Myocardial ATP turnover rates in the in vivo hearts with post-infarction remodeling

Qiang Xiong[1], Pengyuan Zhang[1], Lei Ye[1], Jinfeng Tian[1], Cory Swingen[1], Albert Jang[1], J. Thomas Vanghan[1], and Jianyi Zhang[1]
[1]*Center for Magnetic Resonance Research, University of Minnesota, Minneapolis, Minnesota, United States*

Introduction One of the most unsettled questions in cardiovascular physiology is how the rate of ATP metabolism is regulated in the heart and altered in response to myocardial injury. Conventionally, magnetization saturation transfer (MST) has been widely utilized to measure the ATP synthesis rate [Eqn. 1], where $M_{0,Pi}$ and $M_{ss,Pi}$ stand for fully-relaxed magnetization of inorganic phosphate (Pi) without or with saturation on ATPγ resonance.[1] However, in *in vivo* heart, difficulties in accurate quantification of myocardial inorganic phosphate levels limit the measurement of Pi↔ATP kinetics with conventional MST method. In current study, we developed an indirect MST approach that measures the kinetics of ATP→Pi reaction without quantification of Pi. With the new approach, ATP→Pi reaction rates were measured in normal and diseased hearts based on a swine model of post-infarction left ventricular remodeling.

Methods and Results The flux of ATP→Pi could be indirectly calculated by subtracting the creatine kinase (CK) reaction (ATP↔Phosphocreatine (PCr)) from the total ATP turnover rate. The total ATP turnover rate could be measured from an MST experiment with double saturation on both PCr and Pi peaks. Therefore, the ATP→Pi rate constant ($k_{Pi\rightarrow ATP}$) could be calculated by [Eqn. 2], where $M_{0,ATP\gamma}$ and $M_{ss,ATP\gamma}$ stand for fully relaxed magnetization of ATPγ without or with saturation on both PCr and Pi, and $M_{0,PCr}$ and $M_{ss,PCr}$ stand for fully relaxed magnetization of PCr without or with saturation on ATPγ. [Eqn. 2] does not require Pi quantification and thus avoids the primary barrier for applications in *in vivo* hearts.

$$k_{Pi\rightarrow ATP}=\left(\frac{M_{0,Pi}-M_{ss,Pi}}{M_{ss,Pi}}\right)\Big/T_{1,Pi}^{int} \quad [1] \qquad k_{ATP\rightarrow Pi}=\left(\frac{M_{0,ATP\gamma}-M_{ss,ATP\gamma}}{M_{ss,ATP\gamma}}\right)\Big/T_{1,ATP\gamma}^{int}-\frac{M_{0,PCr}}{M_{0,ATP\gamma}}\left(\frac{M_{0,PCr}-M_{ss,PCr}}{M_{ss,PCr}}\right)\Big/T_{1,PCr}^{int} \quad [2]$$

The new approach was validated on swine skeletal muscle (n=5), where Pi is MR-measurable such that both convention and new MST approaches could be applied. The total ATP turnover rate as calculated from [Eqn. 4] was compared to total ATP production rate (PCr→ATP and Pi→ATP) as measured using conventional MST approach with saturation frequency set on ATPγ resonance. The measurements from both approaches were not significantly different.

The new MST approach was further applied in *in vivo* hearts (Fig. 2A). MR measurements were performed using a 9.4T-64cm-bore magnet interfaced with vnmrj console. Three groups of swine were included in the study: i) normal pigs (Normal, n=11); ii) myocardium-infarcted pigs (MI, n=9, animals experienced myocardial infarction 4 weeks before); iii) myocardium-infarcted pigs treated with stem cell therapy[2] (CELL, n=10, animals experienced identical MI and were transplanted with 4 million cardiovascular cells). Cardiac MRI performed immediately prior to MST experiments revealed a better left ventricular (LV) functional outcome in the CELL group than the MI group ($p<0.05$). LV hemodynamic parameters in MI and CELL animals were similar to measurements in Normal animals both at baseline and during high cardiac workload states (HWL, induced by catecholamine infusion), which suggests that the injured hearts were in the compensated phase of LV remodeling. MST experiments were performed during both baseline and HWL conditions. In Normal hearts, myocardial ATP hydrolysis rate is linearly related to cardiac workload (Fig.2B). The border zone (BZ) myocardium of MI hearts demonstrated a severe reduction in ATP hydrolysis rate (61% reduction in $k_{ATP\rightarrow Pi}$, Fig.2C) as compared to Normal hearts and lost the capacity of up-regulate the $k_{ATP\rightarrow Pi}$ during HWL (Fig. 2C). The abnormality was not present in the remote zone (RZ) of MI hearts or in BZ of CELL hearts. In contrast, the rate constant of CK ($k_{PCr\rightarrow ATP}$) was found similar among all groups at both baseline and HWL conditions. Plots of myocardial $Flux_{ATP\rightarrow Pi}$ against MRI measurements demonstrated significant correlation between the myocardial ATP hydrolysis rate and the severity of post-infarction LV remodeling ($p<0.05$).

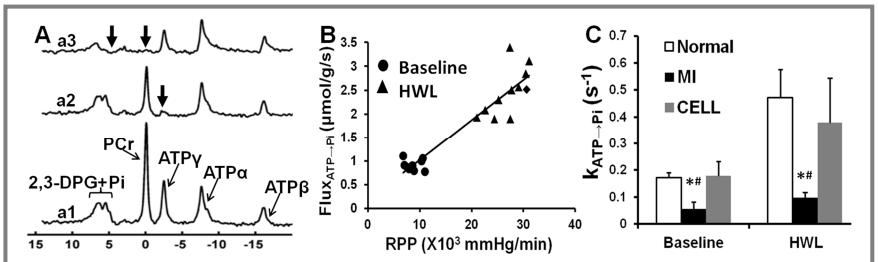

Figure 2. A. MST measurements from in vivo swine heart. **B.** linear relation between myocardial $Flux_{ATP\rightarrow Pi}$ and cardiac workload (RPP: LV rate pressure product). **C.** Summary of $k_{ATP\rightarrow Pi}$ measurements from in vivo swine hearts.

Conclusion With a newly developed MST approach, we demonstrated a heterogeneous abnormality of myocardial ATP hydrolysis (ATP→Pi) rate in post-infarct swine hearts. The myocardial ATP→Pi rate is a sensitive bioenergetic index that is tightly correlated to cardiac workload as well as the severity of post-infarction left ventricular remodeling.

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References [1] Kingsley-Hickman PB, et al. Biochemistry, 1987. [2] Xiong Q, et al. Circ Res, 2012.