 2 fold increase in precision in the measurement of ATP synthase rate while retaining the sensitivity and precision of CK measurement. This may translate to an approximately 4 fold reduction in experimental time. Further improvements are to be expected with additional pulse sequence optimization.

**Acknowledgements:** The authors would like to acknowledge funding from NIH TL1-TR000441, T32-EB007509, F30-HL124894, R01-HL73315.

**References:**

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