Quantitative evaluation of the first order rate constant of creatine-kinase reaction in ovine heart using Magnetization Transfer ^{31}P Magnetic Resonance Spectroscopy (MT-^{31}P-MRS)

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INTRODUCTION: Infants with hypoplastic left heart syndrome (HLHS) have the highest mortality of all congenital heart defects. Their inability to gain weight appropriately may be due to high cardiac energy requirements from their shunt dependent physiology. We hypothesized that the anabolic steroid, oxandrolone would improve cardiac energy utilization. We are modelling HLHS with an 8 mm goretex shunt placed from the aorta to the pulmonary artery inserted prenatally. To quantitatively evaluate cardiac energy utilization, we are using magnetization transfer ^{31}P-MR spectroscopy (MT-^{31}P -MRS) to determine the first order reaction rate of the creatine-kinase (CK) reaction in the heart. For this work, we developed a double tuned ^{1}H/^{31}P coil, ^{1}H/^{31}P TR switch and 1D MT-CSI pulse sequence needed for ^{31}P spectroscopy and measured the k_f values of in-vivo heart of control, shunted, and oxandrolone-treated shunted lambs.

THEORY: CK reaction is essential for the buffering and the rapid regeneration of adenosine triphosphate (ATP) in heart tissue [1]**.** The heart uses this energy to perform its normal function. In CK reaction the CK enzyme catalyzes the reaction, in which ATP is produced from PCr and ADP:

$$PCr^{2-} + ADP^{-} + H^{+} \rightleftarrows Cr + ATP^{2-} \quad \ldots (1)$$

The reaction proceeds in both forward and reverse directions. In MT-^{31}P-MRS, a train of sinc RF pulses with a narrow bandwidth (75 Hz) is applied to suppress ϒ-ATP line, so that the reverse reaction brings no magnetization from ϒ-ATP to PCr. The rate equation of longitudinal magnetization of PCr in the CK reaction is described by the modified Bloch eq. (2). The solution of this first order differential equation for k_f is given by eq. (3) with the apparent relaxation rate $1/T_1^{app}$ in eq. (4). In these equations, M_{PCr}^{ss}, M_{PCr}^{o} M_{PCr}^{Tsat} are respectively the longitudinal magnetizations of PCr for a steady-state with ϒ-ATP peak saturated, the thermal equilibrium magnetization, and the magnetization of the PCr with a MT-suppression time T_{sat}.

$$\frac{dM_{PCr}(t)}{dt} = \frac{M_{PCr}^{o}(t) - M_{PCr}(t)}{T_1} - k_f M_{PCr}(t) \quad \ldots (2)$$

$$k_f = \frac{M_{PCr}^{o} - M_{PCr}^{ss}}{M_{PCr}^{o} T_1^{app}} \quad \ldots (3)$$

$$\frac{1}{T_1^{app}} = \frac{1}{T_1} + k_f = -\frac{\ln(1 - M_{PCr}^{Tsat}/M_{PCr}^{ss})}{T_{sat}} \quad \ldots (4)$$

METHODS: MT-^{31}P-MRS experiments were carried out on three lambs: control, shunted and oxandrolone-treated shunted aged 4 to 6 weeks under an approved University of Utah IACUC using a home built ^{1}H/^{31}P double tuned surface transmit/receive coil and ^{1}H/^{31}P TR switch at a 3T clinical MRI system (Tim-Trio, Siemens Medical Solutions, Erlangen, Germany). A passive RF switch is positioned just before the TR switches to automatically send the RF pulses to either a linear ^{1}H or a quadrature ^{31}P TR switches for ^{1}H MRI or ^{31}P MRS. A CSI-FID pulse sequence was modified to implement 1-dimensional MT-CSI. The ^{31}P coil was designed in quadrature mode to produce the optimal sensitivity at the heart of a one month old lamb [4]. A linear ^{1}H coil was positioned outside the ^{31}P coil for shimming the region of interest and producing the scout images. Fig. 1b shows the timing diagram of MT-31P-CSI with M_z saturation pulse to null the initial magnetization, MT saturation pulses of bandwidth 75 Hz to suppress the ϒ-ATP, OVS pulses to prevent the contamination from the ^{31}P signal at chest wall as shown in Fig. 2a and an adiabatic half passage (AHP) pulse for the excitation. Three experiments were carried out to measure k_f: M_{PCr}^{o} was obtained using TR=20 sec, M_{PCr}^{TR} using three hard RF pulses with flip angle 300˚ as M_z saturation RF and a spoiling gradient pulses followed by MT saturation pulses at -2.7 ppm away from PCr for 2 sec, and M_{PCr}^{ss} was measured with pre-saturation delay of 2.1 sec and MT saturation at -2.7 ppm for 3.5 second [2]. All experiments were performed with receiver bandwidth of 2.5 kHz and 512 data points. The raw data were processed using a home-developed processing software in Python language.

RESULTS: Fig. 2(b~d) are the stacked plots of ^{31}P MR spectra from control, shunted, and oxandrolone treated shunted lambs, respectively. Blue, green and red spectra indicate M_{PCr}^{o} M_{PCr}^{ss}, and M_{PCr}^{TR} respectively. The values of k_f and SNR of the M_{PCr}^{o} peak obtained from these spectra are listed in Table 1. The k_f values are 0.44 sec^{-1}, 0.19 sec^{-1}, and 0.34 sec^{-1} in the normal, shunted, and oxandrolone treated heart, respectively.

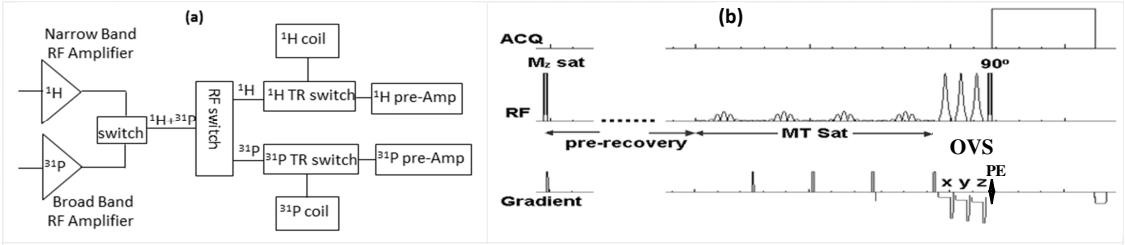
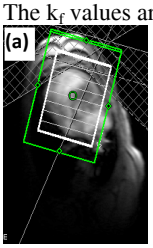
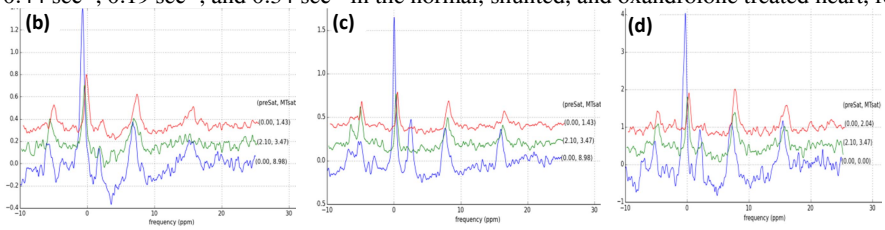


Fig 1: (a) *Block diagram of hardware.* ***(b)*** *Timing diagram of pulse sequence, 1D MT-CSI.*





lamb	k_f (s^{-1})	SNR (M^{0}_{PCR})
normal	0.44	70.0
shunted	0.19	32.0
oxandrolone treated shunted	0.34	29.7

Fig 2: (a) *A scout image with 1D CSI grid, and* ***(b,c,d)*** *in-vivo ^{31}P spectra of shunted, oxandrolone-treated shunted, and control lamb.* ***Table 1:*** *k_f and SNR of M^{o}_{PCr} peak height. Low SNR for shunted heart was caused by the poor shimming.*

DISCUSSIONS and CONCLUSIONS: The coil and TR switch were made optimizing the SNR of the ^{31}P MR signal. AHP pulse was used for the uniform excitation using the surface coil at the volume of lamb's heart. The values of k_f obtained from these initial studies show that the forward reaction rate of CK reaction is increased with the oxandrolone-treatment in the lamb with shunted heart, which may indicate the increase in the rate of energy production. More experiments will be performed.

REFRENCES: [1] Bashir et al., NMR Biomed. 2014;27:663. **[2]** Qiang et al. Am J Physiol. Heart Circ. Physiol. 2009;297:H1010. [3] Jeong et al., NMR Biomed., 2010;24:765**. [4]** Kumar et al., MAGMA, 2008;21:41. **[5]** Neubauer S., N Engl. J Med., 2007;356:1140. **[6]** Degani et al., Biochemistry., 1985;24:5510.

Acknowledgements: This work is supported by NSF CBET 1133908 grant and Primary Children's Hospital Foundation Integrated Science Award.