Brain high-energy phosphates and creatine kinase synthesis rate under graded isoflurane anesthesia: An *in vivo* ^{31}P Magnetization Transfer Study at 11.7 Tesla

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Target Audience Researchers involved in ^{31}P NMR and metabolic rate measurements.

Purpose ^{31}P magnetization transfer (MT) offers a unique, non-invasive tool for directly measuring the creatine kinase (CK) rate of ATP synthesis in vivo. ^{31}P MT measures the forward CK rate ($k_{f,CK}$) by using frequency-selective RF energy to saturate γ-ATP while observing PCr amplitude. ^{31}P MT has been used to measure CK rates under different anesthetics, pharmacologic and functional stimulations[1-5], and in association with stroke[6]. The protocols for these applications of ^{31}P MT ranged from half an hour to ten hours.

This study implemented the accelerated ^{31}P Four Angle Saturation Transfer (FAST)[7] technique to evaluate the brain high-energy phosphates and the forward CK synthesis rate under graded isoflurane anesthesia. High field (11.7 Tesla) and a small sensitive surface coil were used to improve ^{31}P signal sensitivity. BIRP[8] radiofrequency excitation was used to overcome radiofrequency B1 field inhomogeneity associated with the use of surface coil. The temporal resolution of the ^{31}P FAST approach was 5min.

Methods Male Sprague-Dawley rats (n=4, 225-250g) were anesthetized using 2% isoflurane during setup. Animals were secured in a holder with ear and tooth bars. Isoflurane was reduced to 1.2% for 30min prior to beginning data acquisition. MRI was performed on an 11.7T Bruker Biospin Magnet using a dual-tuned (500/202.5 MHz) 2-cm diameter surface coil. The 1H (500MHz) element was used for positioning and shimming prior to ^{31}P NMR. ^{31}P magnetization transfer (MT) data was acquired using the FAST method, where $k_{f,CK}$ was calculated[7] using four spectra acquired with 30° and 60° FA's with and without γ-ATP saturation (TR=1100s, NA=64, DS=6). Accurate FA's throughout the brain were set using BIRP[8] plane rotation adiabatic RF pulses. Narrowband ATP saturation with negligible bleed over was achieved using the BISTRO[9] saturation scheme with eight 50ms hyperbolic secant RF pulses. Total acquisition time for a $k_{f,CK}$ measurement was ~5min. The first ^{31}P data sets were acquired after 30min of exposure to 1.2% isoflurane. The isoflurane was raised to 2% for 30min and ^{31}P measurements were repeated. The ^{31}P measurements were repeated at 1.2% and 2.0% isoflurane with 30min exposures prior to each data set.

Results A typical ^{31}P data set consisting of the four spectra used to calculate $k_{f,CK}$ in the FAST method is shown in **Figure 1**. Spectra were acquired at 60° and 30°, with and without BISTRO[9] saturation of the γ-ATP resonance (-2.3ppm). The pair of spectra acquired without saturation was used to calculate M_0 of PCr. The pair of spectra acquired with saturation was used to calculate M'_0 and T_1^{int} of PCr. The change in PCr signal was robustly detected, allowing for reproducible measurements of the forward CK rate ($k_{f,CK}$).

Under 1.2% isoflurane, the CK rate $k_{f,CK}$ was 0.26±0.02 s^{-1} and the forward metabolic flux $F_{f,CK}$ was 41.0±4.2 μmol/g/min. Under 2.0% isoflurane, $k_{f,CK}$ = 0.16±0.02 s^{-1} and $F_{f,CK}$ = 41.0±4.2 μmol/g/min, corresponding to 38% and 42% reduction, respectively, compared to 1.2% isoflurane. By contrast, the ATP and PCr concentrations were unaltered. After the isoflurane level was returned from 2% to 1.2% for 30 mins, the CK rate recovered slightly and again fell after another 30min exposure to 2.0%, suggesting that 30 mins may not be sufficient for metabolic rate to fully recover and that commonly used isoflurane levels can significantly alter cerebral metabolism.

Discussion Our reported values for the creatine kinase rates under graded isoflurane anesthesia are in general agreement with studies by Sauter and Rudin and Du et al. which had lower temporal resolution. Sauter and Rudin[5] used a conventional ^{31}P saturation transfer method at 4.7T to measure forward CK rate and high-energy phosphate concentrations under 1-2% halothane, thiopental sodium and graded bicuculline (0.4 mg/kg and 0.8 mg/kg) and found $k_{f,CK}$ to be 0.25±0.02 s^{-1}, 0.21±0.03 s^{-1}, 0.30±0.04 s^{-1} and 0.49±0.04 s^{-1}, respectively, in normal animals. $k_{f,CK}$ linearly correlated with EEG activity. Du et al.[1] used variations of the saturation transfer technique at 9.4T and found $k_{f,CK}$ to be 0.24±0.02 s^{-1}, 0.21±0.03 s^{-1}, 0.21±0.02 s^{-1} and 0.19±0.03 s^{-1} for animals anesthetized with 2.0% isoflurane, α-chloralose, low dose pentobarbital and high dose pentobarbital, respectively.

Conclusions This study implemented and employed the ^{31}P FAST technique at 11.7T to evaluate cerebral high-energy phosphates and creatine kinase synthesis rate under graded isoflurane anesthesia. The advantage of the ^{31}P FAST technique is that the measurement of creatine kinase synthesis is made practical using high field and small surface coil, as well as optimized ^{31}P FAST acquisition parameters and radiofrequency pulses that enable robust measurement of the CK synthesis rate. The major findings were: i) the forward creatine kinase rate and the metabolic flux of the rat brain were reliably measured, and ii) changing isoflurane concentration from 1.2% to 2.0% did not change the PCr and ATP concentrations, but significantly decreased the forward creatine kinase synthesis rate and the metabolic flux. This approach has potential applications in studying neurological disorders with metabolic dysfunction. Future studies will incorporate chemical shift imaging and apply to study ischemic stroke and traumatic brain injury.

REFRENCES (1) Du F, et al. PNAS 2008. (2) Shoubridge, EA, et al. FEBS Letters 1982. (3) Chen W, et al. MRM 1997. (4) Mora BN, et al. PNAS 1991. (5) Sauter A and Rudin M, J Biol Chem 1993. (6) Mlynarik V et al. MAGMA 1998.(7) Bottomely PA et al. MRM 2002. (8) Bottomely PA, et al. J Magn Reson., Ser A 1993. (9) de Graaf RA, et al. J Magn Reson, Ser B 1996

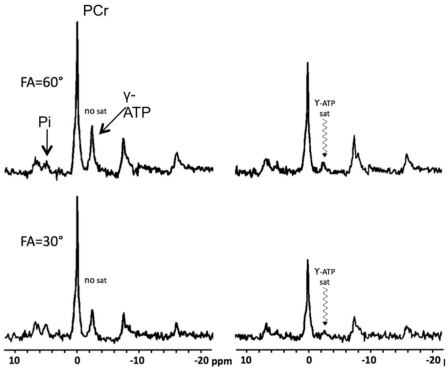

Figure 1. 31P-MT FAST spectra FA's 60° and 30° with and w/o saturation of ϒ-ATP.