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Circ Cardiovasc Imaging. Author manuscript; available in PMC 2012 January 1.

Published in final edited form as:
Circ Cardiovasc Imaging. 2011 January ; 4(1): 42–50. doi:10.1161/CIRCIMAGING.110.959320.

Impaired ATP Kinetics in Failing *in vivo* Mouse Hearts

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Abstract

Background—The hypothesis that the failing heart may be energy starved is supported, in part, by observations of reduced rates of ATP synthesis through the creatine kinase (CK) reaction, the primary myocardial energy reservoir, in heart failure (HF) patients. Although murine models have been used to probe HF pathophysiology, it has not been possible to non-invasively measure the rate of ATP synthesis through CK in the *in vivo* mouse heart. The purpose of this work was to exploit non-invasive spatially-localized magnetic resonance spectroscopy (MRS) techniques to measure ATP flux through CK in *in vivo* mouse hearts and determine the extent of any reductions in murine HF.

Methods and Results—The Triple Repetition Time Saturation Transfer (*TRiST*) MRS method of measuring ATP kinetics was first validated in skeletal muscle, rendering similar results to conventional saturation transfer MRS. In normal mouse hearts the *in vivo* CK pseudo-first-order-rate constant, k_f, was 0.32±0.03 s^{-1} (mean±SD) and the rate of ATP synthesis through CK was 3.16±0.47 μmol/g/s. Thoracic aortic constriction (TAC) reduced k_f by 31% (0.23±0.03 s^{-1}, $p<0.0001$) and ATP synthesis through CK by 51% (1.54±0.25 μmol/g/s, $p<0.0001$), analogous values to those in failing human hearts.

Conclusions—Despite the small size and high murine heart rate, the ATP synthesis rate through CK is similar *in vivo* in murine and human hearts and comparably reduced in HF. Because murine TAC shares fundamental energetic similarities with human HF, this model and new MRS approach promise a powerful means to non-invasively probe altered energetics in HF.

Keywords

energetics; CK flux; hypertrophy; *in vivo* mouse hearts; magnetic resonance spectroscopy

Adenosine 5′-triphosphate (ATP) is the source of chemical energy that maintains intracellular homeostasis and fuels myocardial function. Relatively large rates of ATP synthesis are required to maintain normal cardiac contractile function and it has been hypothesized that inadequate ATP supply contributes to the contractile dysfunction in heart

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Disclosures
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failure (HF)[1]. The observations that pharmacological HF agents that reduce metabolic demand improve outcomes whereas those that increase energetic demands worsen them are consistent with the energy-starvation hypothesis[1–2].

Mouse models offer novel and appealing means to investigate the mechanistic link between altered energy metabolism and contractile function because they allow gene-targeted interventions not currently possible in larger mammalian species and humans. In several important ways murine models mimic functional and energetic aspects of normal human cardiac physiology[3]. Among many murine HF models, thoracic aortic constriction (TAC) is one that results in pressure-overload hypertrophy, progresses to HF, and shares some gross energetic and functional similarities with clinical human HF[4]. One factor that has limited testing of the energy-starvation hypothesis is the inability to non-invasively quantify energy transfer and, in particular, the rate of ATP synthesis in the *in vivo* beating heart.

From mice to humans ^{31}P magnetic resonance spectroscopy (MRS) is the only non-invasive, non-destructive means to quantify the concentrations of cardiac high-energy phosphates, ATP and creatine phosphate (PCr), which are rapidly and reversibly converted by the creatine kinase (CK) reaction, the primary energy reserve of the heart. ^{31}P MRS approaches have identified moderate reductions in cardiac PCr/ATP ratio and minimally reduced [ATP] in patients with HF[5–7]. ^{31}P MRS techniques called saturation transfer (ST) use frequency-selective irradiation pulses and enable measurement of the rate of ATP synthesis through CK in the beating heart. Because conventional ST approaches are extremely time consuming, more rapid ST approaches have been described recently[8] that have enabled, for the first time, measurement of the rate of ATP synthesis through CK in the human heart and detection of 50%–70% reductions in the rate of ATP synthesis through CK in HF[8–10].

The extremely small size (~0.1g) and rapid rate (~600/min) of the mouse heart complicate metabolic measurements, including those with ^{31}P MRS. Most mouse ^{31}P MRS studies have been conducted in isolated, perfused hearts[11–14]. Non-invasive, spatially-localized *in vivo* ^{31}P MRS studies can be performed under physiologic conditions[3–4] and permit repeated studies in the same animals as pathology evolves[4]. Such approaches have largely been limited to measures of cardiac PCr/ATP ratio although more recently *in vivo* measures of the absolute concentrations of murine cardiac [ATP] and [PCr] were described and validated[15]. Unlike the human heart, there have been no reported measures of the rate of ATP synthesis through CK in the *in vivo* mouse heart. The measurements of ATP flux in the mouse heart have been inaccessible because of long times required by conventional ST techniques. In the present study we apply the Triple Repetition Time Saturation Transfer (*TRiST*) method for rapidly measuring ATP flux through CK in the human heart[10] to measure CK reaction kinetics in the *in vivo* mouse heart. There were two aims for this study. First, we sought to adapt and validate the ^{31}P MR *TRiST* method for measuring muscle CK reaction kinetics in the *in vivo* mouse. Once validated, we applied the new ^{31}P MRS saturation transfer approach to test the hypothesis that *in vivo* myocardial CK reaction kinetics are reduced in the TAC model of murine HF.

Methods

The procedure and protocols were reviewed and approved by the Institutional Animal Care and Use Committee of the Johns Hopkins University. Experiments were carried out on a Bruker Biospec MRI/MRS spectrometer equipped with a 4.7T/40 cm Oxford magnet and a 12cm actively shielded gradient set, using a custom built probe assembly as previously described[3–4, 15].

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Skeletal muscle studies

Prior to proceeding to experiments on the heart, the *TRiST* method was validated in a series of non-localized (NL) saturation transfer studies on mouse upper thigh muscle (n=5), where both *TRiST* and conventional saturation transfer measures could be obtained in the same exam within a reasonable amount of total experimental time. The details of these experiments are as follows: conventional NL ST measurements were performed using a set of nine TR values of 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 10.0, and 16.0 s with 128 signal averages (NEX) and 2 dummy scan (DS), except for the two longest TR values, where NEX=64 and DS= 2 were used to keep the experimental time within reasonable limits. Signal amplitudes for these two experiments with NEX= 64 were multiplied by two in order to correct for the reduced number of averages. In addition, two more spectra were obtained with TR=16 s, one with saturating pulse placed equidistant from the PCr signal, but downfield, and the other with no saturation pulse at all. The intensities of the PCr signal were then used to calculate T_1' using the conventional progressive saturation method, as previously explained in reference [10]. For the *TRiST* method, the T_1' was determined with the 2TR method using signal intensity values acquired with TR=1.5 and 16 s from the same data set. The pseudo-first-order rate constant, k_f was determined using the following equation as explained in reference [10].

$$k_f = \frac{1}{T_{1PCr}'} \left(1 - \frac{M_{0PCr}'}{M_{0PCr}}\right) \quad (1)$$

Heart Studies

In vivo MRI/MRS studies were carried out on 11 male control mice (weight 34 ± 5 g) and ten others seven to eight weeks after TAC (weight 36 ± 6 g), as previously described[4,15]. Thoracic aortic construction (TAC) was performed as previously described[16]. Anesthesia was induced with ~2% isoflurane in an induction chamber, and maintained at ~1% in 50/50 air/O_2 mixture delivered through a nose cone, as previously described[3–4,15]. The probe set included a 22-mm ^{1}H MRI and 13-mm ^{31}P MRS coils. MR images were obtained with a ^{1}H MRI FLASH sequence [echo time = 1.5ms; TR =12 ms; and NEX =12]. In the *TRiST* experiment, localized ^{31}P MR spectra were obtained with a one-dimensional chemical shift imaging (1D CSI) sequence (16 mm field of view, 16 phase encoding steps) using modified BIR4 90° adiabatic pulses. Two acquisitions with different untriggered repetition periods (TR=1.5s, NEX=96 and TR=6s, NEX=32) were acquired in the presence of saturating irradiation pulse applied to the exchanging CK moiety, *viz*., γ-phosphate of ATP at −2.5ppm, relative to PCr, and another acquisition, fully relaxed (TR=10s, NEX=16) in the presence of control irradiation applied at +2.5ppm. The measured signal intensities were normalized for different NEX. In order to measure PCr concentration from the acquisition with control saturation, separate studies were performed (n=4) where two cardiac spectra were obtained with 1D CSI and TR=16 s, one with the saturating pulse placed at + 2.5ppm and the other with no saturation pulse at all. Intensities of the PCr signal from these two cardiac spectra were used to determine the average correction factor (1.1) arising from spill-over effects of the RF pulse in order to measure PCr concentration.

After the mouse exams were completed, external phantom experiments were also performed on the same day with the same coil set under comparable conditions in order to calculate the absolute concentrations of PCr and ATP, as described previously[15]. ^{1}H MR Images were analyzed with commercial Paravision software and ^{31}P MR spectral peak areas determined with in-house custom software[17]. Metabolite signal intensities were determined from the integrated peak areas of the PCr and ATP resonances from voxels centered on cardiac muscle as identified from the high-resolution ^{1}H MR images, as described previously[3–4].

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Voxel shifting was performed when necessary to optimize slice alignment with cardiac structures and minimize skeletal muscle contamination of cardiac spectra[18]. Although both heart and chest are curved and the voxels extend in both directions, the surface coil size and profile limit signal contributions from the lateral dimensions and thus more than 93% of signal in cardiac voxels obtained with this approach are from the mouse heart[3]. The same slice was selected to measure the inorganic phosphate peak area in the external phosphate phantom. The [PCr], [ATP] and PCr/ATP ratio were determined for control and TAC mouse hearts as previously explained[15]. T_1' was calculated from the area of the PCr signal with TR_{short} (1.5 sec) and TR_{long} (6 sec) according to the 2TR method[19]. The k_f (s^{-1}) was determined for control and TAC mouse hearts using the *TRiST* method[10] and the rate of ATP synthesis through CK (μmol/g/s) calculated as the product of k_f and [PCr], as previously described[8–9].

To evaluate the degree of hypertrophy and extent of contractile dysfunction induced by TAC in this setting, a separate cine MRI study was performed in some animals using the following protocol. Specifically, left ventricular (LV) function was quantified from multislice FLASH cine MR images (15 frames, TE=1.5ms, TR=7ms, flip angle=45°) by using ECG and respiratory gating, as previous reported[4]. MR images were analyzed with ImageJ software and end-diastolic (EDV), end-systolic volume (ESV), LV mass, and LV ejection fraction (EF) were calculated as previously described[3–4].

CK Activity

After completing the MRS study, mice were sacrificed, the hearts excised and immediately frozen in liquid nitrogen. Ventricular tissue was homogenized in ice-cold potassium phosphate buffer containing 1 mM EDTA and 1 mM β-mercaptoethanol, pH 7.4 (final concentration, 5 mg tissue/mL). An aliquot was removed for protein assay by the method of Lowry et al.[20] using bovine serum albumin as the standard. Subsequently, Triton X-100 was added to the homogenate at a final concentration of 0.1% and total CK activity measured spectrophotometrically (Molecular Devices Sunnyvale, CA) (340nm, 37°C)[21] using a kit (C184-0A) from Catachem and according to the manufacturer's instructions. CK activity was expressed as IU/mg protein. Lung and body weights were determined at the time of sacrifice to assess for pulmonary congestion.

Statistics

A paired *t-test* was performed to compare conventional and *TRiST* measures of T_1' and k_f in the same animals. The PCr/ATP ratio, [PCr], [ATP], T_1', k_f, and CK reaction kinetics of normal and TAC mice heart were compared using non-paired student's *t-test*. Differences were considered statistically significant at $p \leq 0.05$. All results are shown as mean ± SD.

Results

Skeletal Muscle

Because a conventional saturation transfer experiment with spatial-localization could not be conducted in the *in vivo* mouse heart in a time frame that would be tolerated by an anesthetized animal, we validated the faster *TRiST* approach in the *in vivo* mouse with non-localized (NL) studies comparing conventional nine point progressive saturation (PS) measurements with those from *TRiST* in skeletal muscle. A representative high-resolution transverse ^{1}H MR thigh image and corresponding non-localized skeletal muscle ^{31}P MR spectra under different saturating conditions are shown in Figure 1. Figure 2 shows a graph of the PCr peak area versus repetition time of the nine acquisitions and the resulting mono-exponential T_1' fit from one set of *in vivo* data for the skeletal muscle. The mean *in vivo* T_1' in the presence of saturating irradiation on γ-phosphate of ATP for conventional PS and

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TRiST methods were 1.94±0.03 s and 1.95±0.04 s, respectively (Figure 3, n=5, mean difference ~0.5%, p=0.64 by paired comparison). The 9 point PS data acquisition took ~78 min as compared to ~22 min for *TRiST*, a time saving of ~73% for the same measurement. Figure 3 shows that in these non-localized measurements, the *TRiST* method renders the same mean *in vivo* k_f as does the conventional PS approach (0.26±0.01s^{-1} and 0.26±0.01s^{-1}, p=0.56).

Cardiac Muscle

As compared to control animals (n=5), TAC hearts (n=6) exhibited a significant increase in LV mass (90±5 mg vs, 209±27 mg, control vs TAC, respectively, p<0.0001) and the ratio of LV mass (mg)/body mass (g) (2.5±0.2 vs, 6.0±2.0, p<0.005). TAC hearts had significant contractile dysfunction with a reduced LV ejection fraction (70±7% vs, 30±5%, control vs TAC, respectively, p<0.0001) and increased end-systolic volumes (13±3 μl vs, 84±21μl, p<0.0002). This significant degree of contractile dysfunction in TAC hearts was associated with pulmonary congestion as evidenced by an approximate tripling in lung weights (142±14mg vs 370±87mg, control (n=9) vs TAC (n=4), respectively, p<0.0001) and lung/body weight ratios (4.4±0.7 vs 12.4±3.1, p<0.0001), as shown in Figure 4.

A representative high-resolution transverse ^{1}H MR cardiac image and corresponding spatially-localized cardiac ^{31}P MR spectra under different saturating conditions from the anterior wall of the heart are shown in Figure 5. The decrease in the PCr resonance with selective saturation of γ–ATP is directly proportional to the rate of ATP synthesis through the CK reaction in the heart[22]. Figure 6 shows summary data for *in vivo* PCr/ATP ratio, [PCr], [ATP], T_1', k_f, CK flux, and *in vitro* CK activity in control (n=11, heart rate 457±22 min^{-1}) and TAC (n=10, heart rate 451±35 min^{-1}) hearts. The mean *in vivo* PCr/ATP ratio was significantly reduced in TAC (1.66±0.10) as compared to control mice (1.90±0.17, p<0.001), consistent with prior reports[4,15]. The mean *in vivo* [PCr] was reduced by 33% in TAC (6.61±0.59 μmol/g of wet weight) as compared to that of control mice (9.95±1.5 μmol/g of wet weight, p<0.0001). Mean *in vivo* [ATP] was reduced by 26% in TAC (3.59±0.37 μmol/g of wet weight) as compared to that of control mice (4.81±1.06 μmol/g of wet weight, p<0.003). The [PCr] and [ATP] values and their relative reduction with TAC agree with those previously reported in this model[15]. In control and TAC mice, the mean *in vivo* T_1' was 1.64±0.16 s and 2.21±0.27 s, respectively (p<0.0001). Mean *in vivo* cardiac k_f was reduced by 31% in TAC (0.23±0.03 s^{-1}) as compared to that of control mice (0.32±0.03 s^{-1}, p<0.0001) and these values and the relative reduction also agree with those in other species, including humans[8–9, 23–24]. The mean *in vivo* forward rate of ATP synthesis through cardiac CK was 3.16±0.47μmol/g/s in control mice and 1.54±0.25 μmol/g/s in TAC mice (p<0.0001). The absolute values and the ~50% reduction in the failing hearts are similar to those observed in normal and failing human studies[8–9]. *In vitro* CK activity, measured in the same hearts was also significantly decreased in TAC mice (6.06±1.7 IU/mg protein) as compared to that of controls (9.66 ± 1.8 IU/mg protein, p<0.0006). The relationship between cardiac energetic indices derived from MRS (PCr/ATP and ATP flux through CK) and functional/anatomic indices derived from MRI (EF, ESV, mass) are shown in Figure 7.

Discussion

There are five novel aspects to this work. First, these are the first non-invasive, direct measures of ATP synthesis in the *in vivo* mouse heart. They were acquired with a non-invasive ^{31}P magnetic resonance spectroscopy ST method, *TRiST*, initially developed for people, adapted here to mice, and validated here *in vivo* by comparison with conventional ST measures in skeletal muscle. Second, despite the massive difference in size and resting rate between murine and human hearts, the rate of ATP synthesis (~3 μmol/g/s) through CK, the primary myocardial energy reserve, is remarkably similar per gram between these

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species. Third, long-standing pressure-overload and heart failure induced by TAC results in a ~30% reduction in k_f and a ~50% decline in the rate of ATP synthesis through CK. Fourth, the reduction in ATP flux through CK is much greater than the reduction in PCr/ATP ratio or [ATP] suggesting the previously used indices of myocardial energetics based on metabolite pool sizes underestimate the reduction in myofibrillar ATP delivery in failing hearts. Fifth, the 50% reduction in ATP flux through CK observed here in the *in vivo* failing TAC mouse heart is similar to the 50% reduction in patients with dilated cardiomyopathy[8] and the 65% reduction in patients with pressure-overload hypertrophy and heart failure[9], suggesting that this murine model mimics important bioenergetic aspects of human heart failure.

Creatine kinase is the primary energy reserve of the heart, reversibly exchanging a high energy phosphate group between ATP and phosphocreatine (PCr):

$$PCr^{2-} + MgADP^{-} + H^{+} \underset{K_r}{\overset{K_f}{\rightleftharpoons}} MgATP^{2-} + Creatine$$

This reaction rapidly buffers ADP and ATP in cardiac and skeletal muscle and can be a rapid source of ATP during ischemia and burst activity in skeletal muscle[25–29]. The CK reaction has also been postulated to shuttle or transfer high energy phosphates from the mitochondria, where ATP is synthesized, to the cytosol, where it is used, and back for rephosphorylation[30–31]. This spatial buffering function is supported by the smaller size and molecular weight of PCr and creatine, which diffuse more rapidly than ATP and ADP, and by the compartmentalization of separate mitochondrial and cytosolic isoforms of CK, also critical for CK temporal ATP buffering[25–26,30]. There are several derangements in CK during heart failure that may limit ATP transfer and these include reductions in the pool sizes of CK substrates, reduced CK activity, altered isoform distribution and, most importantly, a reduction in the rate of ATP synthesis through CK[1–2, 28,32].

^{31}P MRS is uniquely able to non-invasively and repetitively measure the CK reactants, ATP and PCr, and to quantify the rate of ATP flux through CK in the beating heart[33]. Saturation transfer is a class of MRS techniques that allows quantification of chemical exchange through specific reactions and such techniques were first used to explore cardiac CK kinetics nearly 30 years ago[33]. Conventional magnetization transfer experiments are very time consuming, typically requiring seven or more fully-relaxed acquisitions for a single measurement. In addition, spatial-localization techniques, used to obtain ^{31}P signals from the heart in closed-chest animals, are also time-consuming as compared to simple one-pulse experiments used in open-chest or isolated heart studies. Thus, despite the biological appeal of measuring the rate of ATP synthesis non-invasively in the *in vivo* beating heart under physiologic conditions or as pathology develops, spatially-localized conventional saturation transfer techniques simply require too much time, in some instances many hours, to be easily tolerated by anesthetized animals or by patients. The first measures of ATP flux through CK in the human heart were obtained with a new, more rapid saturation transfer approach that required fewer acquisitions under incompletely relaxed conditions by exploiting acquisitions with precise but different flip angles (four angle saturation transfer, *FAST*)[22]. The *FAST* method required approximately one-seventh the time of conventional saturation transfer and yielded a mean rate of 3.4μmol/g/s for ATP synthesis through CK in the normal human heart[8]. To overcome power limitations at higher, 3T field strengths for patient studies, another rapid approach was developed that varied the repetition time rather than the flip angle and this method, called *TRiST* for triple repetition time saturation transfer, gave similar results for CK kinetics in the human heart as prior FAST reports[22] and offered a 73% time savings over conventional saturation transfer[10]. More recently another rapid ST

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approach was described that uses a strategy of pre-saturation delays numerically optimized for the expected k_f[34]. This method was used to study kinetics in the open-chest dog heart and offered a time savings of up to 82%[34]. All three approaches were validated by comparison to conventional saturation transfer measurements obtained under non-localized conditions. A similar approach for validation was used here in the *in vivo* closed-chest mouse.

The mouse is a commonly used, important model for many human diseases because it allows specific gene-targeted interventions of over-expression or knock-out. In the absence of genetic manipulation, the normal mouse heart exhibits some fundamental similarities to normal human heart in high-energy phosphate metabolism and global left ventricular ejection fraction[3,15]. We adapted *TRiST* to the mouse heart and validated it by direct comparison with conventional ST measures in skeletal muscle. There was no significant difference between the measured T_1' or k_f between the two methods (Figures 1 and 3), although *TRiST* measures took 73% less time. This time saving allowed us to perform image-guided, spatially-localized *TRiST* measurements in the mouse heart under *in vivo* closed-chest conditions despite the small heart weight and rapid heart rates. Although there are large species differences in size and heart rate between mice and humans, the constancy of the cardiac PCr/ATP ratio[3], the absolute concentrations of [PCr] and [ATP][15], myocardial oxygen consumption[11,35], and, as shown in this study for the first time, the rate of ATP synthesis through CK is striking and speaks to the central importance of the ATP reserve offered by CK across species.

The current findings of a significant ~50% decrease in regeneration of myocardial ATP, through CK, in the murine TAC heart (Figure 6) are consistent with the prior reports in larger animal models of heart failure and clinical studies. In hypertrophied canine hearts one year after aortic banding, the *in vivo* rate of ATP synthesis through CK was reduced by 50%, while *in vitro* CK activity was unchanged[36]. Two months of aortic banding in Yorkshire pigs resulted in severe hypertrophy in all animals, and heart failure in half[37]. Hypertrophied swine exhibited a 35% mean reduction in ATP flux through CK, while animals in heart failure showed a 57% reduction in ATP flux through *in vivo* CK and a 48% reduction in *in vitro* CK activity[37]. Thus the current findings of a 50% mean reduction in ATP flux through *in vivo* CK in TAC mouse hearts are consistent with reductions observed in aortic-banded pigs and dogs. Changes in *in vitro* measures of total CK activity sometimes[37] as in this report, but not always[36], are similar to changes in the *in vivo* rate of ATP synthesis through CK. Importantly, the magnitude of the reduction in ATP kinetics observed here in TAC mice is similar to that observed in failing human hearts[8–9]. In patients with non-ischemic dilated cardiomyopathy the mean rate of ATP synthesis through CK was reduced by 50%[8]. In patients with pressure-overload hypertrophy, the rate of ATP synthesis through CK was reduced by 30% in those without HF and by 65% in those with HF symptoms[9]. Thus a significant reduction in the rate of ATP delivery by CK is a common finding in hypertrophied and failing hearts from mice through humans. Because the magnitude of the reduction of *in vivo* CK ATP capacity is similar in TAC mice and failing human hearts, this model and the non-invasive repetitive aspects of the new technique appear to be appealing, important tools to probe energetic hypotheses of heart failure development and progression.

ATP generated by the CK reaction and that from the ATPase, the latter derived mostly from oxidative metabolism, support contractile energy demands and may be limiting under some conditions, especially high-workloads in large animals[38]. Murine myocardial energy demands (per gram tissue/unit time) are likely similar to, or only modestly higher, than those of human hearts[11,35] because the ATP requirements of the 7–9 fold higher murine heart rates are mostly offset by the 6–7 fold lower wall tension in the dramatically smaller murine LV. Since the rate of ATP synthesis through CK is similar in mice and humans, the ATP reserve provided by CK could, in theory, be inadequate at high-workloads and/or in species with

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higher energy demands. Because the high-energy phosphate pools are small compared to their high rate of turnover[8–9,12] and because high-energy phosphates are consumed on a millisecond time scale during muscle contraction [33,39–41], a 50% reduction in the CK energy reservoir in the failing heart represents a significant deficit in temporal, and possibly spatial, ATP delivery[8–9]. However, it is still not known whether in fact a reduction in the rate of ATP delivery by CK of the magnitude measured here, or a reduction in that provided by the ATPase, not studied here, is the cause of LV dysfunction in HF. Additional studies determining the direct contractile consequences of independent manipulations of the rate of ATP delivery through such reactions would be needed to answer that question as well as studies at maximal workload conditions.

There are several limitations that merit mention. First, because significant reductions in k_f were previously observed in failing hearts but not in compensated, hypertrophied hearts in both pigs[37] and patients[9], additional, serial studies are needed in mice at earlier times after TAC to determine whether k_f can distinguish compensated and failing hypertrophied mouse hearts and predict the development of anatomic remodeling and decreased contractile function in this setting. Second, like other *in vivo* saturation transfer techniques this ^{31}P MRS *TRiST* approach applied to the *in vivo* mouse heart, cannot render CK results for the different sub-cellular compartments[42]. It does however reveal the net rate of ATP synthesis through CK[22] and this parameter is significantly altered in HF patients[8–9].

In summary, the first non-invasive, direct, validated measures of ATP synthesis in the *in vivo* mouse heart are reported. Despite the manifold difference in size and resting rate between mouse and human hearts, the rate of ATP synthesis through CK is remarkably similar per gram between these species. The rate of ATP synthesis through CK is reduced by 50% in the murine TAC heart and this reduction is comparable to that previously reported in canine and ovine pressure-overload models and importantly in human heart failure. The ability to non-invasively measure ATP kinetics in the intact, *in vivo* mouse heart promises new insights into the role of bioenergetics in contractile dysfunction and ventricular remodeling in this animal model with readily available genetic manipulations and similarities to human physiology and clinical heart failure.

Energy metabolism fuels ongoing myocardial contraction and altered metabolism may contribute mechanistically to several cardiac diseases including heart failure. ^{31}P magnetic resonance spectroscopy (MRS) is the only means to non-invasively quantify the levels of myocardial high-energy phosphates, ATP and creatine phosphate. Magnetization transfer MRS methods were adapted in recent years to measure the rate of ATP turnover in the human heart. Such MRS techniques, when combined with magnetic resonance imaging (MRI) approaches, offer a powerful means to assess cardiac energy metabolism, structure, mass, and contractile function in the same examination. Murine models are increasingly used to probe molecular mechanisms of human disease because of the possibility of creating specific transgenic lines. However, the small size (~0.1g) and high rate (~600/min) of the mouse heart present significant challenges for non-invasive imaging approaches.

In this paper, the first non-invasive *in vivo* measures of the rate of ATP synthesis through creatine kinase (CK), the major myocardial energy reserve, in the murine heart are reported. They demonstrate that ATP turnover rates in normal and failing mouse hearts are comparable to those reported in human hearts. This validated, non-invasive approach to measuring ATP synthesis rates through CK in the intact mouse can be combined with anatomic and functional measures in the same exam and be repeated over time as pathology develops. The observations demonstrate that murine models recapitulate several fundamental aspects of human myocardial energy metabolism *in vivo* and support

their use to probe specific metabolic contributions to the development and progression of heart failure.

Acknowledgments

Sources of Funding

This work is supported by National Institutes of Health Grants HL-63030 and HL-61912 as well as funds from the Donald W. Reynolds Foundation and the Clarence Doodeman Endowment.

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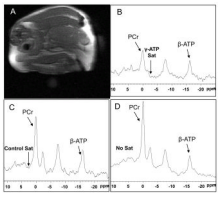

Figure 1.

(A) Typical ^{1}H magnetic resonance image of a mouse at the skeletal muscle with non-localized (B) ^{31}P MR spectrum with γ-phosphate of ATP saturation, (C) control saturation spectrum, (D) no saturation spectrum with TR=16s and NEX=64. PCr; phosphocreatine, β-ATP; β-phosphate of adenosine triphosphate

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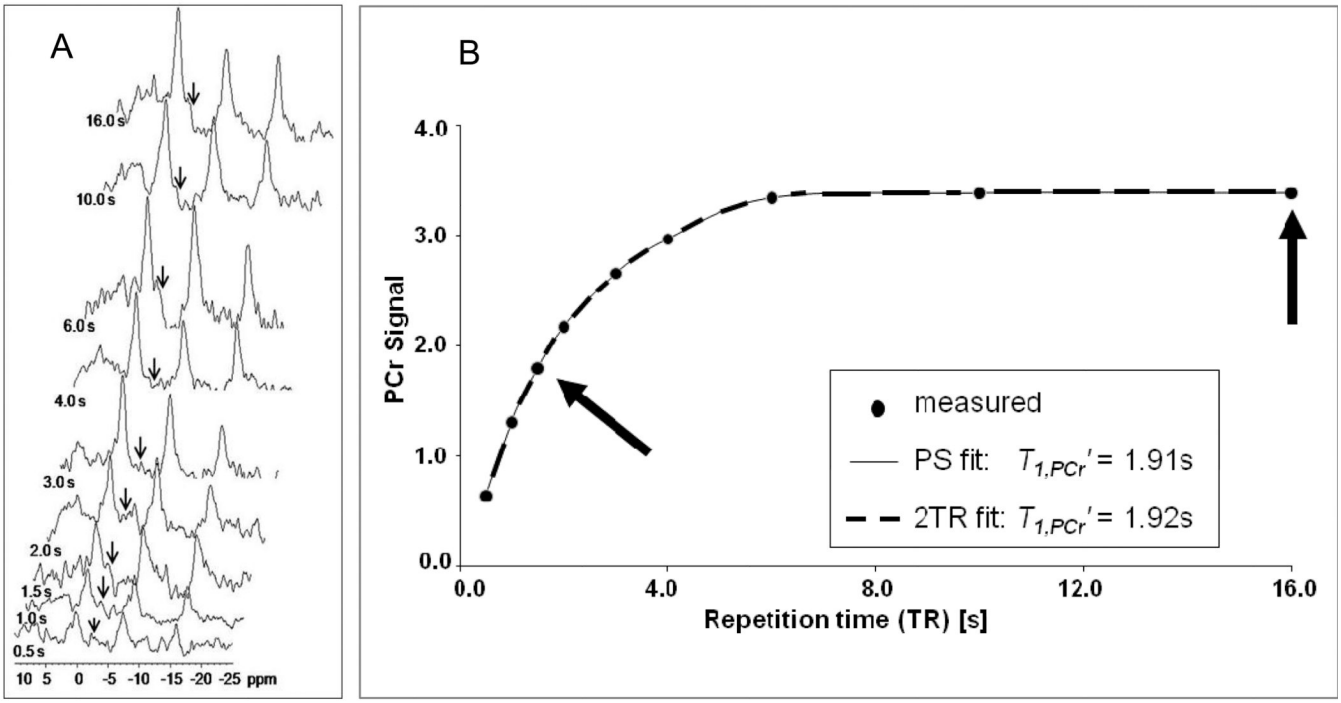

Figure 2.

(A) Stack plot of ^{31}P spectra acquired with γ-phosphate of ATP saturation at different TR (TR values listed to left of each spectrum), (B) PCr signal intensity (a.u.) versus TR (s) during saturation of γ-phosphate of ATP in the skeletal muscle of same representative mouse. T_1' was determined both by conventional saturation transfer analysis fitting all 9 data points (light thin line) and by the 2TR method using only the data points acquired at TR of 1.5s and 16s (dark thick dotted line).

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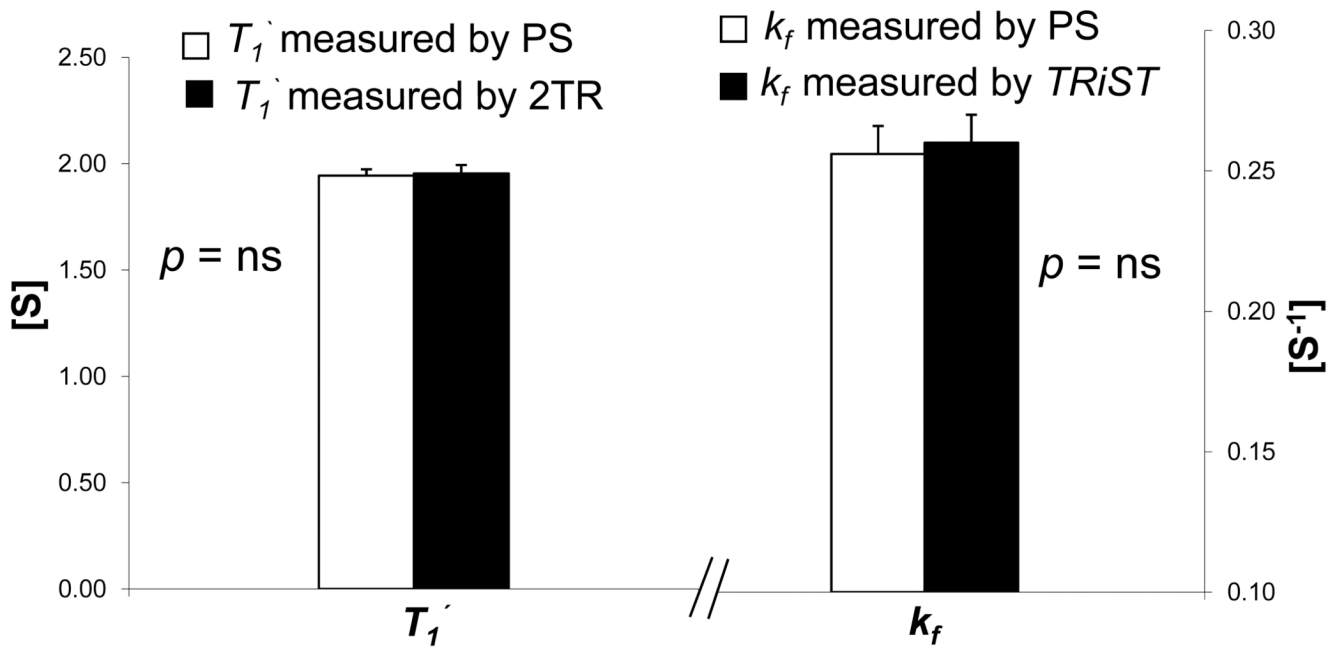

Figure 3.

T_1' and k_f measured non-localized *in vivo* in skeletal muscle (n=5). Conventional PS (white bars) and faster *TRiST* techniques (black bars) produced identical results within experimental error.

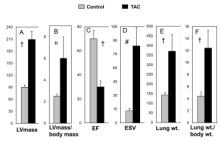

Figure 4.

Cardiac functional parameters for control (gray bars) and TAC (black bars) mice: (A) LV mass (mg), (B) LV mass (mg) / body mass (g), (C) Ejection Fraction (%), (D) End Systolic Volume (μl), (E) Lung weight (mg) and (F) Lung wt. (mg) / body wt. (g) ratio. Control vs TAC mouse †, $p < 0.0001$; וו, $p < 0.005$; #, $p < 0.0002$.

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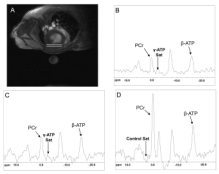

Figure 5.

(A) Typical transverse ^{1}H magnetic resonance image of a mouse at the mid left ventricle with the nominal location of ^{31}P MR cardiac voxel denoted between the white lines (B) ^{31}P MR spectrum with γ-phosphate of ATP saturation with TR=1.5s, NEX=96, (C) γ-phosphate ATP saturation with TR=6s, NEX=32, and (D) control saturation spectrum with TR=10s and NEX=16. PCr; phosphocreatine, β-ATP; β-phosphate of adenosine triphosphate

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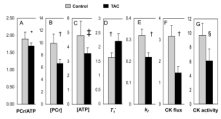

Figure 6.

Cardiac metabolic parameters for control (gray bars) and TAC (black bars) mice: (A) PCr/ATP ratio, (B) PCr concentration ([PCr]) (μmol/g wet weight), (C) ATP concentration ([ATP]) (μmol/g wet weight), (D) T_1' of PCr (s), (E) CK forward pseudo-first-order rate constant, k_f (s^{-1}), (F) the rate of ATP synthesis through CK ("CK flux", μmol/g/s), (G) *in vitro* CK activity (IU/mg protein).

Control vs TAC mouse *, p <0.001; †, p <0.0001; ‡, p <0.003; §, p <0.0006.

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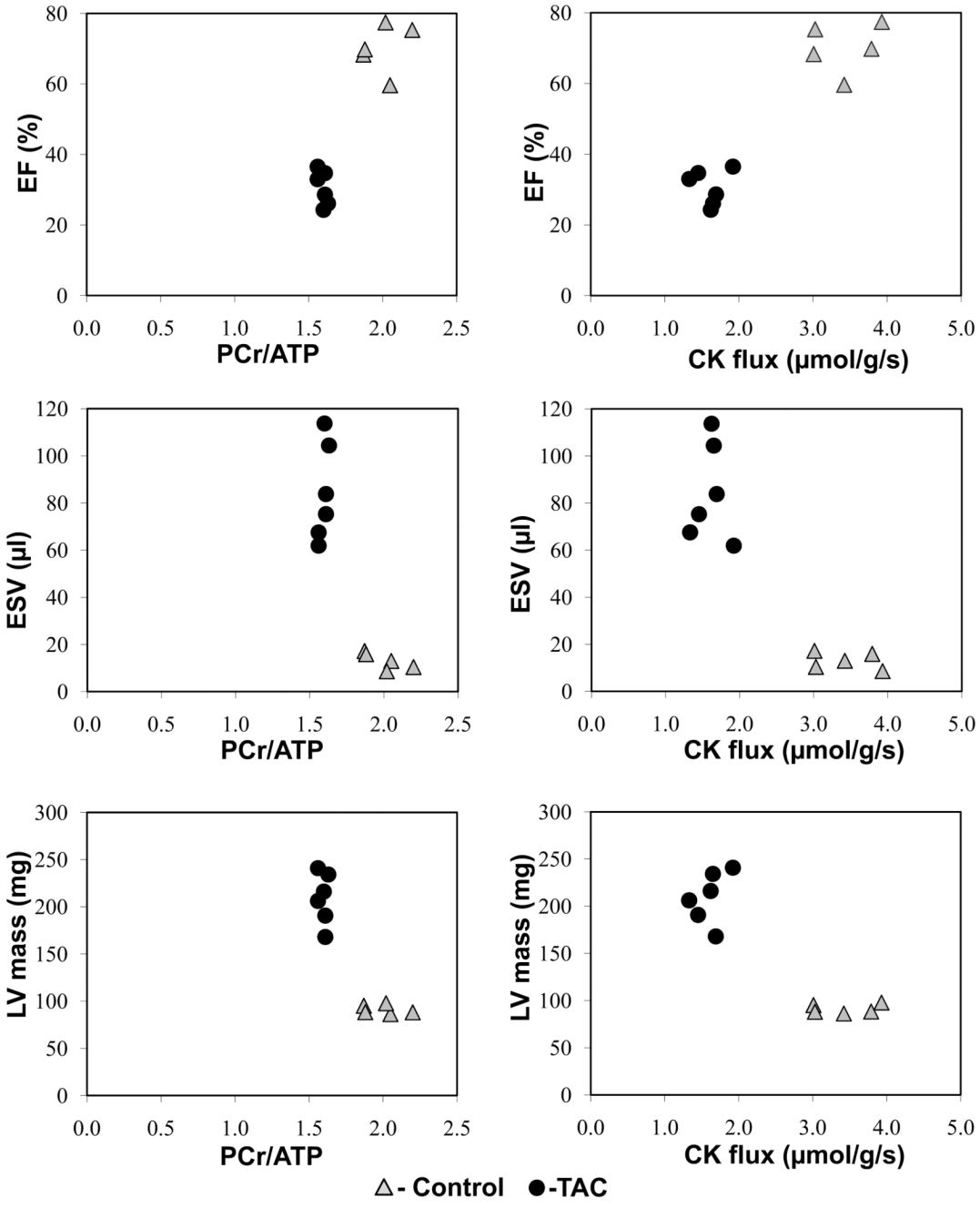

Figure 7.
Cardiac MRS parameters (PCr/ATP ratio, left panel; rate of ATP synthesis through CK "CK flux", µmol/g/s, right panel) versus cardiac MRI parameters (ejection fraction (EF), %; end systolic volume (ESV), µl; and LV mass, mg for control (triangles) and TAC (filled circle) animals.