ATP Production Rate via Creatine Kinase or ATP Synthase

In Vivo

A Novel Superfast Magnetization Saturation Transfer Method

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Rationale: $^{31}$P magnetization saturation transfer (MST) experiment is the most widely used method to study ATP metabolism kinetics. However, its lengthy data acquisition time greatly limits the wide biomedical applications in vivo, especially for studies requiring high spatial and temporal resolutions.

Objective: We aimed to develop a novel superfast MST method that can accurately quantify ATP production rate constants ($k_f$) through creatine kinase (CK) or ATP synthase (ATPase) with 2 spectra.

Methods and Results: The $T_1^{\text{nom}}$ ($T_1$ nominal) method uses a correction factor to compensate the partially relaxed MST experiments, thus allowing measurement of enzyme kinetics with an arbitrary repetition time and flip angle, which consequently reduces the data acquisition time of a transmurally differentiated CK $k_f$ measurement by 91% as compared with the conventional method with spatial localization. The novel $T_1^{\text{nom}}$ method is validated theoretically with numeric simulation, and further verified with in vivo swine hearts, as well as CK and ATPase activities in rat brain at 9.4 Tesla. Importantly, the in vivo data from swine hearts demonstrate, for the first time, that within an observation window of 30 minutes, the inhibition of CK activity by iodoacetamide does not limit left ventricular chamber contractile function.

Conclusions: A novel MST method for superfast examination of enzyme kinetics in vivo has been developed and verified theoretically and experimentally. In the in vivo normal heart, redundant multiple supporting systems of myocardial ATP production, transportation, and utilization exist, such that inhibition of one mechanism does not impair the normal left ventricular contractile performance. (Circ Res. 2011;108:653-663.)

Key Words: heart ■ metabolism ■ magnetic resonance spectroscopy ■ ATP ■ phosphates

The adenosine triphosphate (ATP) metabolism in a living organ is characterized by a chemical exchange network among phosphocreatine (PCr), ATP, and inorganic phosphate (Pi), which is largely controlled by the enzymes creatine kinase (CK) (catalyzing $\text{PCr}\leftrightarrow\text{ADP}$) and ATP synthase (ATPase) (catalyzing $\text{Pi}\leftrightarrow\text{ADP}$).

$$
\begin{align*}
  & k_{f,\text{CK}} \quad \text{PCr} \leftrightarrow \text{ATP} \\
  & k_{r,\text{ATPase}} \quad \text{Pi} \leftrightarrow \text{ATP}
\end{align*}
$$

where $k_f$ and $k_r$ are the pseudo–first-order forward and reverse rate constants for CK and ATPase reactions. Under most in vivo circumstances, a steady-state condition is established, resulting in equal forward and reverse fluxes for both CK and ATPase reactions. Therefore, the kinetics of $\text{PCr}\leftrightarrow\text{ATP}\leftrightarrow\text{Pi}$ chemical exchange can be characterized by 2 forward pseudo–first-order rate constants ($k_{f,\text{CK}}$ for $\text{PCr}\rightarrow\text{ATP}$ and $k_{f,\text{ATPase}}$ for $\text{Pi}\rightarrow\text{ATP}$) and studied by $^{31}$P magnetization saturation transfer (MST) experiment, where ATP$\gamma$ resonance is selectively saturated.1,2

The exchange rates of CK and ATPase reactions have been extensively studied on various organs, such as heart, brain, and skeletal muscle.3–5 Previous studies have suggested that the kinetics of the $\text{PCr}\leftrightarrow\text{ATP}\leftrightarrow\text{Pi}$ exchange network may be associated with the pathological status of the organ. For example, significantly lowered ATP production rates via CK have been observed in association with various heart diseases in both large animal models4,5 and patients.6–8 The cerebral ATP metabolic rate through ATPase has been demonstrated to be tightly coupled to brain activity level in a rat model.9 In addition, the CK activity in the visual cortex of human brain was increased during visual simulation.10 In contrast, in heart it was found that CK forward flux rate was independent from...
the increase of cardiac workloads in response to catecholamine stimulations.5

To compensate the lengthy data acquisition time imposed by conventional MST technique, Bottomley et al proposed a four-angle saturation transfer (FAST) method, allowing rapid in vivo measurement of CK reaction rates with 4 short-repetition time (TR) spectra.11 This method was later used by Weiss et al in patients to examine the myocardial CK reaction kinetics.11 We have recently reported an improved MST method for measuring CK kinetics with as few as 3 spectra,12 the method focused on minimizing the saturation time by optimizing the presaturation delay, which resulted in a significant reduction of repetition time.

In the present study, we demonstrate a novel steady-state MST method (T1 nom) for performing extremely rapid measurements of CK and ATPase kinetics with arbitrary repetition time and flip angle (FA). The accurate quantification of kf under such partial relaxation conditions requires only 2 spectra. The T1 nom method is theoretically validated based on numeric simulation of modified Bloch–McConnell equations that govern the evolution of spin magnetizations during MST experiment. In addition, an optimization strategy for finding the best acquisition parameter range (TR and FA) used in the T1 nom method is provided. The new method is verified experimentally with in vivo measurements of: (1) kf,CK on swine heart model during the process of CK inhibition by iodoacetamide (IAA) infusion; and (2) both kf,CK and kf,ATPase on rat brain model at rest condition. Finally, the T1 nom method was used to measure the myocardial CK forward rate constant with transmural differentiation, demonstrating a reduction of data acquisition time by 91% as compared with a similar study using conventional saturation transfer method.13

Detailed descriptions of different types of T1 are included in the Online Data Supplement, available at http://circres.ahajournals.org.

Theory

kf Calculation of Conventional Steady-State MST Experiment

The evolution of spin magnetizations in the coupled CK and ATPase reactions can be characterized by the modified Bloch–McConnell equations,14,15 as shown below:

\[
\frac{dM_{PCr}(t)}{dt} = \frac{M_{0,PCr} - M_{PCr}(t)}{T_{1,PCr}^{int}} - k_{f,CK}M_{PCr}(t) + k_{f,ATP}M_{ATP}(t)
\]

\[
\frac{dM_{Pi}(t)}{dt} = \frac{M_{0,Pi} - M_{Pi}(t)}{T_{1,Pi}^{int}} - k_{f,ATP}M_{Pi}(t) + k_{f,ATP}M_{ATP}(t)
\]

\[
\frac{dM_{ATP}(t)}{dt} = \frac{M_{0,ATP} - M_{ATP}(t)}{T_{1,ATP}^{int}} + k_{f,CK}M_{PCr}(t) + k_{f,ATP}M_{Pi}(t)
\]

When ATPγ is selectively saturated as applied in MST experiments, Equations 1 through 3 change to:

\[
\frac{dM_{PCr}(t)}{dt} = \frac{M_{0,PCr} - M_{PCr}(t)}{T_{1,PCr}^{int}} - k_{f,CK}M_{PCr}(t)
\]

\[
\frac{dM_{Pi}(t)}{dt} = \frac{M_{0,Pi} - M_{Pi}(t)}{T_{1,Pi}^{int}} - k_{f,ATP}M_{Pi}(t)
\]

\[
M_{ATP}(t) = 0
\]

Equation 4 and 5 are mathematically equivalent; therefore, CK and ATPase reactions are treated together using the same equations in the following discussion. The extent of the reduction of PCr and Pi magnetizations in response to ATPγ saturation is proportional to the forward rate constants:

\[
k_{f,CK(ATPase)} = \frac{M_{0,PCr(Pi)} - M_{0,PCr(Pi)}}{M_{PCr(Pi)}}
\]

where M0 and M0 represent the fully relaxed magnetizations with and without saturation on ATPγ and T1 int is the intrinsic longitudinal relaxation time constant. kf calculation using Equation 7 is called conventional steady-state MST experiment, which requires measurement of 2 fully relaxed spectra.
The new relationship between $k_f$ value and the extent of magnetization reduction in response to ATPγ saturation can be elucidated by numeric simulation with various $k_f$ values and acquisition parameters (Figure 3). The simulation results suggest an approximately linear relationship between $M_r/M_s$ ratio and $k_f$ values under various acquisition conditions. Therefore, based on a simple linear regression, Equation 7 can be reformulated into the following equation for $k_f$ quantification under partial relaxation conditions:

$$
\frac{M_{r,PCr(Pi)}}{M_{s,PCr(Pi)}} = \beta + T_{1,PCr(Pi)}^{\text{nom}} \cdot k_f^{\text{CK}(\text{ATPase})}
$$

where $\beta$ is the intercept (usually within ±5% of 1) and $T_{1,PCr(Pi)}^{\text{nom}}$ is the slope of the line obtained by linear regression of the simulated $M_r/M_s$ versus $k_f$ plot. Equation 8 is similar to the following equation which is the rearrangement of Equation 7 (dashed lines in Figure 3):

$$
\frac{M_{r,PCr(Pi)}}{M_{s,PCr(Pi)}} = 1 + T_{1,PCr(Pi)}^{\text{int}} \cdot k_f^{\text{CK}(\text{ATPase})}
$$

Equation 8 indicates that the partial relaxation effects can be largely accounted for by one empirical parameter $T_{1,PCr(Pi)}^{\text{nom}}$ (means nominal $T_1$ in contrast to intrinsic $T_1$ as in Equation 9). In general, $T_{1,PCr(Pi)}^{\text{nom}}$ is a function of both spin system parameters ($T_{1,PCr(Pi)}^{\text{int}}$ and pool size ratios of metabolites, such as PCr/ATP or Pi/ATP ratio) and acquisition parameters (TR and FA), and it approaches to $T_{1,PCr(Pi)}^{\text{int}}$ as TR increases and/or FA decreases:

$$
T_{1,PCr(Pi)}^{\text{nom}} = f(T_{1,PCr(Pi)}^{\text{int}}, \text{pool size ratio}; \text{TR,FA})
$$

There is no general analytic expression for Equation 10; however, the value of $T_{1,PCr(Pi)}^{\text{nom}}$ can be obtained with linear regression of simulated $M_r/M_s$ versus $k_f$ plot based on Equation 1 through 6. In practice $T_{1,PCr(Pi)}^{\text{nom}}$ and $\beta$ can be empirically determined for specific experimental setup, and then the $k_f$ value can be readily calculated with $M_r$ and $M_s$ measurements according to Equation 8.

**Optimization Strategy for $T_{1,PCr(Pi)}^{\text{nom}}$ Method**

$T_{1,PCr(Pi)}^{\text{nom}}$ method allows $k_f$ calculation with arbitrary repetition time and flip angle. However, the best experimental condition (optimal TR and FA) remains unclear. Here, we provide an optimization strategy to generate the best TR/FA range for $T_{1,PCr(Pi)}^{\text{nom}}$-based $k_f$ measurement and quantification. The goal of optimization is to have the smallest
relative $k_f$ calculation error for a given data acquisition time. Three types of $k_f$ error have been considered in this section. Analytic expression for each type is provided followed by a demonstration of parameter optimization using human brain studies at 7 Tesla.\(^{16}\)

**Type 1 Error: $M_c/M_s$ Versus $k_f$ Nonlinearity**

$M_c/M_s$ versus $k_f$ plot in Figure 3 are not perfectly straight lines. The relative $k_f$ calculation error attributable to nonlinearity is defined as below:

$$\left( \frac{\delta k_f}{k_f}_{\text{NLIN}} \right) = \left| \frac{k_f^{\text{real}} - k_f^{\text{cal}}}{k_f^{\text{real}}} \right|$$

(11)

where $k_f^{\text{real}}$ and $k_f^{\text{cal}}$ stand for actual $k_f$ and $k_f$ calculated from Equation 8, respectively. The deviation attributable to nonlinearity is acquisition condition dependent; thus, the optimization can be performed such that the type 1 error is minimized.

**Type 2 Error: Spectral SNR**

$k_f$ calculation is based on 2 measurements from control ($M_c$) and saturated ($M_s$) spectra (Equation 8), each of which is subject to sampling error attributable to finite spectral SNR. The measurement error of each spectrum would in turn contribute to the final $k_f$ calculation error following error propagation theory.

Assuming a constant total acquisition time ($t$) and intrinsic scanner noise level ($\sigma$), the final $k_f$ relative error attributable to spectral SNR can be expressed as:

$$\left( \frac{\delta k_f}{k_f}_{\text{SNR}} \right) = \frac{M_c/M_s}{M_s/M_c - \beta} \sqrt{\frac{1}{T_1} + \frac{1}{\frac{1}{\alpha} \sqrt{\frac{1+T_1}{T_1}}} \frac{T_1}{\sigma}}$$

(12)

Equation 12 (see deduction in the Online Data Supplement) takes into account both the SNR of each spectrum ($M_c$ and $M_s$) and the sensitivity level of $k_f$ calculation toward spectral errors ($T_1^{\text{nom}}$ value). A normalized type 2 error ($K_{\text{SNR}}$) can be introduced from Equation 13:

$$K_{\text{SNR}} = \frac{\delta k_f}{k_f}_{\text{SNR}} \sqrt{\frac{1+\frac{T_1}{M_c}}{M_s/M_c - \beta}}$$

(13)

Because of the lack of extra information on MR system performance or total data acquisition time, the optimization strategy is based on minimizing $K_{\text{SNR}}$ level.

**Type 3 Error: Flip Angle Inaccuracy**

Flip angle can vary spatially because of $B_1$ field inhomogeneity, especially in the case of surface coil and ultrahigh magnetic field. Such variation can be greatly minimized by using adiabatic pulses (such as BIR4 pulse as used in the present study\(^{21}\)). Therefore, the accuracy of $k_f$ calculation based on the $T_1^{\text{nom}}$ method would be affected by flip angle variation. The relative $k_f$ calculation error attributable to flip angle inaccuracy can be expressed by the following equation (see detailed deduction in the Online Data Supplement):

$$K_{\text{flip}} = - \left( \frac{\delta k_f}{k_f}_{\text{flip}} \right) / \frac{\delta FA}{FA} - \frac{\delta T_1^{\text{nom}}}{T_1^{\text{nom}}}$$

(14)

$K_{\text{flip}}$ is a nondimensional parameter that characterizes the sensitivity level of $k_f$ error attributable to flip angle error, i.e., a smaller absolute $K_{\text{flip}}$ value means the $k_f$ calculation is more robust against flip angle variation. The negative sign in Equation 14 indicates that an underestimation of flip angle would result in overestimation of $k_f$ and vice versa. The optimization strategy thereby is to find the acquisition conditions that lead to a $K_{\text{flip}}$ value below an arbitrary level.
As a demonstration, numeric simulation for each type of $k_f$ error (Equations 11, 13, and 14) have been carried out based on human brain data at 7 Tesla and the results are shown in Figure 4a through 4f. By setting arbitrary cutoff criteria for each type of $k_f$ error, the overall optimized TR and FA range for the $T_{1\text{nom}}$ experiment can be obtained (Figure 4g and 4h, shadowed regions).

Methods

All experiments were performed in accordance with the animal use guidelines of the University of Minnesota, and the experimental protocol was approved by the University of Minnesota Research Animal Resources Committee. The investigation conformed to the NIH Guide for the Care and Use of Laboratory Animals (NIH publication No 85-23.).

In Vivo Swine Heart Studies

Validation of $T_{1\text{nom}}$ method was performed with a creatine kinase inhibition experiment by iodoacetamide (IAA), an irreversible CK inhibitor. Young female Yorkshire swine (≏30 kg, n=8) were used for the study. Iodoacetamide solution (450 mmol/L) was administrated (1 mL/kg per hour, IV), and a complete CK activity inhibition (as evidenced by $M_0\text{PCr}/M_{ss\text{PCr}}$) was usually achieved with a total dose of 0.45 mmol/kg IV. Infusion was paused every 10 minutes, and steady-state MST experiments were performed in both fully and partially relaxed conditions, with interleaved acquisition. Dummy scans were used to enforce steady state for MST experiments with partial relaxation. Five more pigs received an extra catecholamine intervention (dopamine/dobutamine, each of 10 μg/kg/min IV) after complete inhibition of CK. Details of the open-chest surgery preparation and $31P$ MRS have been described previously and are included in the Online Data Supplement.
The $T_{1\text{nom}}$ method was further used to measure myocardial CK activity with transmural differentiation on female Yorkshire pigs ($\approx 40$ kg, $n=4$). The spatially localized measurement was achieved with 1D chemical shift imaging (1D-CSI) sequence. Detailed methods are included in the Online Data Supplement.

To examine whether the left ventricular (LV) contractile function can be maintained when the CK system is completely inhibited, additional 6 swine were used for the cardiac MRI study on a clinical 1.5 Tesla scanner. LV chamber function was measured throughout the process of CK inhibition via iodoacetamide infusion at both basal and high cardiac workload conditions. Detailed cardiac MRI methods are included in the Online Data Supplement.

**In Vivo Rat Brain Studies**

Male Sprague-Dawley rats ($n=5$) were used for brain studies. Details of rat preparation as well as MRS data acquisition have been published previously (Online Data Supplement).

**Results**

**Cardiovascular Physiological Studies Using a Swine Model**

$^{31}$P MR Spectroscopy Data

Intrinsic $T_1$ measurements before and after complete CK inhibition yielded the same results for PCr ($3.2 \pm 0.2$ versus $3.1 \pm 0.2$ s, $P=\text{NS}$; see in Figure 5), suggesting that $T_{1\text{int}}$ value is independent of CK activity and thus it is feasible to apply $T_{1\text{nom}}$ method to calculate the CK activity based on a constant $T_{1\text{int}}$ value.

Figure 6 illustrates the representative spectra from steady-state MST experiments with various acquisition conditions throughout the CK inhibition process. ATP$\gamma$ saturation was achieved by BISTRO saturation pulse train, which has been shown to have negligible spillover effects on the neighboring PCr peak. As CK gets completely inhibited (top to bottom), the PCr magnetization in saturated spectra (Sat.) all approaches that of control spectra (Ctrl.), regardless of acquisition conditions, in agreement with Equation 8 when $k_f$ equals 0, $M_f/M_s$ ratio equals 1.

PCr signals measured with partially relaxed conditions (Figure 6b through 6d) throughout the CK inhibition process were quantified, and the ratio of PCr signals in control and saturated spectra was plotted against the CK $k_f$ value, as measured by conventional steady-state MST experiments (Figure 7). The plot indicates a linear relationship between PCr signal ratios and $k_f$ values, with a slope depending on the acquisition parameters. Also included in Figure 7 (solid lines) are the simulation results with the same parameters as used by the experiment. The experimental results matched the simulation, indicating the validity of the $T_{1\text{nom}}$ method. Notably, the steady-state MST experiments in condition d produced the least $k_f$ measurement error as compared with conditions b and c, consistent with the prediction based on the simulation results using the optimization strategy.

Figure 8 illustrates a typical set of transmurally differentiated measurement of creatine kinase forward flux rate constant ($k_{f,\text{CK}}$) using the $T_{1\text{nom}}$ method in combination of 1D-CSI sequence. The 1D-CSI spectra (Figure 8b) displayed a typical “column” along the phase encoding direction perpendicular to the surface coil plane, as demonstrated by the minimal overlap of the characteristic resonances representing different depths away from the surface coil. Namely, signals are from compounds of: localization phantom (Na$_3$PO$_4$), coil, myocardium characterized by high levels of PCr and ATP, and erythrocytes from the LV cavity blood characterized by 2,3-DPG peaks. The particular setup generated a $T_{1\text{nom}}$ of 1.8 seconds, which was used for $k_f$ calculation according to Equation 8. Figure 8c illustrates the reconstructed spectra demonstrating the spatially localized $k_f$ measurements from the subepi and the subendo layers of LV anterior wall. Based on 4 swine studies, the corresponding $k_f$ values are 0.36±0.03 and 0.40±0.03 sec$^{-1}$ for subepi- and subendomyocardial layers, respectively.

**Hemodynamic, Myocardial Energetics, and MRI Data in Response to CK Inhibition**

The hemodynamic and myocardial energetic data in response to CK inhibition via iodoacetamide infusion are summarized in Online Tables II and III, respectively. Iodoacetamide infusion significantly increased the heart rate ($P<0.05$ versus baseline). However, within an observation window of 30 minutes, both the LV systolic pressure (LVSP) and the high energy phosphate PCr/ATP ratio are maintained despite of complete inhibition of CK activity. In respond to catecholamine stimulation, both the heart rate and LV systolic pressure increased significantly (Online Table III; $P<0.05$ versus IAA).

The LV contractile functions measured by cardiac MRI during baseline and high cardiac workload states with or
without creatine kinase inhibition are summarized in the Online Data Supplement. Representative movies of LV short-axis cine imaging on one heart are also included in the Online Data Supplement. The LV contractile functions in terms of ejection fraction and systolic thickening fraction were not impaired during CK inhibition. Moreover, despite of CK inhibition, the heart can respond to catecholamine stimulation with an increased ejection fraction as noninhibited hearts do (P<0.05 versus IAA; Online Figure I).

Taken together, these data demonstrate that LV contractile performance is maintained when the ATP production rate via CK is inhibited, suggesting existence of multiple and redundant ATP production systems in supporting the chemical energy need of the contractile apparatus.

**In Vivo Rat Brain Studies**

The noninvasive T\textsubscript{1}\textsuperscript{nom} method is further verified on rat brain at 9.4 Tesla with measurements of the CK and ATPase activities at rest condition (Online Figure II). There is no statistically significant difference between the k\textsubscript{f} values measured by conventional (TR=9 seconds, FA=90\textdegree) and T\textsubscript{1}\textsuperscript{nom} (TR=3 seconds, FA=45\textdegree) methods (k\textsubscript{f,CK}: 0.26±0.04 versus 0.24±0.03 sec\textsuperscript{-1}, P=NS; k\textsubscript{f,ATPase}: 0.17±0.06 versus 0.15±0.08 sec\textsuperscript{-1}, P=NS).

**Discussion**

The present work demonstrated a novel and simple method (T\textsubscript{1}\textsuperscript{nom}) to quantify k\textsubscript{f} under partial relaxation conditions, allowing steady-state MST experiments to be performed with arbitrary repetition time and flip angle. The T\textsubscript{1}\textsuperscript{nom} method features with extremely fast k\textsubscript{f} measurement yet simple linear algorithm (Equation 8) for quantification. In addition, the optimization strategy would significantly enhance the performance of the T\textsubscript{1}\textsuperscript{nom} method by minimizing the final k\textsubscript{f} errors. By necessity, the T\textsubscript{1}\textsuperscript{nom} method together with the optimization strategy can greatly facilitate the in vivo enzyme kinetic studies that demand high spatial and temporal resolution.

**Versatility of the T\textsubscript{1}\textsuperscript{nom} Method**

The linear relationship between M\textsubscript{r}/M\textsubscript{s} ratio and k\textsubscript{f} is well maintained throughout a large range of simulated acquisition conditions.

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**Figure 6.** Representative spectra from steady-state MST experiments before, during, and after CK inhibition. Each steady-state MST experiment consisted of 2 spectra, with saturation pulse set at ATP\textgamma frequency (Sat.) or symmetrically opposite site (Ctrl.), as indicated by the bold arrows. Spectra from different columns (a through d) were acquired with different acquisition parameters as indicated by the legend on top. Condition a used full relaxation, representing the conventional method, whereas all the others used partial relaxation, representing the T\textsubscript{1}\textsuperscript{nom} method. Condition d represents an optimized condition according to the optimization strategy (similar to Figure 4g).
parameters (Figure 3). More extensive simulation suggested that this linear relationship holds in general regardless of pool size ratio or intrinsic $T_1$ values, suggesting the $T_1^{\text{nom}}$ method as a versatile tool for kinetic studies independent of experimental setup.

In the present study, the $T_1^{\text{nom}}$ method is theoretically demonstrated based on the human brain study at 7 Tesla (3-site exchange model, PCR$\rightarrow$ATP$\rightarrow$Pi) and further experimentally verified on an in vivo swine heart model for measuring myocardial CK forward reaction rate constant at 9.4 Tesla (2-site model, PCR$\rightarrow$ATP). The 2-site model is preferably used for myocardial bioenergetic studies because the Pi resonance is largely overlapped by the 2,3-diphosphoglycerate peaks from blood and thus difficult to quantify unless spatial localization is used. When applied to the 2-site exchange model, ATP$\rightarrow$Pi reaction (corresponding to Equations 3 and 6) was ignored during the numeric simulation process ($M_2/M_1$ versus $k_f$; Figure 3) for finding the $T_1^{\text{nom}}$ value. Therefore, the $T_1^{\text{nom}}$ method–based $k_f$ calculation is readily applicable to both two- and three-site models, as supported by the good agreement between experimental and simulation results shown in Figures 7 and Online Figure II.

### Validity of the Methodology

The $T_1^{\text{nom}}$ method can be considered as an improved version of conventional steady-state MST technique. The extensive previous studies on CK and ATPase kinetics have suggested that the intrinsic $T_1$ is constant among subjects regardless of physiological and pathological conditions. This is consistent with the observation in the present study that $T_1^{\text{nom}}$ is a constant among subjects and independent of reaction rate change throughout CK inhibition process. The intrinsic $T_1 (T_1^{\text{int}})$ characterizes the relaxation process of a spin population to reestablish the thermal equilibrium distribution (spin–lattice relaxation).

Therefore, in a defined magnetic field of a given organ of interest, the $T_1^{\text{int}}$ of a compound is a constant, which should only reflect its characteristic molecular tumbling rate. However, the reported $T_1^{\text{int}}$ value does vary because of different magnetic fields, species, organs, pulse and pulse sequences, and acquisition parameters. Therefore, it is always recommended to be cautious when using the $T_1^{\text{int}}$ value from literature. In a rare case where a biological system has no prior report of its $T_1^{\text{int}}$, a direct $T_1^{\text{int}}$ measurement of a few healthy subjects should be performed before the application of the $T_1^{\text{nom}}$ method.

The $T_1^{\text{nom}}$ method is highly robust to the variation of pool size ratio of metabolites, such as PCR/ATP ratio for CK reaction and Pi/ATP ratio for ATPase reaction. For the acquisition parameters within the optimized region as shown in Figure 4g and 4h, the relative $k_i$ measurement error attributable to a variation of pool size ratios of metabolite is less than one-eighth of the variation level itself, i.e., a change of PCR/ATP ratio of 40% would result in only a 5% of $k_i$ measurement error using the $T_1^{\text{nom}}$ method. Finally, in the case of large change of pool size ratios of metabolites, an iteration approach can be used to correct for the originally assumed pool size ratio based on $M_0$ measurement (Online Figure III). The iteration approach is based on the assumption that the change in pool size ratio is proportionally reflected in the magnetization ($M_0$) ratio measured in control spectra as long as the intrinsic $T_1$ in the 2 statuses are the same.

![Figure 7](image_url)

**Figure 7.** Quantification of steady-state MST results from the MRS data used in Figure 6. PCR signal ratio from conditions b through d were plotted against $k_f$ value, which was calculated according to Equation 7 from condition a. Solid lines were generated from simulation with the same parameters as in conditions b through d.

![Figure 8](image_url)

**Figure 8.** Transmural measurement of CK forward flux rate constant ($k_{f,\text{CK}}$) using the $T_1^{\text{nom}}$ method in combination of 1D-CSI sequence. a, Schematic view of the experimental setup. A phantom tube (200 µL of 0.5 mol/L Na$_3$PO$_4$ in water) was sutured onto the surface coil as a spatial reference. b, Representative 1D-CSI spectra with (1DCSI Sat, saturation pulse indicated by gray band) and without (1DCSI Ctrl) saturation on ATP$_y$ resonance for transmural measurement of $k_{f,\text{CK}}$. Total data acquisition time is 13.6 minutes with a spatial resolution of 2.4 mm. Acquisition parameters are: field of view (FOV)=40 mm; phase-encoding steps=17; TR=3 seconds; FA=90°; NEX=8. c, Reconstructed transmural spectra from 1D-CSI data for actual $k_f$ calculation. A 9-term Fourier series windowing algorithm was used for the spectra reconstruction to increase the spectral signal-to-noise ratio. The reconstructed voxel can be arbitrarily shifted. $T_1^{\text{nom}}$ is calculated to be 1.8 seconds based on the acquisition parameters. The $k_{f,\text{CK}}$ values from 4 pig studies are 0.36±0.03 sec$^{-1}$ and 0.40±0.03 sec$^{-1}$ for the epi and the endo layers of left ventricle, respectively.
Enhanced Performance From Optimization Strategy

The performance of $k_t$ measurement using the $T_1^{\text{nom}}$ method would be greatly enhanced by the optimization strategy, which is based on the $k_t$ error analysis to generate the best acquisition parameter range (TR and FA), that are most relevant to the longitudinal relaxation processing.

Type 1 error, as defined by Equation 11, represents the accuracy of $k_t$ calculation using the $T_1^{\text{nom}}$ method. As shown in Figure 4a and 4b, the type 1 error for human brain studies at 7 Tesla is below 1% for most acquisition conditions. Similar type 1 error levels were observed from numeric simulations with parameters that are characteristic of heart and skeletal muscle. Those simulation results again demonstrated the versatility of the $T_1^{\text{nom}}$ method for measuring enzyme activity on various organs.

The type 2 error specifically addresses the spectral SNR issue. For MR experiment with partial relaxation, the spectral SNR per unit acquisition time would be maximized if the flip angle is chosen at the Ernst angle that is determined by TR and the longitudinal relaxation time of spin. When chemical exchange is involved, the Ernst angle also depends on the reaction rate. Therefore, the Ernst angle for control and saturated spectrum would be different. However, applying different flip angles for $M_c$ and $M_s$ measurements would render the spectrum comparison less intuitive and the $k_t$ calculation more prone to flip angle inaccuracy. In the present approach, instead, both spectra are acquired with a same flip angle that is globally optimized according to error propagation theory (Equation 13). Because the acquisition parameters are identical for both $M_c$ and $M_s$ spectra, any measurement error attributable to flip angle variation would be cancelled out in Equation 8 for $k_t$ calculation and the only residual effect would be the change of $T_1^{\text{nom}}$ value, which is taken into account as the type 3 error. Even though none of the $M_c$ or $M_s$ measurements is acquired exactly at its Ernst angle, the overall performance from this globally optimized flip angle is still substantially better than the conventional steady-state MST. Taking human brain studies at 7 Tesla for instance (same parameter as used in Figure 4), the $T_1^{\text{nom}}$ method can easily achieve a level of <1% type 1 error. The same type 1 error level would require a TR of 16 seconds for the conventional MST methods (99% full relaxation, FA = 90°). Should such an experiment be performed under an optimized condition using the $T_1^{\text{nom}}$ method (eg, TR = 2 seconds, FA = 45°), an 88% reduction of total acquisition time could be achieved assuming a same number of signal averaging (NEX).

Type 3 error deals with the residual effects of flip angle inaccuracy on the final $k_t$ calculation error. As demonstrated in Figure 4e and 4f, the spin system becomes more robust against flip angle variation as flip angle decreases or TR increases. This result is consistent with the previous simulation results showing that $T_1^{\text{nom}}$ approaches to $T_1^{\text{int}}$ as flip angle decreases or TR increases (Figure 3). Therefore, based on the analysis of type 3 error (Figure 4e and 4f), we can compensate the impact of flip angle variation to an arbitrary level at an expense of reduced SNR per unit time. This is advantageous over some other rapid saturation transfer methods which use multiple flip angles for calculating the $k_t$ and thus more vulnerable to flip angle variation, such as FAST method.

The superior performance of the $T_1^{\text{nom}}$ method is demonstrated by the transmutally differentiated measurement of $k_{t,CK}$ (Figure 8). The total data acquisition time using the $T_1^{\text{nom}}$ method in combination of 1D-CSI sequence (2 sets of spectra, 17 phase encoding steps, NEX = 8, TR = 3 seconds) is 13.6 minutes. In contrast, a similar transmutally differentiated $k_{t,CK}$ measurement performed by Robitaille et al using a conventional saturation transfer method took 153.6 minutes (8 sets of spectra, 18 phase encoding steps, NEX = 8 and TR = 8 seconds) to accomplish the data acquisition. Therefore, the present study demonstrates that using the $T_1^{\text{nom}}$ method results in a reduction of data acquisition time by 91.2% as compared with the conventional saturation transfer method.

LV Contractile Function in Relation to CK Inhibition in the In Vivo Heart

On inhibition of creatine kinase via iodoacetamide infusion, the LV function and systemic hemodynamic did not change within an observation window of 30 minutes (Online Tables), the high energy phosphate PCr/ATP ratio was preserved (Online Table III), and the Pi level did not increase (Figure 6). Collectively, the present study demonstrates for the first time that a normal LV chamber contractile function can be maintained in the presence of complete inhibition of CK activity in normal in vivo heart under basal and high cardiac work states. This finding is surprising, and raises a significant question of what a significant role CK plays in the cascade of ATP production, transportation and utilization. In the normal in vivo heart, the ATP production rate via creatine kinase exceeds that of the mitochondria ATPase by an order of magnitude. Therefore, it is possible that a small amount of residual CK activity may be sufficient to support normal LV function in a relatively short term. In the present study, it is possible that a residual undetectable creatine kinase activity of 5% (or less) remained at 30 minutes after the...
IAA infusion initiation. Based on the signal to noise ratio (Figure 6), a 5% of residual CK activity could be at noise level. This small fraction of residual creatine kinase-derived ATP, along with other ATP sources, could be sufficient in supporting cardiac contractile function in the short term.

The severity of CK kinetics change is related to the chronic cardiac pathological changes such as severity of the LV hypertrophy or LV dysfunction. However, the mechanisms of these relationships are still unknown. The results of the present study demonstrate that an acute severe inhibition of ATP production rate via CK does not impair the LV regional or global contractile function. The finding of normal LV chamber function with complete inhibition of CK activity in the present study is in agreement with the previous observation using engineered mice, which demonstrated a normal growth and LV chamber function in mice with double knockout of the muscle (M/MtCK−/− mice).28 Taken together, these data suggest that in normal heart under in vivo conditions, redundant supporting systems exist to maintain an important organ function. These data also suggest that in the failing hearts that are usually severely hypertrophied, the redundant supporting systems such as CK, mitochondrial electron transport system and ATPase, may all be impaired. Consequently, the severity of the alterations of each of these systems is related to the severity of the LV dysfunction such as being observed earlier.6,7,29–31

In summary, we have demonstrated a novel steady-state MST method (T1 nom), together with an optimization strategy, that allows accurate kT measurement under partially relaxed acquisition conditions. The new method features an unprecedented fast kT measurement yet simple linear algorithm for quantification. This method enables broad applications for in vivo enzyme kinetic studies that require high spatial or temporal resolution. Using this novel NMR methods and an established swine model, these data demonstrate that acute inhibition of CK activity does not limit LV chamber function in the in vivo heart.

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Disclosures

None.

References

Novelty and Significance

What Is Known?
- The mechanisms and relationships between the alterations of myocardial creatine kinase (CK) and left ventricular (LV) contractile dysfunction in failing heart remain undefined.
- Although the 31P MR-based magnetization saturation transfer (MST) can measure the activity of the 2 most important energetic enzymes: CK and ATP synthase (ATPase), the conventional MST technique suffers from the lengthy acquisition time such that an in vivo transmurally differentiated enzyme activity measurement could not be obtained in the in vivo failing hearts.

What New Information Does This Article Contribute?
- A novel MST method was established theoretically with mathematical and numeric simulation and was verified with in vivo measurements of CK activity of swine hearts, as well as CK and ATPase activities of rat brain at 9.4 Tesla magnetic field.
- This novel MST method enables in vivo transmural differentiation studies to examine the CK activity with an unprecedented short data acquisition time.
- The in vivo swine study demonstrates that the acute inhibition of CK activity does not limit LV chamber function, suggesting that redundant multiple supporting systems of myocardial ATP production, transportation, and utilization exist in the heart.

This study describes a novel MST method that enables noninvasive in vivo studies for superfast measurements of enzyme kinetics in vivo. By applying this method to an in vivo swine model of myocardial CK inhibition by iodoacetamide, it was found that the acute inhibition of CK activity does not limit LV chamber function, suggesting that there are redundant multiple supporting systems of myocardial ATP production, transportation, and utilization in the heart, such that inhibition of one mechanism does not impair normal LV contractile performance.
ATP Production Rate via Creatine Kinase or ATP Synthase In Vivo: A Novel Superfast Magnetization Saturation Transfer Method
Qiang Xiong, Fei Du, Xiaohong Zhu, Pengyuan Zhang, Piradeep Suntharalingam, Joseph Ippolito, Forum D. Kamdar, Wei Chen and Jianyi Zhang

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ATP production rate via CK or ATP synthase in vivo: a novel superfast magnetization saturation transfer method
**ONLINE SUPPLEMENTAL THEORY**

**k_f calculation of progressive MST method**

Without knowledge of intrinsic T_1 (T_1\text{int}), the k_f can be calculated from the following equation:

\[
k_{f,\text{CK(ATPase)}} = \left(\frac{M_{0,\text{PCr(Pi)}} - M_{\text{ss,PCr(Pi)}}}{M_{0,\text{PCr(Pi)}}}\right) / T_{\text{app,PCr(Pi)}} \quad [S1]
\]

where M_{ss} and M_0 represent the fully relaxed magnetizations with and without saturation on ATP\gamma and T_{app} is the apparent longitudinal relaxation time constant during ATP\gamma saturation. T_{app} can be measured with progressive MST method, which employs multiple data acquisitions (5~7 spectra) with progressively prolonged saturation time (t_{sat}) on ATP\gamma followed by curve fitting of the PCr (Pi) signals to the following equation:

\[
M_{\text{PCr(Pi)}}(t_{sat}) = \left(\frac{M_{0,\text{PCr(Pi)}} - M_{\text{ss,PCr(Pi)}}}{M_{0,\text{PCr(Pi)}}}\right) \exp\left(\frac{-t_{sat}}{T_{\text{app,PCr(Pi)}}}\right) + M_{\text{ss,PCr(Pi)}} \quad [S2]
\]

Since T_{\text{app}} is related to T_{\text{int}} according to the following equation:

\[
T_{\text{app,PCr(Pi)}}^{-1} = T_{\text{int,PCr(Pi)}}^{-1} + k_{f,\text{CK(ATPase)}} \quad [S3]
\]

progressive MST is the gold standard for measuring intrinsic T_1 values when chemical exchange is involved.

**Deduction of Equation [12] (type 2 error)**

For a given data acquisition time and a specific experimental setup, the relative error in magnetization measurement is inversely proportional to the spectral SNR:

\[
\left(\frac{\delta M}{M}\right)_{\text{SNR}} = \frac{\sigma}{M} \sqrt{\frac{TR}{t}} \quad [S4]
\]
where $\sigma$ and $t$ stand for intrinsic scanner noise level and total acquisition time, respectively.

The final relative $k_f$ calculation error due to spectral SNR can be expressed as follow based on error propagation theory:

$$\left( \frac{\delta k_f}{k_f} \right)_{\text{SNR}} = \frac{\delta (M_c/M_s) - \beta}{M_c/M_s - \beta} \left( \frac{\delta (M_c)}{M_c} \right)^2 + \left( \frac{\delta (M_s)}{M_s} \right)^2 \left[ S5 \right]$$

where $\beta$ stands for the intercept as defined in Equation [8]. Since both $M_c$ and $M_s$ are obtained with the same acquisition parameters, we have:

$$\left( \frac{\delta M_c}{M_c} \right)_{\text{SNR}} = \frac{M_s}{M_c} \left[ S6 \right]$$

Combining Equations [S4] to [S6] yields:

$$\left( \frac{\delta k_f}{k_f} \right)_{\text{SNR}} = \frac{M_c/M_s}{M_c/M_s - \beta} \sqrt{\frac{M_c}{M_s}} \frac{\sqrt{TR \sqrt{t}}}{\sigma} \left[ 12 \right]$$

**Deduction of Equation [14] (type 3 error)**

The relative $k_f$ calculation error due to flip angle inaccuracy can be expressed by the following equation:

$$\left( \frac{\delta k_f}{k_f} \right)_{\text{flip}} = 1 \frac{\partial k_f}{\partial FA} \delta FA = 1 \frac{k_f}{k_f} \left( \frac{\partial T_1^{\text{nom}}}{\partial FA} \frac{\partial k_f}{\partial T_1^{\text{nom}}} + \frac{\partial k_f}{\partial \beta} \frac{\partial \beta}{\partial FA} \right) \delta FA \left[ S7 \right]$$

where $k_f$ is treated as a function of slope ($T_1^{\text{nom}}$) and intercept ($\beta$) according to Equation [8], which in turn are functions of flip angle (FA). Since the intercept ($\beta$) in $T_1^{\text{nom}}$ method is always close to 1, Equation [S7] can be simplified as below:

$$\left( \frac{\delta k_f}{k_f} \right)_{\text{flip}} \approx \frac{1}{k_f} \frac{\partial k_f}{\partial T_1^{\text{nom}}} \frac{\partial T_1^{\text{nom}}}{\partial FA} \delta FA \left[ S8 \right]$$
Combining Equations [8] and [S8] we have:

\[
\left( \frac{\delta k_f}{k_f} \right)_{\text{flip}} = -\frac{\partial T_{1 \text{nom}}}{\partial FA} \frac{FA}{T_{1 \text{nom}}} \frac{\delta FA}{FA} \quad [S9]
\]

where the \((\delta FA/FA)\) represents the relative error in flip angle and can be absorbed into the left hand side of Equation [S9]:

\[
K_{\text{flip}} = -\left( \frac{\delta k_f}{k_f} \right)_{\text{flip}} \frac{\delta FA}{FA} = \frac{\partial T_{1 \text{nom}}}{\partial FA} \frac{FA}{T_{1 \text{nom}}} \quad [14]
\]

**Summary of T1s in the Literature**

**Online Table I. Summary of different types of T1**

<table>
<thead>
<tr>
<th>Symbols</th>
<th>Actual physical meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>(T_1^\text{int})</td>
<td>Intrinsic (T_1) of a spin</td>
</tr>
<tr>
<td>(T_1^\text{mix})</td>
<td>Apparent (T_1) of a spin when chemical exchange is involved</td>
</tr>
<tr>
<td>(T_1^\text{app})</td>
<td>Apparent (T_1) of a spin when the counterpart spin is saturated</td>
</tr>
<tr>
<td>(T_1^\text{nom})</td>
<td>Nominal (T_1) of a spin when saturation transfer experiment is performed in partial relaxation conditions</td>
</tr>
</tbody>
</table>

Basically, \(T_1^\text{int}\) is an intrinsic property of a spin and it is only determined by the magnetic field and the spin’s intrinsic tumbling rate \(^1\). The \(T_1^\text{int}\) can be directly measured using inversion recovery experiment if the spin is not involved in any chemical exchange. However, when chemical exchange is involved, the measured value is defined as \(T_1^\text{mix}\). The \(T_1^\text{mix}\) is an approximation, because the actual recovery of spin magnetizations under chemical exchange is not an exact exponential curve. \(T_1^\text{mix}\) is determined by \(T_1^\text{int}\), chemical exchange rate and the
pool size ratios of the exchanging metabolites. The relationship can be solved numerically based on equations [1-3]. In saturation transfer experiments, when the other spin which is undergoing chemical exchange with the spin of interest is continuously saturated, the chemical exchange decouples and the measured (either progressive saturation or inversion saturation transfer experiments) $T_1$ is defined as $T_1^{\text{app}}$. $T_1^{\text{app}}$ is determined by $T_1^{\text{int}}$ and the chemical exchange rate only. The relation between $T_1^{\text{app}}$ and $T_1^{\text{int}}$ can be analytically expressed according to Equation [S3]. In most enzyme kinetic studies using saturation transfer experiments, the direct measured $T_1$ always refers to $T_1^{\text{app}}$. Those experiments also yield $k_f$ value according to Equation [S1] as introduced previously. Based on the $k_f$ and the $T_1^{\text{app}}$ measurements, the intrinsic $T_1$ can be readily deducted using Equation [S3]. This is actually the gold standard for calculating the $T_1^{\text{int}}$ when chemical exchange is present. However, the $T_1^{\text{int}}$ calculated is shown to be independent of: (1) chemical exchange rate, (2) metabolites' level, (3) physiological and pathological conditions. Therefore, information of $T_1^{\text{int}}$ from literature review, prior experiences or preliminary experiments can be reliably used. $T_1^{\text{nom}}$, initially introduced in the current work, can be considered as the product of $T_1^{\text{int}}$ and a correction factor which compensates the saturation transfer experiment performed in the partially relaxed conditions. $T_1^{\text{nom}}$ is determined by $T_1^{\text{int}}$ and the pool size ratio of exchanging metabolites as well as the acquisition parameters but is independent of chemical exchange rate.
ONLINE SUPPLEMENTAL METHODS

Cardiovascular Physiologic Studies Using a Swine Model

Open-chest surgery preparation for $^{31}$P MR spectroscopy

Young female Yorkshire swine (~30 kg, n=8) were anesthetized with 2% isoflurane and ventilated with supplemental oxygen on a respirator. Polyvinyl chloride catheters (3 mm OD) were inserted into the ascending aorta (through the left external carotid artery) and left ventricle (through the apical dimple) for hemodynamic monitoring. The heart was exposed via a sternotomy and suspended in a pericardial cradle. The LV catheter was introduced. Ventilation rate, volume, and inspired oxygen content were adjusted to maintain physiological values for arterial PO$_2$, PCO$_2$, and pH. Aortic and LV pressures were continuously monitored throughout the study.

In vivo $^{31}$P MR spectroscopy experiment

Measurements were performed in a 650 mm bore 9.4 Tesla magnet interfaced with a Vnmrj console (Varian, CA). Radiofrequency transmission and MRS signal detection were performed with a 28 mm-diameter double tuned ($^1$H and $^{31}$P) surface coil sutured directly to the epicardium of LV anterior wall. The coil was cemented to a sheet of silicone rubber 0.7 mm in thickness and approximately 20% larger in diameter than the coil itself. The proton signal from water detected with the surface coil was used to homogenize the magnetic field and to adjust the position of the animal in the magnet so that the coil was at or near the magnetic isocenter. $^{31}$P MR spectra were acquired with adiabatic BIR4 RF pulses for
excitation to minimize flip angle variation due to B$_1$ inhomogeneity from surface coil. Selective saturation on $\gamma$-adenosine triphosphate (ATP$_\gamma$) was achieved with $B_1$-insensitive train to obliterate signal (BISTRO) as previously reported $^2,^3$. Chemical shifts were measured relative to PCr, which was assigned a chemical shift of 0 ppm. Before and after complete inhibition of creatine kinase reaction, intrinsic T$_1$ values were measured using progressive MST and inversion recovery methods, respectively.

Transmurally differentiated measurement of CK forward flux rate constant ($k_f$)

T$_1$ nom method was further employed to measure the cardiac CK activity with transmural differentiation on female Yorkshire pigs (~40 kg, n=4). Experimental preparations were performed with identical setup as described in the previous section except that a one dimensional chemical shift imaging (1D-CSI) sequence was utilized for transmural differentiation. Parameters for 1D-CSI experiment are: Field of view = 40 mm, phase encoding steps=17, TE=620 $\mu$s, TR=3 sec, FA=90°, NEX=8. Phase encoding direction was chosen to be perpendicular to the surface coil plane based on scout cardiac MRI. 1D-CSI data acquisition was gated with both respiratory and cardiac cycle using a model 1025 monitoring and gating system (SAII instruments, NY) to minimize motion artifacts. A total of two sets of 1D-CSI data were acquired with or without saturation on ATP$_\gamma$ resonance. A Fourier series windowing (FSW) algorithm $^4$ was employed for post-acquisition data reconstruction which can improve the spectral signal to noise ratio. The reconstructed voxel can be arbitrarily shifted. In current study, a 9-term FSW (hann function) reconstruction was
performed which yielded three spectra corresponding to the epi-, mid- and endo-layer of the myocardium with minimal overlap between layers. Transmural $k_f$ was calculated from the epi- and the endo- spectra using a $T_1^{\text{nom}}$ value of 1.8 sec.

*Magnetic resonance imaging (MRI) methods*

Female Yorkshire pigs (~30kg, n=6) were employed to examine the cardiac contractile function during the experimental conditions of: a) baseline (BL), b) high cardiac workload (HWL) secondary to catecholamine stimulation (dobutamine and dopamine, each of 10 $\mu$g/kg/min, iv), c) re-baseline after iodoacetamide infusion to inhibit CK (IAA, 0.45 mmol/kg, iv), and d) high cardiac workload after iodoacetamide infusion (IAA+HWL, dobutamine and dopamine, each of 10 $\mu$g/kg/min, iv). Data acquisition time was 15 minutes for each experimental condition. Detailed MRI methods have been published previously \(^5\). Briefly, MRI was performed on a 1.5 Tesla clinical scanner (Siemens Sontata, Siemens Medical Systems, Islen NJ) using a phased-array 4-channel surface coil and ECG gating. Animals were anesthetized with 2% isoflurane and positioned in a supine position within the scanner. The MRI protocol consisted of: 1) localizing scouts to identify the long- and short-axis of the heart, 2) short- and long-axis cine for the measurement of global cardiac function. Steady-state free precession “True-FISP” cine imaging used the following MR parameters: TR=3.1 ms, TE=1.6 ms, FA=79°, matrix size=256 x 120, field of view=340 mm x 265 mm, slice thickness=6 mm (4 mm gap between slices) and 16-20 phases were acquired across the cardiac cycle. Global function and regional wall thickness data were computed from the short-axis cine images.
using MASS (Medis Medical Imaging Systems, Leiden, The Netherlands) for the manual segmentation of the endocardial and epicardial surfaces at both end-diastole (ED) and end-systole (ED) from base to apex.

**Complete Non-invasive Brain Studies using a Rat Model**

Details of rat preparation as well as MRS data acquisition have been published previously. Briefly, five male Sprague-Dawley rats were anesthetized with 2% isoflurane and the femoral arteries of rats were catheterized for blood sampling and physiology monitoring. *In vivo* MR experiments were conducted at a 9.4 Tesla horizontal animal magnet (Magnex Scientific) interfaced to a Varian INOVA console. A dual RF surface-coil probe consisting of a butterfly-shape $^1$H surface coil and an elliptical-shape $^{31}$P surface coil was used for acquiring anatomy images, $B_0$ shimming and *in vivo* $^{31}$P spectra, respectively. A BISTRO saturation pulse train scheme and a BIR4 pulse were used to saturate ATPγ resonance and to read out $^{31}$P signal, respectively. Both fully (TR=10 sec, FA=90°) and partially (TR=3 sec, FA=45°) relaxed steady-state saturation transfer experiments were performed with 256 signal averages.
ONLINE SUPPLEMENTAL RESULTS

To examine whether the LV chamber function and regional systolic thickening fraction can be maintained when the CK system is completely inhibited, additional 6 swine were examined on a 1.5 Tesla clinical magnet non-invasively with an external coil. The animals were lightly anesthetized with a close-chest preparation, and were supported by a respirator. The cardiac contractile performance data during baseline, and during high cardiac workstates with or without creatine kinase inhibition are summarized in Online Figure III. Representative movies of LV short-axis cine imaging are also available in Online Supplemental Materials. The MRI data showed no significant difference in terms of LV ejection fraction and thickening fraction before and after complete inhibition of CK activity. Further, after complete inhibition of CK activity, the LV can respond to catecholamine stimulation in a similar pattern as the non-inhibited hearts do. Taken together, the preservation of LV contractile performance when the ATP production rate via CK is inhibited suggested multiple and redundant ATP production systems in supporting the chemical energy demands of the contractile apparatus.
### Online Table II. Hemodynamic data in response to CK inhibition

<table>
<thead>
<tr>
<th></th>
<th>N=8</th>
<th>HR (bpm)</th>
<th>LVSP (mmHg)</th>
<th>LVEDP (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>104±13</td>
<td>102±6</td>
<td>7±2</td>
<td></td>
</tr>
<tr>
<td>CK inhibition (30 min)</td>
<td>124±11*</td>
<td>99±7</td>
<td>7±3</td>
<td></td>
</tr>
<tr>
<td>CK inhibition (40 min)</td>
<td>135±12*</td>
<td>90±9</td>
<td>7±4</td>
<td></td>
</tr>
<tr>
<td>CK inhibition (60 min)</td>
<td>105±11</td>
<td>70±6*</td>
<td>7±2</td>
<td></td>
</tr>
</tbody>
</table>

Data are mean±SD; *, p<0.05 vs Baseline; LVSP, left ventricular systolic pressure; LVEDP, LV end diastolic pressure. CK inhibition was achieved with iodoacetamide infusion (0.45 mmol/kg iv).
Online Table III. Preservation of high energy phosphates ratio despite of CK inhibition at both baseline and high workload (HWL) conditions

<table>
<thead>
<tr>
<th></th>
<th>N=5</th>
<th>HR (bpm)</th>
<th>LVSP (mmHg)</th>
<th>PCr/ATP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>96±10</td>
<td>103±3</td>
<td>2.1±0.2</td>
<td></td>
</tr>
<tr>
<td>IAA</td>
<td>120±10*</td>
<td>98±8</td>
<td>2.3±0.3</td>
<td></td>
</tr>
<tr>
<td>IAA+HWL</td>
<td>176±12*†</td>
<td>133±7*†</td>
<td>2.4±0.3</td>
<td></td>
</tr>
</tbody>
</table>

Data are mean±SD; *, p<0.05 vs Baseline; †, p<0.05 vs IAA. IAA, CK inhibition by iodoacetamide infusion (0.45 mmol/kg, iv). IAA+HWL, CK inhibition at high cardiac workload condition (dobutamine and dopamine infusion, each 10 μg/kg/min, iv).
Online Figure I. LV contractile function (a, ejection fraction (TF), and b, systolic thickening fraction (TF)) in response to creatine kinase inhibition (IAA) under basal (BL) and high cardiac workload (HWL) conditions. Creatine kinase inhibition was achieved by iv infusion of iodoacetamide (IAA, 0.45 mmol/kg). HWL condition was achieved using catecholamine stimulation (dopamine/dobutamine, each of 10 µg/kg/min iv).
Online Figure II. Non-invasive CK (grey) and ATPase (black) activity measurements in rat brain using both conventional (TR=9 sec, FA=90°) and T1\textsuperscript{nom} (TR=3 sec, FA=45°) steady-state MST methods (NEX=256). There is no statistical difference between the measurements. The T1\textsuperscript{nom} and β (Equation [8]) for CK and ATPase reactions of rat brain at 9.4 Tesla are: T1\textsubscript{CK}\textsuperscript{nom} = 3.30 s, β\textsubscript{CK}=1.00; T1\textsubscript{ATPase}\textsuperscript{nom}=3.50 s, β\textsubscript{ATPase}=1.00. The parameters for calculating the T1\textsuperscript{nom} and β are taken from reference \cite{6}: pool size ratio of PCr : ATP\gamma : Pi = 1:0.55:0.14, intrinsic T1 for PCr, ATP\gamma and Pi are 3.83, 1.24 and 4.03 sec, respectively.
Online Figure III. Iteration approach for finding the pool size ratio without fully relaxed measurements.

1. Make an initial estimation of pool size ratio of metabolites (\(M_{0,PCr}/M_{0,ATP}\)) either based on literature, previous experiences or baseline measurement (for intervention studies).

2. According to intrinsic T1 values and the acquisition parameters (TR and FA), calculate the \(T_{1,\text{int}}\), TR and FA using numerical simulation.

3. Perform steady-state MST experiment with the selected TR and FA to get \(M'_{c,PCr}\), \(M'_{c,ATP}\), and \(M'_{s,PCr}\) measurements.

4. Calculate \(k_f\) based on \(M'_{c,PCr}\) and \(M'_{s,PCr}\) using \(T_{1,\text{nom}}\) method (Equation [8]).

5. Feed the \(k_f\) value into numerical simulation, and calculate the simulated control spectrum (\(M_{c,PCr}\) and \(M_{c,ATP}\)).

6. Update the initial estimation of pool size ratio of metabolites based on Equation [15], the new pool size ratio of metabolites is:
\[
\frac{M'_{0,PCr}/M'_{0,ATP}}{M_{0,PCr}/M_{0,ATP}} = \left(\frac{M_{0,PCr}/M_{0,ATP}}{M_{c,PCr}/M_{c,ATP}}\right) \times \left(\frac{M'_{c,PCr}/M'_{c,ATP}}{M_{c,PCr}/M_{c,ATP}}\right).
\]

7. Utilize the updated metabolites ratio \(M'_{0,PCr}/M'_{0,ATP}\) to calculate the new \(T_{1,\text{nom}}\) and \(\beta\), and then new \(k_f\).

The iteration stops when simulated \(M_{c,PCr}/M_{c,ATP}\) is close enough to the measured \(M'_{c,PCr}/M'_{c,ATP}\).
REFERENCES


