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Transmurally Differentiated Measurement of ATP Hydrolysis Rates in the *in vivo* Porcine Hearts

Albert Jang^{a,b,c}, Qiang Xiong^a, Pengyuan Zhang^a, and Jianyi Zhang^{a,d}

^a Department of Medicine, Cardiovascular Division, University of Minnesota

^b Department of Electrical and Computer Engineering, University of Minnesota

^c Center for Magnetic Resonance Research and Department of Radiology, University of Minnesota

^d Department of Biomedical Engineering, School of Medicine, School of Engineering UAB | The University of Alabama at Birmingham, Birmingham, AL 35294

Abstract

Purpose—Compare the transmural distribution of forward creatine kinase reaction ($k_{f,CK}$) and ATP hydrolysis rate ($k_{f,ATPase}$) in the myocardium of normal porcine heart. Rate constants were extracted from partially relaxed spectra by applying the T_1^{nom} method, effectively reducing data acquisition time by up to an order of magnitude.

Theory and Methods— T_1^{nom} method for double saturation of PCr and Pi is introduced and validated through simulations. Bioenergetics was measured *in vivo* utilizing one-dimensional chemical shift imaging (1D-CSI) magnetic resonance ^{31}P spectroscopy.

Results—At basal conditions, there was no significant difference between subepicardial layers (EPI) vs. the subendocardial layers (ENDO) for both $flux_{f,CK}$ and $flux_{f,ATPase}$. At high cardiac workload (HWL), where the rate pressure product increased 2.6-fold, PCr/ATP ratio and $flux_{f,CK}$ showed no significant change in both EPI and ENDO layers, while $flux_{f,ATPase}$ increased significantly (baseline: 1.11 ± 0.12 and 1.12 ± 0.13 $\mu\text{mol/s/g}$, EPI and ENDO, respectively; to HWL: 2.35 ± 0.27 and 2.21 ± 0.08 $\mu\text{mol/s/g}$, EPI and ENDO respectively, each $p < 0.01$ vs. baseline).

Conclusion—In the normal heart, increase of cardiac work state is accompanied by an increase in ATP hydrolysis rate with no changes in CK flux rate. There are no significant differences between EPI vs. ENDO concerning the ATP hydrolysis rate or CK flux rate in both baseline and high cardiac work states.

Keywords

Heart; magnetic resonance spectroscopy; saturation transfer; high-energy phosphate; ATP hydrolysis rate

INTRODUCTION

Adverse changes in cellular ATP metabolism are known to contribute to the progression of myocardial dysfunction in patients with left ventricular hypertrophy and heart failure(1-8). Until recently, attempts to test this hypothesis have been largely unsuccessful due to techniques for monitoring ATP hydrolysis ($\text{ATP} \rightarrow \text{ADP} + \text{Pi}$) in the hearts of living animals being unavailable. In principle, the rate of ATP hydrolysis can be measured via phosphorous-31 magnetic resonance spectroscopy (^{31}P MRS) magnetization saturation transfer (^{31}P MRS-MST) experiments. However, the conventional approach requires quantification of inorganic phosphate (Pi) levels, which is difficult to achieve, because Pi levels in the myocardium are low, and the resonance peaks of Pi and 2,3-diphosphoglycerate (2,3-DPG) from the erythrocytes overlap in the ^{31}P MR spectrum (9). We have very recently developed a novel ^{31}P MRS-MST method that bypasses this need to quantify Pi levels when measuring the ATP hydrolysis rate, and have used this approach to show that the rate of myocardial ATP hydrolysis at the peri-scar border zone (BZ) is significantly decreased in the hearts of swine with experimentally induced ischemia reperfusion injury(10). Our results also indicated that the functional improvements associated with the administration of cell therapy to injured swine hearts were accompanied by reduction of LV wall stress and improvement in BZ ATP hydrolysis rate(10).

In hearts, although the inner (ENDO, subendocardial) and outer (EPI, subepicardial) layers are known to differ substantially in perfusion, metabolism and contractile activity(11,12), few cardiovascular investigations have been able to evaluate how the properties of myocardial metabolism and the response to myocardial injury may vary across the thickness of the ventricular wall. Some evidence suggests that these variations may be accompanied by differences in cellular energetics. For example, flow-limiting coronary stenosis appears to reduce cellular energy levels more substantially in the ENDO than in the EPI(11), which may suggest that the inner layer is more vulnerable to ischemia, while the induction of a high cardiac work state via catecholamine infusion led to more prominent increases in blood flow and energy consumption in the subepicardium with a uniform distribution across the LV wall of the myocardial phosphorylation potential(13-15). Observations can also vary depending on the method used to elevate the heart rate: stenosis with dobutamine-induced elevations reduced energy levels in both layers, with the greatest decline observed in the EPI layers, whereas combining stenosis with pacing-induced tachycardia reduced energy levels more pronouncedly in the ENDO layers(16).

It remains a significant clinical question why the inner layers of the myocardium across the left ventricle are more susceptible to an ischemia insult(17). Although these series of bioenergetic evaluations convincingly shows that the myocardial ischemic and oxidative stress responses are not uniform across the thickness of the left ventricular (LV) wall, the observations were generally limited to measurements of the steady state levels of myocardial high-energy-phosphate (HEP), rather than the kinetics of the ATP turnover rate. The transmurally differentiated evaluations of the myocardial ATP turnover rate is important for examining the mechanisms of myocardial ischemia or metabolic diseases. Difficulty in measuring myocardial inorganic phosphate has been a major barrier in examining the transmural gradient of the reserve of myocardial ATP hydrolysis rate, and whether this

gradient contributes to the progression cardiac failure in hearts with compensated hypertrophy(18-20), a subject still of much debate. Thus, for the experiments presented in this report, we used ^{31}P MRS-MST to compare the EPI and ENDO ATP hydrolysis rates under both baseline and catecholamine induced high cardiac workload states.

We also introduce a modified version of our ^{31}P MRS protocol that enables us to perform our rate calculations from partially relaxed spectra: T_1^{nom} method, which was recently established(21). The implementation of T_1^{nom} effectively reduced the time required for data acquisition by up to an order of magnitude (for details, see online supporting materials).

^{31}P MRS-MST Assessments of Myocardial ATP Turnover Rates from Partially Relaxed Spectra

THEORY

Cellular ATP levels are maintained by the activity of creatine kinase (CK), which catalyzes the interconversion of PCr and ATP, and by cellular ATP synthases (ATPase), which catalyzes the metabolic pathways responsible for ATP production ($\text{ADP} + \text{Pi} \rightarrow \text{ATP}$) and consumption ($\text{ATP} \rightarrow \text{ADP} + \text{Pi}$). Thus, the kinetics of ATP metabolism can be summarized with the following model:

where k_f and k_r are pseudo first-order rate constants for the indicated forward and reverse reactions, respectively. Because the $\text{PCr} \rightleftharpoons \text{ATP}$ reaction is at equilibrium under steady-state condition, the ATP hydrolysis rate ($\text{ATP} \rightarrow \text{ADP} + \text{Pi}$) can be calculated by subtracting the flux of the $\text{ATP} \rightarrow \text{PCr}$ reaction from the flux of the combined $\text{ATP} \rightarrow \text{Pi}$ and $\text{ATP} \rightarrow \text{PCr}$ reactions $\text{flux}_{\text{ATP,total}}$ (i.e., $\text{flux}_{r,ATPase} = \text{flux}_{\text{ATP,total}} - \text{flux}_{f,CK}$)(10). The fluxes, in turn, can be calculated from the concentrations of the reactants and from MRS measurements of the corresponding rate constants, which are determined via the following equations:

$$k_{\text{ATP,total}} = \left(\frac{M_{o,ATP}}{M_{ss,ATP}} - 1 \right) / T_{1,ATP}^{int} \quad [1]$$

$$k_{f,CK} = \left(\frac{M_{o,PCr}}{M_{ss,PCr}} - 1 \right) / T_{1,PCr}^{int} \quad [2]$$

$$k_{r,ATPase} = \left(\frac{M_{o,ATP}}{M_{ss,ATP}} - 1 \right) / T_{1,ATP}^{int} - \frac{M_{o,PCr}}{M_{o,ATP}} k_{f,CK} \quad [3]$$

where M_{ss} and M_o are the fully relaxed magnetizations with (M_{ss}) and without (M_o) saturation, and T_1^{int} is the intrinsic longitudinal relaxation time. T_1^{int} is exceptionally long for PCr and ATP and, consequently, the acquisition time required for accurate saturation of

$k_{f,CK}$ and $k_{r,ATPase}$ can be prohibitive, particularly when attempting to differentiate between measurements obtained in the epicardial and endocardial layers of the ventricular wall. To decrease this unrealistic acquisition time, T_1^{nom} method can be employed. By determining a correction factor, T_1^{nom} enables calculating the rate constants from partially relaxed spectra, thereby dramatically reducing the acquisition time. The validity of using this method is presented in the following.

In the case of double saturating PCr and Pi, mathematically, the Bloch McConnell equations for ATP become equivalent to the case of single saturation of ATP for PCr(Pi),

$$\begin{aligned}\frac{dM_{PCr(Pi)}(t)}{dt} &= \left(\frac{M_{o,PCr(Pi)} - M_{PCr(Pi)}(t)}{T_{1,PCr(Pi)}^{int}} \right) - k_{f,CK(ATPase)} M_{PCr(Pi)}(t) \\ \frac{dM_{ATP}(t)}{dt} &= \left(\frac{M_{o,ATP} - M_{ATP}(t)}{T_{1,ATP}^{int}} \right) - k_{ATP,total} M_{ATP}(t)\end{aligned}\quad [4]$$

where $k_{ATP,total} = k_{f,CK} + k_{r,ATPase}$. Hence, the same linear relation between the partially relaxed magnetization without and with saturation ratio (M_c/M_s), and rate constant $k_{ATP,total}$ is observed, and is therefore plausible to apply the T_1^{nom} method to ATP in a partially relaxed state for double saturation. This was verified for the B_1 -Insensitive TR_{in} to Obliterate signal (BISTRO) pulse sequence(22), a train of pulses interleaved with crusher gradients used to selectively saturate a target frequency prior to excitation, which is then followed by a delay (d_1) as shown in Fig. 1. Spin parameters PCr:ATP:Pi = 1:0.48:0.20, $T_{1,PCr}^{int} = 3.2s$, $T_{1,ATP}^{int} = 1.1s$, $T_{1,Pi}^{int} = 3s$ were numerically simulated for flip angle (FA) of 90° , repetition time (TR) of 2.5s, and delay of 0.052s (Fig. 2). Under partially relaxed state, the longitudinal magnetization of PCr, ATP and Pi reach a steady state in both without (Fig. 2a) and with (Fig. 2b) PCr-Pi double saturation. Parallel to ATP single saturation(21), the empirical T_1^{nom} parameters is a function of spin system parameters (T_1^{int} and pool size ratios of metabolites) and pulse sequence acquisition parameters, and intercepts the M_c/M_s -axis at 1. Based on this, in steady-state, the equations used to calculate T_1^{nom} for ATP and PCr(Pi) from partially relaxed MR spectra are:

$$T_{1,PCr(Pi)}^{nom} = \left(\frac{M_{c,PCr(Pi)}}{M_{s,PCr(Pi)}} - 1 \right) / k_{f,CK(ATPase)} \quad [5]$$

$$T_{1,ATP}^{nom} = \left(\frac{M_{c,ATP}}{M_{s,ATP}} - 1 \right) / k_{ATP,total} \quad [6]$$

and when this correction factor is incorporated into equations [1] - [3], the modified equations for calculating $k_{f,CK}$ and $k_{r,ATPase}$ from partially relaxed spectra become:

$$k_{f,CK} = \left(\frac{M_{c,PCr}}{M_{s,PCr}} - 1 \right) / T_{1,PCr}^{nom} \quad [7]$$

$$k_{r,ATPase} = \left(\frac{M_{c,ATP}}{M_{s,ATP}} - 1 \right) / T_{1,ATP}^{nom} - \frac{M_{o,PCr}}{M_{o,ATP}} k_{f,CK} \quad [8]$$

where M_s and M_c are the partially relaxed magnetizations with (M_s) and without (M_c) saturation, respectively.

METHODS

All experimental protocols were approved by the University of Minnesota Research Animal Resources Committee. Experimental and animal maintenance procedures were in agreement with the Animal Use Guidelines of the University of Minnesota and consistent with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (NIH publication No. 85-23).

Animal Preparation

Female Yorkshire swine (~21 kg) were anesthetized with inhaled 2% isoflurane, intubated, and ventilated with a respirator and supplemental oxygen; the animal's arterial blood pressure, left ventricle pressure, and blood oxygen levels were monitored throughout all surgical and MRS procedures. A left thoracotomy was performed to expose the heart, and polyvinyl chloride catheters (3 mm outer diameter) were inserted into the ascending aorta (through the left external carotid artery) and LV (through the apical dimple) for hemodynamic monitoring and drug administration. A double-tuned (^1H and ^{31}P), 28-mm diameter surface coil was sutured on to the epicardium over the anterior wall of the left ventricle; then, the coil was tuned and matched, and the animal was placed on a cradle and inserted into the magnet bore. MR experiments were performed under both baseline cardiac workload and high cardiac workload which was induced via intravenous infusion of dopamine and dobutamine (10 $\mu\text{g/kg}$ per minute each) from a syringe pump (PHD 2000; Harvard Apparatus, Holliston, MA, USA); high-workload assessments were performed ~10 minutes after infusion was initiated.

MR Spectroscopic Imaging

MR spectra were obtained with a 9.4T, 65-cm bore magnet (Magnex Scientific, Oxford, UK) interfaced with a Vnmrj console (Agilent Technologies, Santa Clara, CA, USA). Gradient echo (GRE) images were obtained and used both for positioning the probe to the isocenter of the magnet and for planning acquisition of the chemical-shift imaging (CSI) sequence; B_0 shimming was performed via proton signal detection, and the center frequency offset between PCr and ATP was determined from global ^{31}P spectra acquired with a 1 ms adiabatic pulse to ensure uniform spin rotations within the sensitive volume of the surface coil. After acquisition of fully relaxed global control spectra (TR = 12s, nt = 16, SW = 10kHz), magnetization-saturation-transfer experiments were performed in the absence of saturation, with ATP saturation and with both PCr and Pi simultaneous saturation (TR = 6.8s, nt = 16, d_1 = 1.51s, and SW = 10kHz). Saturation was achieved by using the BISTRO pulse sequence(22). ATP was excited with a frequency selective hyperbolic secant pulse, and PCr and Pi were simultaneously saturated with a composite pulse consisting of two distinct

hyperbolic secant pulses with different excitation frequencies; the composite pulse was validated via Bloch simulations to ensure that adjacent peaks were not significantly excited. The carrier frequency for saturation and excitation was positioned between the chemical-shift frequencies of PCr and ATP. Having these measurements at hand, calculation of the rate constants $k_{f,CK}$ and $k_{ATP,total}$ using equations [1] - [3] assuming $T_{1,PCr}^{int} = 3.2s$ and $T_{1,ATP}^{int} = 1.1s(10)$ was carried out.

Each epicardial and endocardial measurement was calculated from three sets of 1D-CSI spectra: 1) spectrum obtained in the absence of saturation ($M_{c,PCr}$ and $M_{c,ATP}$), 2) “single-saturation” spectrum ($M_{s,PCr}$) obtained with saturation applied at the ATP resonance frequency, and 3) “double-saturation” spectrum ($M_{s,ATP}$) obtained with saturation applied at both the PCr and Pi resonance frequencies (FOV 4 cm, $d_1 = 0.05174s$, nPE 17, SW = 10kHz, nt = 12). T_1^{nom} was experimentally obtained as follows: 3 additional global spectra, control, single-, and double-saturation, were acquired using identical sequence parameters that were used in CSI, yielding global $M_{c,PCr}/M_{c,ATP}$, $M_{s,PCr}$, and $M_{s,ATP}$ in partially relaxed states, respectively. To accommodate for the recovery of longitudinal relaxation that occurs during the CSI phase encode period, a delay equivalent to the phase encode time ($\tau_{PE} = 536\mu sec$) was inserted between excitation and acquisition to compensate. Combining these measurements with the global rate constants obtained from above, T_1^{nom} for both PCr and ATP were calculated using equations [5] and [6]. These T_1^{nom} values were then incorporated to the measurements obtained from the 3 CSI MST experiments to calculate $k_{f,CK}$ and $k_{r,ATPase}$ using equations [7] and [8] for each transmural layer. PCr/ATP ratio obtained from the control spectra under these conditions were used to calculate the normalization factor between fully relaxed and partially relaxed states by dividing it by the fully relaxed ratio initially obtained. A flow chart depicting this process is given in Figure 3. Motion artifacts were minimized by gating NMR acquisition to coincide with both the diastolic state of the cardiac cycle and the expiration state of the respiratory cycle. Because excitation flip angle was 90° , no dummy scans were needed to achieve steady-state. To ensure that the delay between the last BISTRO pulse and the excitation pulse was minimized (thereby maximizing saturation), an optimal TR was determined from assessments of the animal's heart and respiratory rates, and the number of BISTRO pulses was adjusted accordingly. In our studies, the optimal TR ranged from 2.369 - 3.525s, $T_{1,ATP}^{nom}$ and $T_{1,PCr}^{nom}$ ranged from 0.18 - 0.53s and 1.11 - 2.15s, respectively, at baseline condition, resulting in a typical study lasting on average 45 - 60min.

MR Data Analysis

CSI data were reconstructed using in-house software programmed in MATLAB (MathWorks, Inc., Natick, Massachusetts, USA). The transmural series of spectra ($N = 17$) was averaged with 6 Fourier-series windows(23) to generate representative epicardial and endocardial spectra with no voxel in between, resulting in a voxel width of 0.67cm. It was confirmed through numerical simulations of the slice profile constructed from Fourier-series windows that the adjacent voxel signal contamination was less than 3%. Resonance peaks were integrated and quantified using the data analysis toolkit provided by the VnmrJ software. The ATP hydrolysis rate ($flux_{r,ATPase} = [ATP]k_{r,ATPase}$) was calculated based on an ATP concentration of $5.3 \mu mol/g$ (as determined in a previous report)(24,25) and the PCr

concentration required to calculate the CK flux rate ($flux_{f,CK} = [PCr]k_{f,CK}$) was extracted based on the ratio between the magnitudes of the PCr and ATP resonances from the control spectrum of each transmural layer.

Data are presented as mean±standard deviation (SD) and were evaluated for significance via student T test (unpaired, 2 tailed); the Bonferroni correction was used when more than two groups were being compared. A p-value less than 0.05 was considered significant.

RESULTS

Hemodynamic Data

Hemodynamic data during each experimental condition are summarized in Table 1. During high cardiac workload condition, the heart rate (HR), LV systolic pressure (LVSP) and rate pressure product (RPP) were significantly increased in response to catecholamine stimulation. Application of dopamine and dobutamine stimulation increased rate pressure product (mmHg/min) by 260% compared to baseline conditions ($p < 0.05$, Table 1).

Validity of Applying T_1^{nom} Method to PCr-Pi Double Saturation

Numerical simulations were carried out to confirm partially relaxed steady-state magnetization without and with PCr-Pi double saturation (Figure 2a and 2b). For the given parameters, all three spins (PCr, ATP and Pi) attained partially relaxed steady-state after 1 repetition cycle for both no saturation (Figure 2a) and double saturation (Figure 2b). Based on this, the $k_{ATP,total}$ versus ATP M_c/M_s ratio was simulated for various flip angles (Figure 2c) and repetition times (Figure 2d). Depending on flip angle (FA) and TR, the slope, T_1^{nom} , varies while the linear relationship between M_c/M_s and $k_{ATP,total}$ is maintained. This corroborates that in addition to T_1^{nom} being a function of spin system parameters (T_1^{int} and relative ratio of metabolites), it is also a function of acquisition parameters (TR and FA). Increasing TR and/or decreasing FA results in partially relaxed steady-state magnetization approaching fully relaxed steady-state, with T_1^{nom} approaching T_1^{int} .

³¹P MR Spectroscopy

At basal conditions, there was no significant difference between subepicardium and subendocardium (Table 2) for both $flux_{f,CK}$ (4.4 ± 0.53 vs. 4.57 ± 0.84 , $\mu\text{mol/g/s}$, EPI and ENDO, respectively) and $flux_{r,ATPase}$ (1.11 ± 0.12 vs. 1.12 ± 0.13 , $\mu\text{mol/g/s}$, EPI and ENDO, respectively). Under high workload where rate pressure product increased 2.6-fold, $flux_{f,CK}$ showed no significant change in both subepi- and subendo-layers (EPI: 4.52 ± 0.47 ; ENDO: 3.68 ± 0.16 , $p = \text{NS}$ vs. baseline), while $flux_{r,ATPase}$ increased significantly (EPI: 2.35 ± 0.27 ; ENDO: 2.21 ± 0.08 , each $p < 0.01$ vs. baseline).

DISCUSSION

MVO_2 calculation

In the present study, myocardial oxygen consumption (MVO_2) was calculated by taking the arteriovenous oxygen difference and multiplying it by the rate of myocardial blood flow (MBF) taken from our previous report using the identical porcine open chest model and

radioactive microspheres(26). Blood samples were drawn from the aortic and coronary vein catheters into heparin-filled syringes during both surgical preparation and MR imaging. PO_2 and pH were measured using a calibrated blood gas analyzer (RAPIDLab 248, Siemens Healthcare, Erlangen Germany). Since the rate pressure product (RPP, heart rate $\times$ LVSP) is linear with respect to MVO_2 , (27) under high workload conditions, the MBF was scaled based on the RPP increase going from baseline to high workload state. Table 1 summarizes the calculated myocardial oxygen consumption. Myocardial blood flow of $0.92 \text{ ml min}^{-1} \text{ g}^{-1}$ was used for the EPI layers and $0.95 \text{ ml min}^{-1} \text{ g}^{-1}$ was used for the ENDO layers. In high cardiac workload state, MBF was scaled based on increase in RPP. Based on this, going from basal to high workload condition, both RPP and MVO_2 rate are more than doubled in both the EPI and ENDO layers.

In dogs, using FLAX-ISIS and RAPP-ISIS, we have previously reported a significant transmural gradient in the myocardial PCr to ATP ratio across LV wall of the normal heart, with slightly but significantly lower PCr/ATP in the inner layer of the LV wall(16,26,28). The concept that the inner layer myocardium of LV is more susceptible to an ischemia insult metabolically is based on the fact that inner layer works twice as much as the outer layer(29) and myocardial blood flow ceases during systole as a result of the back pressure during the systole(30). This concept was later demonstrated experimentally in a study where myocardial ischemia was induced by a partial coronary artery stenosis produced by a hydraulic occluder, with the coronary perfusion pressure monitored using an intra-coronary pressure transducer and regional myocardial blood flow measured using microspheres(12). In this experimental setting, the myocardial PCr/ATP ratio was measured continuously while the severity of coronary stenosis was progressively increased by tightening the hydraulic occluder. The present study uses 1D-CSI, which uses a spatially linear gradient field to spatially encode. Compared to FLAX-ISIS and RAPP-ISIS, which utilizes the B_1 gradient of a surface coil to spatially encode, 1D-CSI results in a uniform spatial resolution (identical voxel size) whereas FLAX-ISIS and RAPP-ISIS results in a non-uniform spatial resolution due to the B_1 gradient being spatially nonlinear. The present data indicates that in the normal porcine heart at baseline, there is no significant transmural gradient across the LV wall in terms of PCr/ATP, CK forward flux rate, and ATP hydrolysis rate (Table 2). The reason for the discrepancy between the present study and previous reports that used canine models(12,24,27) remain unclear. It could be simply be due to the small size of the study group, or the difference in species between the porcine and canine hearts.

ATP turnover Rates in the Subepicardial and Subendocardial Layers of the LV Wall Spectra were generated for the epicardial and endocardial layers of the LV wall in the absence of saturation, with ATP saturated, and with both PCr and Pi saturated (Figure 4); notably, the resonance for 2,3-DPG was considerably more prominent in endocardial spectra than in epicardial spectra, which can be explained by the presence of blood in the LV chamber. Please note that all spectra shown were obtained from short TR and represent partially relaxed states. In both LV layers, the PCr peak was lower in the ATP-saturated spectrum than in the unsaturated spectrum, while the ATP peak declined in response to simultaneous PCr and Pi saturation. The ATP hydrolysis rate ($flux_{r,ATPase} = [ATP]k_{r,ATPase}$) and CK flux rate ($flux_{f,CK} = [PCr]k_{f,CK}$) was extracted as described in the methods section. Significant differences were not observed between the epicardial and endocardial layers for either flux

rate, and $flux_{f,CK}$ did not change significantly when the cardiac workload was increased (Table 2). However, $flux_{f,ATPase}$ in both regions was significantly greater during periods of high cardiac workload in comparison with baseline condition, which is consistent with the concept that ATP consumption increases when RPP increases.

CONCLUSION

A novel method in measuring myocardial ATP hydrolysis rates with transmural differentiation in the *in vivo* heart has been demonstrated. The findings in the present study indicate that there are no significant differences between EPI vs. ENDO concerning the ATP hydrolysis rate or CK flux rate, in both baseline and high cardiac work states of normal porcine model. The change of the basal to high cardiac work states is accompanied by a linear increase of ATP hydrolysis rate with no changes in CK flux rate.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

1. Conway MA, Bottomley PA, Ouwerkerk R, Radda GK, Rajagopalan B. Mitral regurgitation: impaired systolic function, eccentric hypertrophy, and increased severity are linked to lower phosphocreatine/ATP ratios in humans. *Circulation*. 1998; 97(17):1716–1723. [PubMed: 9591766]
2. Hardy CJ, Weiss RG, Bottomley PA, Gerstenblith G. Altered myocardial high- energy phosphate metabolites in patients with dilated cardiomyopathy. *Am Heart J*. 1991; 122(3 Pt 1):795–801. [PubMed: 1877457]
3. Smith CS, Bottomley PA, Schulman SP, Gerstenblith G, Weiss RG. Altered creatine kinase adenosine triphosphate kinetics in failing hypertrophied human myocardium. *Circulation*. 2006; 114(11):1151–1158. [PubMed: 16952984]
4. Weiss RG, Bottomley PA, Hardy CJ, Gerstenblith G. Regional myocardial metabolism of high-energy phosphates during isometric exercise in patients with coronary artery disease. *N Engl J Med*. 1990; 323(23):1593–1600. [PubMed: 2233948]
5. Weiss RG, Gerstenblith G, Bottomley PA. ATP flux through creatine kinase in the normal, stressed, and failing human heart. *Proceedings of the National Academy of Sciences of the United States of America*. 2005; 102(3):808–813. [PubMed: 15647364]
6. Liao R, Nascimben L, Friedrich J, Gwathmey JK, Ingwall JS. Decreased energy reserve in an animal model of dilated cardiomyopathy. Relationship to contractile performance. *Circulation research*. 1996; 78(5):893–902. [PubMed: 8620610]
7. Neubauer S, Horn M, Cramer M, Harre K, Newell JB, Peters W, Pabst T, Ertl G, Hahn D, Ingwall JS, Kochsiek K. Myocardial phosphocreatine-to-ATP ratio is a predictor of mortality in patients with dilated cardiomyopathy. *Circulation*. 1997; 96(7):2190–2196. [PubMed: 9337189]
8. Saupe KW, Spindler M, Tian R, Ingwall JS. Impaired cardiac energetics in mice lacking muscle-specific isoenzymes of creatine kinase. *Circulation research*. 1998; 82(8):898–907. [PubMed: 9576109]
9. Balaban RS, Kantor HL, Katz LA, Briggs RW. Relation between work and phosphate metabolite in the *in vivo* paced mammalian heart. *Science*. 1986; 232(4754):1121–1123. [PubMed: 3704638]

10. Xiong Q, Ye L, Zhang P, Lepley M, Tian J, Li J, Zhang L, Swingen C, Vaughan JT, Kaufman DS, Zhang J. Functional consequences of human induced pluripotent stem cell therapy: myocardial ATP turnover rate in the in vivo swine heart with postinfarction remodeling. *Circulation*. 2013; 127(9):997–1008. [PubMed: 23371930]
11. Path G, Robitaille PM, Merkle H, Tristani M, Zhang J, Garwood M, From AH, Bache RJ, Ugurbil K. Correlation between transmural high energy phosphate levels and myocardial blood flow in the presence of graded coronary stenosis. *Circulation research*. 1990; 67(3):660–673. [PubMed: 2397574]
12. Zhang J, Ugurbil K, From AH, Bache RJ. Myocardial oxygenation and high- energy phosphate levels during graded coronary hypoperfusion. *Am J Physiol Heart Circ Physiol*. 2001; 280(1):H318–326. [PubMed: 11123247]
13. Zhang J, Duncker DJ, Xu Y, Zhang Y, Path G, Merkle H, Hendrich K, From AH, Bache RJ, Ugurbil K. Transmural bioenergetic responses of normal myocardium to high workstates. *Am J Physiol*. 1995; 268(5 Pt 2):H1891–1905. [PubMed: 7771539]
14. Zhang J, From AH, Ugurbil K, Bache RJ. Myocardial oxygenation and high- energy phosphate levels during KATP channel blockade. *Am J Physiol Heart Circ Physiol*. 2003; 285(4):H1420–1427. [PubMed: 12805030]
15. Zhang J, Path G, Chepuri V, Homans DC, Merkle H, Hendrich K, Ugurbil K, Bache RJ, From AH. Effects of dobutamine on myocardial blood flow, contractile function, and bioenergetic responses distal to coronary stenosis: implications with regard to dobutamine stress testing. *Am Heart J*. 1995; 129(2):330–342. [PubMed: 7832107]
16. Gong G, Ugurbil K, Zhang J. Transmural metabolic heterogeneity at high cardiac work states. *Am J Physiol*. 1999; 277(1 Pt 2):H236–242. [PubMed: 10409202]
17. Jennings RB, Reimer KA, Hill ML, Mayer SE. Total ischemia in dog hearts, in vitro. 1. Comparison of high energy phosphate production, utilization, and depletion, and of adenine nucleotide catabolism in total ischemia in vitro vs. severe ischemia in vivo. *Circulation research*. 1981; 49(4):892–900. [PubMed: 7273360]
18. Ugurbil K. Magnetization transfer measurements of creatine kinase and ATPase rates in intact hearts. *Circulation*. 1985; 72(5 Pt 2):IV94–96. [PubMed: 2932267]
19. Jeffrey FM, Storey CJ, Nunnally RL, Malloy CR. Effect of ischemia on NMR detection of phosphorylated metabolites in the intact rat heart. *Biochemistry*. 1989; 28(13):5323–5326. [PubMed: 2775710]
20. Humphrey SM, Garlick PB. NMR-visible ATP and Pi in normoxic and reperfused rat hearts: a quantitative study. *Am J Physiol*. 1991; 260(1 Pt 2):H6–12. [PubMed: 1992810]
21. Xiong Q, Du F, Zhu X, Zhang P, Suntharalingam P, Ippolito J, Kamdar FD, Chen W, Zhang J. ATP production rate via creatine kinase or ATP synthase in vivo: a novel superfast magnetization saturation transfer method. *Circulation research*. 2011; 108(6):653–663. [PubMed: 21293002]
22. de Graaf RA, Luo Y, Garwood M, Nicolay K. B1-Insensitive, Single-Shot Localization and Water. *J Magn Reson B*. 1996; 113(1):35–45. [PubMed: 8888589]
23. Garwood M, Schleich T, Ross BD, Matson GB, Winters WD. A Modified Rotating Frame Experiment Based on a Fourier Series Window Function. Application to in vivo Spatially Localized NMR Spectroscopy. *J Magn Reson*. 1985; 65(2):239–251.
24. Zhang J, Merkle H, Hendrich K, Garwood M, From AH, Ugurbil K, Bache RJ. Bioenergetic abnormalities associated with severe left ventricular hypertrophy. *J Clin Invest*. 1993; 92(2):993–1003. [PubMed: 8349829]
25. Ye L, Haider HK, Jiang S, Tan RS, Ge R, Law PK, Sim EKW. Improved angiogenic response in pig heart following ischaemic injury using human skeletal myoblast simultaneously expressing VEGF165 and angiopoietin-1. *European Journal of Heart Failure*. 2007; 9(1):15–22. [PubMed: 16829190]
26. Zhang J, Wilke N, Wang Y, Zhang Y, Wang C, Eijgelshoven MH, Cho YK, Murakami Y, Ugurbil K, Bache RJ, From AH. Functional and bioenergetic consequences of postinfarction left ventricular remodeling in a new porcine model. MRI and 31 P-MRS study. *Circulation*. 1996; 94(5):1089–1100. [PubMed: 8790051]

27. Zhang J, Murakami Y, Zhang Y, Cho YK, Ye Y, Gong G, Bache RJ, Ugurbil K, From AH. Oxygen delivery does not limit cardiac performance during high work states. *Am J Physiol.* 1999; 277(1 Pt 2):H50–57. [PubMed: 10409181]
28. Zhang J, Duncker DJ, Ya X, Zhang Y, Pavsek T, Wei H, Merkle H, Ugurbil K, From AH, Bache RJ. Effect of left ventricular hypertrophy secondary to chronic pressure overload on transmural myocardial 2-deoxyglucose uptake. A 31P NMR spectroscopic study. *Circulation.* 1995; 92(5): 1274–1283. [PubMed: 7648676]
29. Homans DC, Asinger R, Elsperger KJ, Erlien D, Sublett E, Mikell F, Bache RJ. Regional function and perfusion at the lateral border of ischemic myocardium. *Circulation.* 1985; 71(5):1038–1047. [PubMed: 3986974]
30. Duncker DJ, Zhang J, Bache RJ. Coronary pressure-flow relation in left ventricular hypertrophy. Importance of changes in back pressure versus changes in minimum resistance. *Circulation research.* 1993; 72(3):579–587. [PubMed: 8431985]

Figure 1. BISTRO pulse sequence used in partially relaxed MST experiment

A train of adiabatic full-passage (AFP) pulses interleaved with crusher gradients achieves selective saturation of target frequency. This is sequentially followed by excitation, phase encode, and readout. Length of excitation pulse is assumed to be negligible ($T_p \approx 0$). For the control spectrum, the power of the pulse train is turned off.

Figure 2. Numerical simulation of the T_1^{nom} method for PCr-Pi double saturation

Longitudinal magnetizations (a) without saturation (M_c) and (b) with PCr-Pi double saturation (M_s) as a function of number of excitations (n_{EX}) achieve steady-state. Spin system parameters used: PCr:ATP:Pi = 1:0.48:0.2; $T_{1,PCr}^{int} = 3.2s$, $T_{1,ATP}^{int} = 1.1s$ and $T_{1,Pi}^{int} = 3.0s$. Chemical exchange parameters: $k_{f,CK} = 0.3 \text{ sec}^{-1}$; $k_{f,ATPase} = 0.18 \text{ sec}^{-1}$. Acquisition parameters: TR = 2.5s, FA = 90° , $d_1 = 0.052s$. Simulated M_c/M_s ratio versus $k_{ATP,total} = k_{r,CK} + k_{r,ATPase}$ dependence on (c) FA and (d) TR. $k_{f,CK}$ was swept from 0~0.6 sec^{-1} , with $k_{f,ATPase} = 0$ to show that the intercept occurs at 1. Fully relaxed state is indicated by dashed line (M_0/M_{ss}) in which the slope is equal to $T_{1,ATP}^{int}$. Sweeping $k_{f,CK}$ with $k_{f,ATPase} = 0$ produces identical result. Same spin system parameters and delay were used. Linear relationship between M_c/M_s and $k_{ATP,total}$ as observed in the single ATP saturation case confirms that the T_1^{nom} method can be applied for double saturation.

Figure 3. Flow chart of applying T_1^{nom} method to extract transmural rate constants

Fully relaxed global magnetization-saturation-transfer experiments (top left) are performed in the absence of saturation, with ATP saturation and with both PCr and Pi simultaneous saturation, yielding global rate constants $k_{f,CK}$ and $k_{ATP,total}$. Partially relaxed global spectra (top right) control, single-, and double-saturation, yield global $M_{c,PCr}/M_{c,ATP}$, $M_{s,PCr}$, and $M_{s,ATP}$ in partially relaxed states, respectively. These global measurements are combined to calculate T_1^{nom} for both PCr and ATP equations [5] and [6]. The obtained T_1^{nom} values and the normalization factor are then incorporated with the measurements obtained from the 3 partially relaxed transmural spectra (bottom) to calculate $k_{f,CK}$ and $k_{r,ATPase}$ using equations [7] and [8] for each transmural layer.

Figure 4. ^{31}P MRS-MST assessments of myocardial ATP turnover rates from partially relaxed spectra

Spectra were generated for the epicardial (EPI) and endocardial (ENDO) layers of the LV wall with ATP saturated (green arrows), in the absence of saturation, and with both PCr (blue arrows) and Pi (red arrows) saturated. The Fourier-series window profile produced minimal (<3%) overlap between neighboring voxels. Changes in the intensities (black double arrows) of the resonances for PCr in the ATP-saturated spectra and for ATP in the PCr/Pi double-saturated spectra were used to calculate the fluxes of the ATP→PCr and ATP→Pi reactions. Note, it is the difference in relative amount (spectra integral), not spectra height, which is used to extract PCr and calculate rates.

Figure 5. Correlation of flux rates with baseline and high work states of normal porcine hearts (a) Hydrolysis flux rate ($flux_{r,ATPase}$) vs. rate pressure product (RPP) under baseline and high cardiac work states show that an increase in cardiac workload is accompanied by an increase in ATP hydrolysis rate for both EPI and ENDO layers. There was no significant difference between EPI vs. ENDO for $flux_{r,ATPase}$ and (b) CK flux rate ($flux_{f,CK}$) in both baseline and high cardiac work states.

Table 1

Data are presented as mean±SEM.

	HR (beats/min)	LVSP (mmHg)	RPP (mmHg/min×1000)	AV diff (ml/dl)	<i>MVO</i> ₂ (ml·O ₂ /min/100 gram)	
					EPI	ENDO
Baseline	96.1±4.93	90.6±2.27	8.71±0.48	11.1±0.89	10.21±0.82	10.55±0.85
High Cardiac Workload	194±7.50 *	116±4.55 *	22.40±0.77 *	11.7±0.87	27.57±1.93 *	28.47±2.00 *

RPP (mmHg/min×1000) is calculated as the product of heart rate and LVSP. *MVO*₂ (ml·O₂/min/100 gram of myocardium) is calculated as the product of arteriovenous oxygen difference (AV diff) and myocardial blood flow (MBF). AV diff (ml/dl) was assumed to be same in both EPI and ENDO layers. Basal MBF of 0.92 ml min⁻¹ g⁻¹ in the EPI layers and 0.95 ml min⁻¹ g⁻¹ in the ENDO layers were taken from a previous report using the same in vivo model(26). In high cardiac workload state, MBF was increased proportional to increase in RPP. n = 9 for measurements (HR/LVSP/RPP) at both baseline and high cardiac workload; n = 9 and n = 6 for AV diff/*MVO*₂ at baseline and high cardiac work state, respectively.

*p<0.01 vs. baseline.

Table 2

Data are calculated based on $[ATP] = 5.3 \mu\text{mol/g}$ and are presented as mean $\pm$ SE.

	Baseline		High Cardiac Workload	
	EPI	ENDO	EPI	ENDO
PCr/ATP	2.02 $\pm$ 0.05	2.2 $\pm$ 0.05	2.21 $\pm$ 0.09	2.44 $\pm$ 0.11
$k_{t,CK} (s^{-1})$	0.40 $\pm$ 0.04	0.38 $\pm$ 0.06	0.37 $\pm$ 0.06	0.29 $\pm$ 0.01
$flux_{t,CK} (\mu\text{mol g}^{-1} s^{-1})$	4.4 $\pm$ 0.53	4.57 $\pm$ 0.84	4.52 $\pm$ 0.47	3.68 $\pm$ 0.16
$k_{r,ATPase} (s^{-1})$	0.21 $\pm$ 0.02	0.21 $\pm$ 0.02	0.44 $\pm$ 0.05 *	0.42 $\pm$ 0.02 *
$flux_{r,ATPase} (\mu\text{mol g}^{-1} s^{-1})$	1.11 $\pm$ 0.12	1.12 $\pm$ 0.13	2.35 $\pm$ 0.27 *	2.21 $\pm$ 0.08 *

PCr/ATP ratios (n = 8 for baseline and n = 7 for HWL) were obtained using partially relaxed spectra that were normalized back to its fully relaxed equivalent state as described in the methods section. n = 6 for baseline flux rates and n = 5 for high cardiac workload flux rates. EPI, subepicardial; ENDO, subendocardial

*p<0.01 vs. baseline.