Implementation of FAST-MRS in mouse permits the rapid assessment of muscle ATP synthesis in-vivo.

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Introduction

Conventional saturation-transfer (CST) has proven to be a useful technique for measuring the rates of metabolic exchange reactions in a variety of preparations. We have used it effectively in both rats and humans to investigate muscle energy production in-vivo, and found that rates of ATP synthesis (V_{ATP}) are modulated by pharmacological uncoupling of the mitochondrion (1), aging (2) and in insulin-resistant offspring of type 2 diabetic patients (3,4). The existence of numerous transgenic mouse models with modulated metabolism offers scope for many new avenues of investigation. To date, the application of CST in mouse has been restricted due to the small amount of muscle tissue available for MRS, necessitating very long experimental durations (~8 hours) or that time-consuming elements (eg T_1' calibration) of the CST experiment are eliminated (5). The Four-Angle Saturation Transfer (FAST) technique (6) has been proposed as an alternative to CST and may permit more rapid assessment of V_{ATP} in mouse muscle. This technique dispenses with an independent T_1' calibration by acquiring saturation-transfer spectra at two different pulse-angles – the T1 of a metabolite of interest can be calculated indirectly from the ratio of it's signal at the two pulse-angles, precise knowledge of those pulse angles and the T_R. Here, we demonstrate that FAST is a valid alternative to CST for measuring V_{ATP} in mouse muscle in-vivo.

Methods

Experiments were performed on a 9.4T Magnex magnet interfaced to a Bruker Biospec console. Mice were anesthetized with isoflurane and underwent continuous physiological monitoring. The hindlimb muscles were positioned directly under a 15mm double-turn ^{31}P surface coil; scout images and shimming were performed using 25mm diameter quadrature ^{1}H coils. ^{31}P spectra for CST were acquired using a custom-written pulse sequence with frequency-selective saturation of the γATP peak (M') or with saturation at a downfield frequency equidistant from P_i (M_0), using the following parameters: 1msec AHP excitation pulse (centered between P_i and γATP), 10sec 'soft' saturation pulse, sweep width = 8kHz, 1024 complex points, effective T_R = 10sec, 64 transients. FAST spectra were acquired using 1msec adiabatic BIR4 pulses (7) for excitation ($\alpha = 90^o$, $\beta = 30^o$) and a 2sec pulse for γATP or symmetric saturation. The T_1 of P_i under conditions of γATP saturation (T_1') was measured in each animal using an 8-point inversion-recovery calibration with γATP saturation prior to and during the inversion delay. Each block of CST or FAST spectra was repeated twice prior to, and twice after the T_1' calibration. Fully-relaxed ^{31}P spectra (AHP excitation, T_R = 25 sec, 32 transients) were obtained to determine metabolite concentrations in-vivo, absolute [ATP] was determined by high resolution ^{31}P-MRS of freeze clamped tissue extracts.

Results

A cross sectional mouse hindlimb image is shown in Fig 1; typical muscle ^{31}P saturation-transfer spectra (90^o AHP excitation) are shown in Fig 2. Rates of ATP synthesis obtained using either CST or FAST were equivalent (Table 1).

Fig 1.

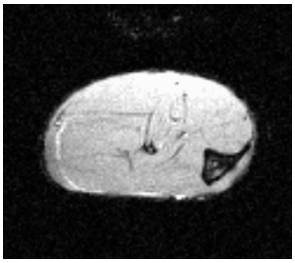

Fig 2.

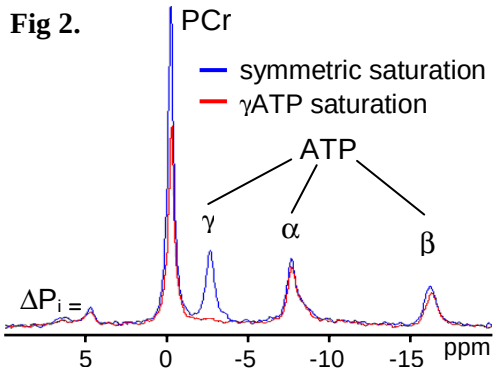

Table 1.

	CST (n = 6)	FAST (n = 6)	p-value
M'/M_0	0.756 ± 0.007	0.756 ± 0.017	0.99
T_1' (sec^{-1})	3.48 ± 0.04	3.45 ± 0.25	0.89
k' (sec^{-1})	0.070 ± 0.002	0.072 ± 0.003	0.63
V_{ATP} (μmol/g/min)	6.08 ± 0.27	6.24 ± 0.42	0.75

Conclusions

FAST offers a practical alternative to CST for measuring rates of V_{ATP} in mouse muscle in-vivo, and decreases the duration of an in-vivo experiment from approximately 8 hours to less than 2.

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