In vivo Murine Cardiac PCr and ATP Concentrations Measured by Magnetic Resonance Imaging and Spectroscopy

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SYNOPSIS: An i*n vivo* ^{1}H MRI and ^{31}P MR spectroscopic method is proposed and validated for the measurement of *in vivo* high energy phosphate metabolite (PCr and ATP) concentrations in normal (n=7) and thoracic aorta constriction (TAC) (n=10) mouse hearts. The *in vivo* MR results for [ATP] are in good agreement with those obtained using an *in vitro* luminescent assay on perchloric acid extracts of the same hearts.

INTRODUCTION: Normal energy phosphate metabolism is critical for cardiac function and viability[1,2]. The combination of ^{31}P magnetic resonance spectroscopy (MRS) and magnetic resonance imaging (MRI) is uniquely able to assess the critical relationship between metabolism and function in the *in vivo* beating heart. ^{31}P MRS/MRI studies in heart failure (HF) patients have documented reductions in the ratio of PCr/ATP, the concentrations of [ATP] and [PCr], and in ATP flux through the creatine kinase reaction[3, 4]. Mouse models of HF offer a novel, appealing means to investigate the mechanistic links between altered energy metabolism and function because they allow gene-targeted metabolic interventions and mimic energetic aspects of human HF[5]. Although PCr/ATP measures have been validated in the *in vivo* mouse heart[5,6], there have been no methods validated for measuring [ATP] or [PCr]. Thus there were two aims for this study. First, we sought to adapt human ^{31}P MRS methods for quantifying [PCr] and [ATP][7] to the *in vivo* mouse heart and validate them by comparison with conventional invasive biochemical measures from a luminescent assay (LA). Second, we used the new ^{31}P MRS method to test the hypothesis that *in vivo* myocardial [ATP] is reduced in the TAC model of murine HF.

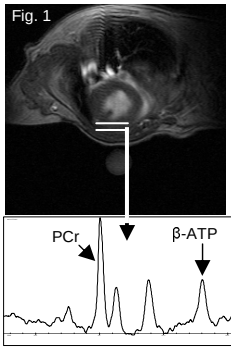
MATERIALS AND METHODS: Experiments were carried out on a Bruker Biospec MRI/MRS spectrometer equipped with a 4.7T/40cm Oxford magnet and a 12cm (inner diameter) actively shielded gradient set, as previously described[5, 6]. Studies were first performed on mice then, on the same day, on an external phantom using comparable protocols. More specifically, *in vivo* MRI/MRS studies were carried out on 7 control and 10 mice four weeks after TAC (weight 28-33gm). Anesthesia was induced by ~1% isoflurane, as previously described[5,6]. The probe set included 22-mm ^{1}H MRI and 13-mm ^{31}P MRS coils. MRI were obtained with ^{1}H MRI FLASH sequence (TE =1.5ms, TR =12ms, NEX =12) and spatially-localized ^{31}P MRS with a one-dimensional chemical shift imaging (1D SCI) sequence (16 mm F.O.V.,16 phase encoding steps, NEX = 64, TR = 2000ms) using modified BIR4 90° adiabatic pulses. After completing the MRI/MRS study, the mice were sacrificed and the hearts immediately frozen in liquid nitrogen and later extracted with perchloric acid for *in vitro* measurement of [PCR] and [ATP] with a luciferase enzyme LA[8]. An external phantom, consisting of a 5mm NMR tube filled with 0.15M phosphate solution (combination of 0.075M NaH_2PO_4 and 0.075M Na_2HPO_4) and 40µM $NiCl_2$ was positioned at the center of the ^{31}P surface coil, like the mouse hearts, and studied with the same MRI/MRS protocol, except that NEX = 2, TR = 8000ms. ^{1}H MR Images were analyzed with Paravision software and ^{31}P MR spectra peak areas determined with in-house custom software.

To calculate cardiac metabolite concentrations we first measured T1-dependent correction factors (C_f) for fully relaxed conditions by comparison of acquisitions with different TR = 2, 4, 8, 10, 12 sec for PCr and ATP separately. The concentration of metabolite [C_c], in one slice of a ^{31}P 1D CSI cardiac data set is given by following formula:

$$C_c = \frac{S_c \times C_p \times V_p \times NEX_p \times RG_p}{S_p \times V_c \times NEX_c \times C_f \times RG_c}$$

where S_p and S_c are the signal intensities of phantom and cardiac metabolites, respectively, C_p and C_c, the concentration of the phantom and cardiac metabolites, V_p and V_c, are the volumes of phantom and cardiac muscle in the same slice, NEX_p and NEX_c, are the number of acquisitions for each phantom and cardiac study, C_f is the saturation correction factor, and RG_p and RG_c, are the receiver gains for phantom and cardiac studies respectively.

RESULTS: Representative images and spatially-localized ^{31}P spectra are shown for the mouse (Fig.1) and the phantom (Fig.2). The results of the ^{31}P MRS and LA methods are summarized in Table 1. The mean *in vivo* [PCr] and [ATP] of roughly 10 and 5 µmol/g, respectively, agree with prior *in vitro* measures[9]. Critically, there is no significant difference in [ATP] determined by MRS and LA (p=ns). [PCr] is significantly higher by MRS (p<0.006) but this is likely due to the well recognized rapid degradation of PCr during sacrifice. [PCr] is significantly decreased in TAC mice as compared to controls by both MRS (p<0.001) as well as LA (p< 0.0002). [ATP] is significantly decreased in TAC mice by MRS (p<0.03) as well as LA (p<0.03).



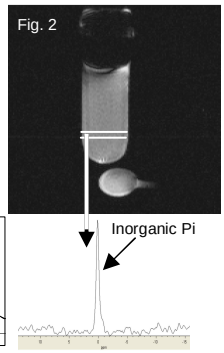


Fig. 1: Axial ^{1}H MRI of mouse (top) at the left ventricle (LV) and corresponding cardiac ^{31}P spectrum (bottom).
Fig. 2: ^{1}H MRI of phantom containing inorganic phosphate (top) and corresponding ^{31}P spectrum (bottom).

DISCUSSION: We present a novel, convenient, and accurate method of measuring cardiac ^{31}P metabolite concentrations in the *in vivo* mouse heart from 1D CSI ^{31}P MRS spectra. Our [ATP] results are consistent with the literature[9] and with conventional measures. This new MRS method does not add significantly to the time of an anesthetized mouse study. Although these are tissue rather than intracellular concentrations, tissue measures are obtained in human heart and are used, in part, to calculate ATP flux through the creatine kinase reaction. Cardiac creatine kinase [ATP] is reduced after TAC in the *in vivo* mouse heart.

Table 1. PCr and ATP concentrations obtained (µmol/g wet wt.) by MRS and LA method (Mean ± S.D.)

	MRS method		Enzyme luminescent assay	
Metabolites	Control	TAC	Control	TAC
[PCr]	10.4 ± 1.4	6.7 ± 2.0	8.1 ± 0.7	5.0 ± 1.3
[ATP]	4.99 ± 0.9	4.0 ± 0.8	4.3 ± 0.7	3.3 ± 0.8

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