An Improved Magnetization Saturation Transfer Approach---T_1^{nom} for Rapidly Measuring and Quantifying CK Activity in the Rat Brain

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Introduction: The chemical exchange reactions (*PCr↔ATP↔Pi*) catalyzed by the creatine kinase (CK) and ATPase enzymes play key roles in maintaining brain function. *In vivo* ^{31}P-MRS combined with magnetization saturation transfer (ST) approach provides a unique tool for assessing the reaction rate constants (k_f) and metabolic fluxes. The accurate k_f quantification usually requires a very long repetition time (*tr*) for approaching a steady-state saturation condition, however, it suffers from low detection sensitivity and limited utility for biomedical application. This problem could be partially overcome by shortening *tr*, allowing more signal averages. However, it makes k_f quantification complicated due to so-called saturation effect caused by short *tr* (but not k_f). Therefore, it is crucial to develop different approaches able to rapidly measuring and quantifying CK and ATPase enzyme activities *in vivo*. Several methods have been proposed [1-2]. In this work, we present an improved magnetization ST approach---T_1^{nom} by correcting the partial saturation effect with short *tr*. This approach was tested in the rat brain.

Method and Experiment: Magnetization saturation transfer method illustrated in Fig. 1 has been popularly used to measure chemical exchange fluxes of reactions among *PCr, ATP and Pi*. In the case of a frequency-selective RF pulse train with a long saturation time (t_{sat}) for completely saturating the γ-ATP resonance and the magnetization of PCr or Pi reaches a steady-state, the unidirectional forward chemical exchange constant (k_f) can be deduced by Eq.1 [3, 4], in which M^0 and M^* are magnetization of PCr or Pi at thermal equilibrium without saturation and steady-state with saturation, respectively; T_1^{int} is the intrinsic relaxation time of PCr or Pi. This method is well known as the steady-state magnetization saturation transfer. For the convenience, we rewrite Eq. 1 as Eq. 2. But it is worth to reminding that Eq. 1 only holds under full relaxation condition. In order to improve SNR, a short *tr* is desired. Generally the magnetizations in the presence (M_s) and absence of saturation (control, M_c), as well as their ratio are the function of many given experimental parameters and it was indicated by Eq. 3.

$$k_f = \frac{M^0 - M^*}{M^*} \times \frac{1}{T_1^{int}} \quad (1) \qquad \frac{M^0}{M^*} = k_f T_1^{int} + 1 \quad (2)$$

$$\frac{M_c}{M_s} = f(M^0_{Pcr}, M^0_{\gamma-ATP}, M^0_{Pi}, T^{int}_{1Pcr}, T^{int}_{1\gamma-ATP}, T^{int}_{1Pi}, k_f^{CK}, k_f^{ATPase}, tr, t_{sat}, \beta) \quad (3)$$

In Eq. 3, chemical equilibrium conditions of CK and ATPase reactions are applied. Therefore, the reverse chemical exchange constants of CK and ATPase are explicitly dependent on the forward chemical exchange constants, and not shown in the equation. T_1^{intr} and M^0 values of three chemically coupled spins (PCr, γ-ATP and Pi) have been measured in a previous study [5]. Therein, we can simulate the magnetizations ratios of M_c and M_s as function of chemical exchange rate constants and repetition times using the modified Bloch-McConeell equations [3] and known parameters in Eq. 3. The purpose is to obtain k_f with any specific *tr* and flip angle (β).

All the *in vivo* ^{31}P experiments were conducted at 9.4T animal scanner located at the University of Minnesota. A dual RF surface-coil probe consisting of a butterfly-shape ^{1}H surface coil and an elliptical-shape ^{31}P surface coil was used for acquiring anatomy images, B_0 shimming and *in vivo* ^{31}P spectra. The pulse sequence was illustrated in Fig.1. A BISTRO saturation pulse train scheme and an adiabatic pulse, BIR4 with 45° flip angle (BIR4-45), were used to saturate γ-ATP resonance and to read out ^{31}P signal, respectively. To test the performance of adiabatic pulse, a phantom of inorganic phosphate solution ([Pi] =0.1M, pH=7.0) was prepared. Five male Sprague-Dawley rats anesthetized with 2% isoflurane were used to acquire *in vivo* ^{31}P-MRS with two different repetition times (1 and 3s) and 256 signal averages. The femoral arteries of rats were catheterized for blood sampling and physiology monitoring. All surgical procedures were approved by the Institutional animal Care and Use Committee of the University of Minnesota.

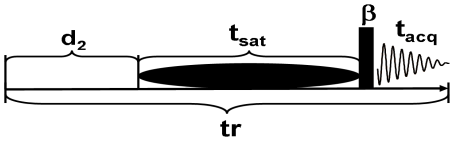

Fig. 1 NMR pulse sequence for magnetization saturation transfer experiments. The control and saturated spectra were acquired by saturation pulse trains (black strip) off and on, respectively.

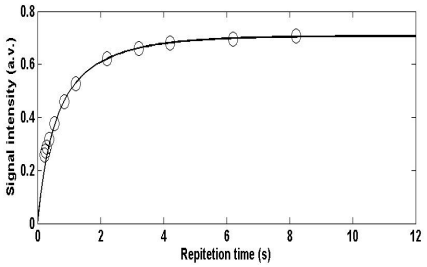

Fig. 2 Relative signal intensity (circles) generated by BIR4-45 readout pulse with different *tr* values. The signal intensity of Pi in phantom solution was normalized to sin(45°) at full relaxation condition. The dark line was predicted by the partial saturation effect with T_1=1.94 s, which was measured using the conventional inversion recovery method.

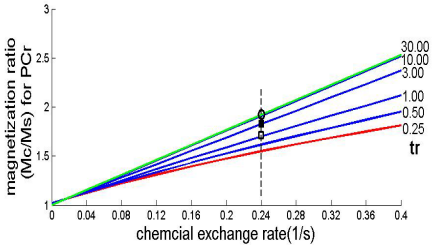

Fig. 3 Dependence of magnetization ratios between control (M_c) and saturation (M_s) on the rate constants (k_f=0-0.4 s^{-1}) and repetition time (*tr*=0.25-30 s) simulated by the Bloch-equations. Flip angle (β=45°) and d_2=0 were applied for the simulations. The magnetization ratios indicated by circle, solid and blank squares were measured with *tr* = 12, 3 and 1s, respectively.

Results and Discussions: An accurate flip angle is critical for k_f quantification. We have tested the performance of BIR4-45 pulse and results were demonstrated in Fig. 2, showing an excellent pulse behavior. The simulated magnetization ratios of M_c and M_s with varied *tr* were shown in Fig. 3. Obviously when $tr \geq 4T_1^{mix}$ (such as tr =10 or 30 s, or near full relaxation condition), the ratio of M_c/M_s is a linear function of k_f, and the relation approaches Eq. 2 with the slop of T_1^{int}. Thus, k_f can be deduced as long as T_1^{intr} is available. This conventional strategy has been applied to determine the k_f values in the rat brain at 9.4T [5]. For instance, from the previously measured T_1^{int} of PCr (*i.e.* the slop of the green line in Fig. 3) and the magnetization ratio (the circle in Fig. 3), k_f of CK was deduced as 0.24 s^{-1} [5]. When *tr* is short, however, the ratio of M_c/M_s will be governed not only by k_f and T_1^{int} but also by the repetition time, flip angle and saturation time. In the rat brain with the same animal preparation as the previous study [5], the ratios of M_c/M_s measured with two different *tr* values (1 and 3 s) in the present study show a good agreement with the predicted values indicated in Fig. 3. In principle, k_f can be estimated from simulations with any arbitrary parameter setup. However, it is more interesting that a simple, linear function following Eq. 4 is still satisfied at a wide range of *tr*. Eq. 4 is very similar to Eq. 1 except that T_1^{nom} is the nominal T_1^{int} indicated by Eq. 5 with a correction factor. The T_1^{nom} value and Eq. 4 can be applied to determine k_f using two spectra (for measuring the M_c/M_s ratio) acquired with desired *tr* and β.

$$\frac{M_c}{M_s} = k_f T_1^{nom} + a \quad (4) \qquad T_1^{nom} = f(tr, \beta, t_{sat}) \times T_1^{int} \quad (5)$$

In conclusion, T_1^{nom} approach should provide a simple quantification algorithm to rapidly measure chemical exchange fluxes *in vivo*.

Reference: 1. Bottomley, et al. MRM 2002; 2. Xiong, *et al. AJP* 2009; 3. Du, et al. MRM 2007; 4. Frosen, et al, *JCP* 1963; 5. Du *et al. PNAS* 2008.

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