# Additional file 1

# Cardiac Work is Related to Creatine Kinase Energy Supply in Human Heart Failure

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## Protocol for measuring creatine kinase (CK) metabolites and energy supply

The magnetic resonance spectroscopy (MRS) studies were performed on a *Philips Healthcare* (Best, the Netherlands) 3 Tesla broadband *Achieva* magnetic resonance imaging (MRI) scanner using a 17-cm/8-cm phosphorus (31P) transmit/receive surface coil set with an embedded coil marker described previously[1]. Absolute concentrations of phosphocreatine, [PCr], and adenosine triphosphate, [ATP], were determined by 31P MRS using an external concentration referencing method that included corrections for coil loading, relaxation, heart motion, tissue volume, and coil sensitivity variations within voxels[2]. The CK reaction rate, *kf* was measured using triple repetition-time saturation-transfer (TRiST)[3]. The patient MRS protocol comprised the following steps (Fig. 1):

(1) Position the subject prone in the MRI scanner with the left ventricle above the 31P MRS coils, as confirmed by scout proton (1H) MRI.

(2) Shim the magnetic field homogeneity over the heart (water linewidth≈20 Hz)[4].

(3) Acquire coronal 1H MRI data for subsequent segmentation of cardiac tissue into MRS volume elements (voxels) for metabolite quantification.

(4) Switch to 31P MRS and acquire a fully-relaxed, cardiac-triggered (end-systole) one-dimensional (1D) spatially-localized, surface-coil detected chemical shift imaging (CSI) data from the chest and heart (16-phase-encodes; acquisitions per encode, NA=2; 1-cm resolution; repetition period TR≥15 s; echo time TE=1.4 ms; bandwidth=3 kHz) using adiabatic half-passage excitation (AHP; 5-ms tan/tanh-modulation; 7kHz frequency-sweep cycling). This acquisition is used to measure metabolite concentrations[1].

(5) Apply the 31P MRS TRiST method using three 1-cm resolution 1D CSI sequences applied with an amplitude-modulated frequency-selective saturation pulse train prior to AHP excitation[3,5]. The three sequences employ: (i) frequency-selective saturation of the γ-phosphate resonance of ATP (γ-ATP, -2.5 ppm relative to PCr) acquired at a TR of two heart-beats (~1.7 s; NA=18); (ii) the same saturation of the γ-ATP resonance but acquired with a cardiac-gated TR~10 s (NA=8); and (iii) frequency-selective saturation applied at +2.5 ppm acquired with a cardiac-gated TR~16 s (NA=2) as a control[6].

(6) To calibrate the MRS signal strength for measuring metabolite concentrations, the subject was replaced by a 6-cm diameter cylindrical concentration reference (30 mM NaH2PO4), and scanned with the same MRS protocol as Step 4 (but with TR=8 s). The three-dimensional (3D) spatial sensitivity profile of the 31P coil set was determined separately from a 3D CSI scan from a large phantom of 600 mM NaH2PO4 [2].

## MRS data analysis

MRS data were quantified to determine [PCr], [ATP] and *kf* from the corresponding MRS peak areas as detailed previously[2,3]. The ratio of the MRS signals from the coil marker measured in the spectra from the patient in Step 4 and from the reference phantom data from Step 6 were used to account for differences in MRS coil loading. Cardiac PCr was considered the primary endpoint for each MRS study because it is needed to determine [PCr], *kf,* and hence the CK flux which is given by the product, *kf*[PCr]. The signal-to-noise ratio (SNR) of PCr in cardiac spectra ranged from 8-40. Voxels with SNR <8 in spectra acquired for measuring [PCr] were generally not quantified because further reductions in SNR due to saturation transfer (Step 5i or 5ii) would the preclude *kf,* and CK flux measurements. The areas of the PCr, γ-ATP and blood 2,3-diphosphoglycerate (DPG) peaks in cardiac voxels in the data from Step 4, and that of the phosphate peak in the corresponding voxels from the concentration reference, were fitted using ‘Circle Fit’[7]. The ATP peak areas were corrected for blood ATP contamination by subtracting 15% of the DPG signal[8].

 The volume of cardiac tissue contributing to each spectrum was determined by co-registering the cardiac MRIs from each subject with the reference phantom and the coil sensitivity profiles, using the embedded coil marker[2]. The myocardial tissue present in each MRS voxel was segmented by two different users (REG, PAB) who contoured the chamber walls in the coronal MRIs acquired in Step 3, and the two results averaged. The segmented tissue maps were multiplied by the coil sensitivity profile, and this process repeated for a segmented image of the concentration reference. The ratio of the resultant maps from the concentration reference and from the subject were used to provide a partial volume correction for each MRS voxel. The ratio of the metabolite peak areas to that of the reference in the same voxel was then multiplied by the partial volume correction factor and by the known reference concentration, to yield the metabolite concentration in mmol/liter of wet tissue[2]. This result was converted to mmol/kg wet weight using a cardiac specific gravity, SG=1.03[9].

 The forward CK rate *kf* in sec-1, was determined from the steady-state PCr signal heights in the same cardiac voxels acquired in Step 5[3]. *kf* was calculated from:

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| --- | --- |
| *kf =* 0.0052(*M0`/M0*-0.8730)(*Q*+27.5332)(*T1`*-6.0647) | (A1) |

derived from a Bloch equation analysis that included the effects of spillover saturation[6,10]. Here, M0`, M0 and T1` are the PCr signals and the 31P MRS spin-lattice relaxation time of PCr obtained from the spectra acquired in Step 5(i-iii), and *Q* is the ratio of the PCr signal from Step 5(iii) to that from Step 4. The CK flux in mmol/(kg wt. sec) was calculated from the product, *kf*∙[PCr], and converted to *Système International* units of Watts/kg wet-tissue weight (W/kg) by multiplying by the free-energy of ATP hydrolysis, ΔGATP=60 kJ/mol. The latter is the mean of 59-61 kJ/mol determined noninvasively in healthy and HF patients using the same methodology[11,12].

## Measuring cardiac stroke work

Cine MRI is a “gold standard” for noninvasive volumetry[13–15], and for pressure volume (PV)-loop measurements in large animals[16–21] and humans[19,21], wherein conductance catheters require volume calibration using an imaging modality. The PV-work, *w*(*t*), was evaluated from the pressure, *P(t),* and temporal (*t*) volume change, *∆V*, as:

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| --- | --- |
|  | (A2) |

where the minus sign associates cardiac contraction with positive muscle work. The mechanical stroke work, SW, is the integral of Eq. (A2) over one cardiac cycle. The instantaneous mechanical power (or rate of energy consumption) is the derivative of Eq. (A2)[22]:

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| --- | --- |
|  | (A3) |

Cine MRI and blood pressure (BP) measurements were performed with patients positioned either supine and scanned with 6- or 32-channel cardiac array coils, or prone using the scanner’s body MRI coil. These studies were performed as an add-on (Step 6) to the MRS protocol or as a separate exam, depending on patient preference and availability (Fig. 1). After shimming and performing scout MRI, double-oblique short-axis retrospective cardiac-gated MRI was performed in breath-held acquisitions using balanced steady-state free-precession (SSFP; 8-12 slices; TR =3.5 ms; echo time TE =1.8 ms; 30 cardiac phases; slice thickness =8 mm; slice gap =2mm; SENSE factor =2 for coil arrays; 1.6x1.3 mm in-plane scan resolution; 256x256 matrix; 1-2 slices/breath-hold; 15-25 min total MRI exam time). Systolic and diastolic BP were measured from an upper-arm cuff using an *In vivo Systems* (Orlando, FL) MRI-compatible pressure monitoring system before and after cine MRI, and averaged for PV-loop calculations.

Note that cardiac work is also converted to kinetic energy carried by the blood leaving the ventricles. Although the blood’s speed during contraction can reach ≈1m/s, the average is only ≈20cm/s[23]. For an average stroke volume of less than 100ml[24], this corresponds to only ≈2mJ which is minute (~0.2%) compared to the pump energy (≈1J), and was therefore neglected.

## MRI data analysis

The inner and outer contours of the left ventricle were manually delineated in all short-axis MRI sections at all time points. The left ventricular (LV) mass (LVM) was calculated from the difference between the two contours at end diastole summed over all slices and multiplied by SG. The LV blood volume was calculated at each cardiac phase by summing the blood volumes in all adjacent myocardial sections, and its derivative determined after temporal filtering to mitigate the effects of noise. The volume change was multiplied by the pressure waveform to calculate the rate of mechanical power consumption, per Eq. A3.

The stroke work (SW) was obtained from the integral of the PV work. The ‘potential energy’ (PE) was estimated from the line connecting the end-systolic PV-point in the PV-loops (Fig. 2), with an unstressed volume V0, assumed negligible[21]. The total mechanical energy (PVA) was estimated by the sum, SW+PE.

The effect of assuming a square pressure waveform was simulated by comparing a half-sinusoid pressure waveform during systole, starting and ending at the mean systolic and diastolic BP, respectively, as listed in Table 1 of the main manuscript. With a similar half-sinusoidal waveform for the volume, the square pressure waveform underestimated the peak power of the sinusoidal wave by 10.5%, and might thus be considered conservative. The effect on average power of neglecting diastolic pressure was investigated by linearly varying end-diastolic pressure (EDP) up to 20mmHg. The variations in EDP affected the average SW estimated from the square waveform model by -5.3% to 2.5% for Psys≥110mmHg (per Table 1).

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