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Quantifying human high-energy phosphate metabolite concentrations at 3T with partial volume and sensitivity corrections

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Abstract

Practical noninvasive methods for measuring absolute metabolite concentrations are key to assessing depletion of myocardial metabolite pools which occurs with several cardiac diseases including infarction and heart failure. Localized magnetic resonance spectroscopy (MRS) offers unique noninvasive access to many metabolites, but is often confounded by nonuniform sensitivity and partial volume effects in the large, poorly-defined voxels commonly used for detecting low-concentration metabolites with surface coils. These problems are exacerbated at higher magnetic field-strengths by greater radio frequency (RF) field inhomogeneity and differences in RF penetration with heteronuclear concentration referencing. An example is the ³¹P measurement of cardiac adenosine triphosphate (ATP) and phosphocreatine (PCr) concentrations, which although central to cardiac energetics, have not been measured at field-strengths above 1.5T. Here, practical acquisition and analysis protocols are presented for quantifying [PCr] and [ATP] with one-dimensionally resolved surface coil spectra and concentration referencing at 3T. The effects of non-uniform sensitivity and partial tissue volumes are addressed at 3T by applying MRI-based three-dimensional sensitivity-weighting and tissue segmentation. The method is validated at 3T in phantoms of different sizes and concentrations, and used to measure [PCr] and [ATP] in healthy subjects. In calf-muscle (n=8), [PCr]=24.7±3.4 (mean±SD) and [ATP]=5.7±1.3 μmol/g wet wt, while [PCr]=10.4±1.5 and [ATP]=6.0±1.1 μmol/g in heart (n=18), consistent with previous reports at lower fields. The method enables for the first time, the efficient, semi-automated quantification of high energy phosphate metabolites in humans at 3T with non-uniform excitation and detection.

Keywords

quantification; metabolism; phosphorus; high-field; human heart; 3Tesla

INTRODUCTION

Phosphocreatine (PCr) is the main energy reservoir in both skeletal and cardiac muscle. PCr supplies adenosine triphosphate (ATP) which fuels muscle contraction, through the creatine kinase (CK) reaction. Localized phosphorus (^{31}P) MRS can measure PCr and ATP in the heart (1), and alone has enabled evaluation of stress-induced ischemic changes in patients with coronary disease (2,3) and ATP turnover through the CK reaction in normal, stressed, cardiomyopathic, failing, and infarcted human hearts (4–6). Measurements of the absolute cardiac metabolite concentrations in patients with myocardial infarction (MI) often reveal large reductions in both [PCr] and [ATP] compared to healthy myocardium, but normal PCr/ATP ratios (6,7). In addition, myocardial [PCr] is reduced in heart failure (HF) (4,5,8), and is needed for determining the CK flux. Indeed, the CK ATP flux is significantly reduced in both MI and HF (4–6).

Importantly, all those studies were performed in MRI/MRS scanners operating at 1.5 Tesla (T) using surface transmit and receive coils (1–8). To date, there are no reports of human cardiac high-energy phosphate metabolite concentrations measured by MRS at 3T or higher. With commercial 3T MRI/MRS scanners now widespread and initial studies suggesting significant performance advantages in signal-to-noise ratio (SNR) and spectral resolution (9,10), we sought to translate the methodology for measuring cardiac [PCr] and [ATP] from 1.5 to 3T. Our goal was to develop and validate a protocol for measuring metabolite concentrations in about 10 min that could be included in a practical patient exam of about an hour that included scout and other 3T functional cardiac MRI, or MRS measurements of CK reaction-rate (and flux) (11). The protocol should at least perform comparably to that used for patient studies done at 1.5 T (4–6).

The standard method of measuring metabolite concentrations is to compare the metabolite signal with that of a concentration reference, and correct the result for relaxation, volume size, surface coil loading, and sensitivity differences between the metabolite voxel and the reference voxel (12–14). The latter is particularly troublesome if the localized volume or voxel size is large and the spatial sensitivity varies significantly across the volume, as may occur in whole-volume image-selected in-vivo spectroscopy (ISIS) (7,15,16), one-dimensional (1D) chemical shift imaging (CSI) (1–3), or depth resolved surface coil spectroscopy (DRESS) (17,18). To avoid these problems at 1.5T, the water ^1H MRS signal was previously used from the same tissue acquired with the same pulse sequence, as a concentration reference (13). The ^1H signal was detected by the ^{31}P surface coil without retuning it, to ensure an optimum ^{31}P performance and that both ^{31}P and ^1H spectra were acquired with the same sensitivity profiles. The method required calibration of the ratio of the ^{31}P signal per phosphate to the ^1H signal per proton, as well as knowledge of the tissue water content, which appeared to be fairly constant for heart (13).

Unfortunately at 3T, a single tuned coil can suffer large variations in sensitivity between the ^1H and ^{31}P MRS frequencies due to differences in tissue RF penetration and phase-shifts at the two frequencies. In fact, our initial experience suggested that the water- ^1H reference ^{31}P -detect quantification method may not be feasible at 3T. Solutions such as double-tuning the ^{31}P coil at 3T, degrades the SNR relative to the best achievable,

counteracting in part the advantages of higher field; while the use of a separate ^1H coil results in different spatial sensitivity distributions for the tissue and the reference acquisitions, introducing further errors or correction requirements.

Here instead, we use separate ^{31}P acquisitions from a concentration reference, an embedded coil-loading phantom, and direct mapping of the ^{31}P MRS field-of-view (FOV) onto coronal and axial scout ^1H MRIs that are co-registered with the coil's three-dimensional (3D) sensitivity map. The tissues generating the average metabolite signal (heart muscle) for each MRS voxel—here, a 1D CSI slice—are segmented on the ^1H MRIs, *weighted by the sensitivity profile determined at each image pixel within each slice*, and integrated to obtain the signal per unit volume or mass present in the voxel or slice. These metabolite measures are then corrected for relaxation and coil loading, and normalized by the reference signal from the same MRS slice to yield metabolite concentrations in $\mu\text{mol/g}$ wet wt. The novelty of this work is that, to our knowledge, it is the first to quantify human cardiac ^{31}P metabolite concentrations above 1.5T—at 3T where RF field non-uniformity is much more problematic. It is also the first to address the problem using point-by-point coronal MRI-based segmentation and sensitivity weighting, and to apply it to the case of 1D localized MRS where surface coil detection provides poorly-defined localization in the other two dimensions.

Our application utilizes 1D CSI localization and frequency-sweep-cycled (FSC) adiabatic half passage (AHP) excitation pulses to provide a uniform flip-angle over the ^{31}P MRS FOV and defined spectral bandwidth using a separate 3T surface excitation coil (10). The pulse excitation uniformity performance versus the distance from the coil is validated by both numerical simulations of the Bloch equations and in a phantom experiment. The coil sensitivity profile is measured with 3D CSI in a uniform phantom, and a protocol is developed to provide the various experimental factors affecting concentration measurements. Image analysis is integrated into a user-friendly software interface that displays the coronal images for segmentation, automatically weights the metabolite signal-generating tissue by the sensitivity profile, and computes the metabolite concentrations from tabulated MRS metabolite peak areas. The approach is validated on phantoms containing phosphate at known, physiological concentrations, then used to measure [PCr] and [ATP] in healthy human calf and cardiac muscle. The results are compared to literature values measured at 1.5T.

METHODS

Concentration measurement protocol

In general, the procedure developed for measuring skeletal or cardiac metabolite concentrations from ^{31}P 1D CSI MRS would use human protocol steps (A1–A4), and calibration steps (A5 and A6) as follows:

- A1.** The subject was positioned in the MRI scanner, with the heart or muscle above a ^{31}P MRS probe with separate transmit and receive surface coils placed flat on the scanner bed (10). All of our studies employed a Philips Healthcare (Best, The

Netherlands) 3T broadband *Achieva* scanner. A dual-turn (diameters, 17cm and 11cm) ^{31}P transmit coil was positioned 2cm below the surface of a 8-cm ^{31}P receiver coil as described in Ref. (10). A 1–2cm diameter spherical phantom filled with NaH_2PO_4 and fixed to the coil housing was used regularly to compensate for differences in ^{31}P coil loading. A vitamin-E pill fixed near the center of the receiver coil was used as a coil marker to co-register the ^1H MR images. All ^1H MRI was performed using the scanner's body coil.

- A2.** Scout MRI was performed to confirm coil location relative to the heart or muscle. A set of coronal sections through the muscle or the chest and anterior myocardium was prescribed and acquired for tissue segmentation. A 3-plane orientation, ungated turbo-fast field echo was used (FFE; 20 1-cm thick slices per orientation; in-plane image resolution $\sim 1.76\text{mm}$; total scan-time, 24s). Other muscle or cardiac MRI may be acquired, for example, cine MRI to measure ejection fraction etc in cardiac studies. The cine MRI data may be used to correct for differences in tissues volumes for MRS data acquired at different times in the cardiac cycle.
- A3.** The region of interest (eg, the heart), was shimmed to $\sim 20\text{Hz}$ ^1H line-width using MRI-guided 2nd-order shimming (19).
- A4.** A 1D ^{31}P CSI MRS sequence was applied. The FOV was adjusted to include the region of sensitivity with the metabolite of interest (MoI) and the 1–2cm spherical loading phantom. We used a 1-cm resolution, 16-step 1D CSI sequence with 5ms duration (τ) tan/tanh-modulated AHP FSC 90° excitation pulses (maximum frequency sweep $f_{\text{max}} = 7\text{kHz}$; acquisition delay, $1.4\text{ms} \ll T_2$, the spin-spin relaxation time) (10). For [PCr] and [ATP] measurements, the ^{31}P MRS frequency was tuned midway between the PCr and γ -ATP resonances (determined from a non-localized (NL) acquisition). Two free induction decays were acquired per gradient step (NA=2; sampled with 512 or 1024 points; receiver bandwidth, BW = 3kHz). We set the repetition period $\text{TR} > 2.5T_1$, the spin-lattice relaxation time of PCr, to minimize or eliminate saturation corrections.
- A5.** With the subject removed from the scanner, a $\sim 6\text{cm}$ diameter cylindrical phantom filled with a 30mM NaH_2PO_4 solution as a concentration reference (^{31}P $T_2 > 350\text{ms}$), was placed on the ^{31}P coil set, imaged and shimmed. A 1D ^{31}P CSI MRS sequence was applied with the same FOV as in Step A4.
- A6.** The 3D sensitivity of the ^{31}P coil set was required to correct for variations that occur within the tissue intersecting the 1D CSI

slices. In our studies, this was determined in a separate 3D CSI scan (resolution, $2 \times 2 \times 1 \text{ cm}^3$; FOV= $40 \times 40 \times 20 \text{ cm}^3$; TR=0.625s; NA=2) performed on a uniform $\sim 32 \times 32 \times 14 \text{ cm}^3$ phantom filled with 600mM NaH_2PO_4 and doped with NiCl_2 to achieve a T_1 of 0.4s.

Steps A5 and A6 need only be performed once for the period over which the coil and scanner are stable.

Range determination

To determine the effective range or FOV over which the ^{31}P MRS AHP excitation was uniform in Steps A4–A6, we simulated the evolution of the magnetization vector numerically on a personal computer (PC) in *Matlab* (The Mathworks, Natick, MA, USA) using the Bloch equations with both rotation and relaxation during the AHP pulses (10). AHP pulses were digitized at $5\mu\text{s}$ intervals and applied at TR=16s. The adiabaticity threshold for the transverse RF field, B_1 , was computed for PCr and γ -ATP assuming T_1 and T_2 values of 5.8s/350ms and 3.1s/70ms respectively, consistent with prior studies (10,20,21). The analysis showed that over a BW of $\pm 100\text{Hz}$ ($\pm 1.9\text{ppm}$) centered between PCr and γ -ATP, a B_1 of $25\mu\text{T}$ should suffice to measure >95% of the magnetization for both moieties. Because $T_2 = 350\text{ms}$ for the 30mM reference phantom, no T_2 correction was needed. For the present studies on the Philips 3T scanner with a 4kW (nominal rating) broadband RF power amplifier, this permitted measurements in the heart for depths of up to $\sim 8\text{--}9\text{cm}$ from the surface of the receiver coil, based on computations of B_1 for the transmit coil on a homogeneous sample with a physiologically comparable RF dielectric constant of 80, and conductivity of 0.65S/m (10).

Calculating metabolite concentrations

The metabolite concentration in the n^{th} 1D CSI slice in mmol/kg wet wt. was calculated from the following equation:

$$[\text{Metabolite}]_{\text{muscle}}^n = \frac{S_{\text{muscle}}^n \cdot \varepsilon_{\text{ref}} \cdot \sum_{i,j} w_{ij}^{\text{ref}} C_{ij}^n}{S_{\text{ref}}^n \cdot \varepsilon_{\text{muscle}} \cdot \rho \cdot \sum_{i,j} w_{ij}^{\text{muscle}} C_{ij}^n} \times F_L \times F_m \times [P]_{\text{ref}}^n. \quad [1]$$

Here $e = 1 - e^{-TR/T_1}$ is a saturation factor, and S is the fitted area of the corresponding spectral peak, with subscripts *ref* and *muscle* denoting the concentration reference (from Step A5) and tissue of interest (from Step A4), respectively. C_{ij}^n is the receiver coil sensitivity at a pixel location (i, j) in the MRI that lies coplanar with the n^{th} 1D CSI slice (from Step A6), and $w_{ij} = 1$ or 0 depending on whether or not there is metabolite-bearing tissue at that location. The summation of $w_{ij} C_{ij}^n$ overall i and j in the sensitive region of the detection coil is equal to the volume generating MRS signal in the n^{th} slice, weighted by the coil sensitivity at each point in that slice. F_L accounts for differences in coil loading between the reference and subject scans acquired in Steps A4 and A5. F_m is a factor used to account for temporal variations in the amount of tissue occupying the MRS slice when MRS and

MRI are acquired at different times, for example, during the cardiac cycle. Further details on deriving F_L , F_m , w_{ij} , C_{ij} are provided below. $[P]$ is the concentration of phosphate in the reference phantom in mM, and ρ is the specific gravity of tissue, taken as 1.03 for the heart muscle and 1.04 for the calf (12).

The MRS peak areas, S , can be determined by any standard MRS processing method. Owing to its robustness for *in vivo* ^{31}P MRS studies, we used “circle fit” which avoids subjective phase and baseline corrections by fitting a circle to each peak in the un-phased spectrum as it appears on a 3D plot (real vs. imaginary vs. chemical shift) from the viewpoint of the chemical shift axis (22). The γ -ATP resonance was used for quantifying [ATP]. We generated the sensitivity maps that define C_{ij}^n by interpolating the peak areas of the 3D CSI spectra acquired in Step A6 using Matlab.

Segmentation and analysis

In our studies, a PC *Matlab* based user interface was constructed to perform the metabolite quantification embodied by Eq. [1]. The software has the following 7 steps:

- B1.** Both the subject and reference phantom scout images were loaded, along with text files listing the fitted peak areas of the metabolites of interest and the concentration reference. The embedded coil marker was identified in the ^1H scout images of the subject and of the phantom. The subject and phantom images were co-registered with the MRS slices.
- B2.** The loading factor, F_L , was determined from the ratio of the MRS signal from the coil marker measured in the phantom, to that in the subject(s).
- B3.** The partial saturation correction factors, e_{ref} and e_{muscle} , were applied to the quantified reference and metabolite signals, based on previously measured T_1 values (10). A correction was also applied to the cardiac ATP signal to account for contamination by blood ATP in the ventricular chambers, based on 15% of the measured blood 2,3-diphosphoglycerate (DPG) signal, as described previously (23,24).
- B4.** In general, the images used for segmentation (Step A2) should either be triggered at the same cardiac phase as that used for spectral acquisition (Step A4), or extracted from cine MRI. However, if either MRS or scout MRI are un-gated or triggered at different cardiac phases the images can still be used for segmentation by correcting for differences in tissue volume via a compensation factor F_m . F_m is determined by the ratio of the average myocardium muscle present in each MRS slice to that at the MRS cardiac phase determined from additional MRI acquisitions.

- B5.** A coronal slice was selected from the subject's images for quantification, for example, in the heart. The myocardium in the MRS slice overlaying the image was segmented by contouring the chamber walls seeded by the user. In our studies, to minimize potential user bias, the tissue segmentation was done by two different users and the results averaged in the heart.
- B6.** The segmented tissue was co-registered with the coil sensitivity map from Step A6, and multiplied by it on a pixel-by-pixel basis, to obtain the $w_{ij}C_{ij}^n$ in Eq. [1]. For this purpose, the in-plane sensitivity map, which is a smooth spatial function, was interpolated to the same resolution of the MRI. The process was repeated on a segmented image of the concentration reference from Step A5. The $w_{ij}C_{ij}^n$ were integrated over the entire volume of the slice in the subject and reference phantom, to obtain the sensitivity-weighted ratio of the tissue volume present in the MRS slice to that in the concentration reference.
- B7.** The ratio of the co-registered quantified metabolite peak areas in the selected slice to the reference signal in the same slice was calculated, and multiplied by the reference concentration to complete the concentration determination. The concentration measurements, segmentation masks, F_L , F_{mr} and the locations of the coil marker were saved to a text file.

Figure 1 depicts a flow chart summarizing the acquisition and processing steps required for determining metabolite concentrations in the presence of nonuniform sensitivity across the MRS slices.

EXPERIMENTS

Phantom studies

We validated the concentration measurement protocol for ^{31}P MRS 1D CSI in 12 different cylindrical phantoms of different sizes and concentrations, immersed in a saline tank to provide human-equivalent coil loading. The phantoms had diameters of 3.7cm, 5.7cm, and 9.4cm, and contained NaH_2PO_4 at concentrations of 5, 10, 20 and 30mM. Their ^{31}P T_1 values were measured using NL progressive saturation experiment for the saturation correction (the ϵ in Eq. [1]). The 5.7cm diameter phantom with 30mM concentration was used as the external reference. Step A4 was performed with $\text{TR}=8\text{s}$. The pulse bandwidth was verified by repeat 1DCSI performed on resonance and at $\pm 100\text{Hz}$ in an external reference phantom.

Human studies

Quantification of the high-energy phosphates PCr and ATP in 1D CSI spectra from calf muscle and heart was then performed in human studies approved by the Johns Hopkins Institutional Review Board and subjects provided written informed consent. The calf muscle

studies were performed on 8 healthy volunteers (age=38±9 yrs; n=3 women) lying supine with the gastrocnemius/soleus muscle positioned on the ^{31}P surface coil set. After scout MRI, shimming, 1D CSI was applied per Step A4 with TR=25s to ensure complete relaxation (assuming muscle T_1 s of 6.8 and 5.4s for PCr and γ -ATP respectively (10)).

The cardiac measurements were performed on 18 healthy volunteers (age=39±17 yrs; n=7 women) who were positioned prone on the ^{31}P coils to minimize breathing motion and the distance between the coil and the anterior myocardium. Axial cine images were acquired during free breathing to determine the trigger delay for end-systole acquisition, and for providing the F_m correction factor (per Steps A2 and B4 above), where appropriate. Cardiac-triggered 1D CSI was performed with TR≈16s to minimize partial saturation and an average TR value of 16.2s was used for relaxation correction as determined in 16 volunteers from the scanner's physiology log (16.2±0.1s). The total acquisition time was 9min. The measured 3T cardiac metabolite T_1 s of 5.8 and 3.1s (10) were used to correct PCr and γ -ATP for any remaining partial saturation.

RESULTS

Protocol and analysis steps A1–A6 and B1–B7 were generally implemented as described. Figure 2 is a screen shot demonstrating the PC-based user interface being applied to segment cardiac MRIs and quantify [PCr] and [ATP] in ^{31}P 1D CSI spectra acquired from a healthy subject.

Phantom studies

Figure 3 summarizes the results from the phantom studies. Fig. 3(a) plots the calculated concentration for the 5.7cm diameter cylindrical phantom filled with 5, 10, 20 and 30mM NaH_2PO_4 as a function of depth. The results show that concentration measurements can be obtained up to depths of ~9cm, consistent with the analysis of the useful range of the excitation field. Fig. 3(b) plots the mean and standard deviation of 8 measurements of the phosphate concentration at depths of 3.5–9.5cm for the three different phantom sizes, as a function of the known concentration. The results show agreement with a mean absolute error of ~7% over the physiological range of concentrations of the intended application, independent of phantom size or diameter. The off-resonance experiment confirmed the excitation BW of ±100Hz up to a depth of ~9cm, consistent with the simulations.

Human studies

Figure 4 plots [PCr] and [ATP] as a function of depth in the calf muscle of the 8 healthy volunteers. On average the calculated values are almost independent of depth consistent with prior studies at 1.5T (13). Table 1 shows that the average measured concentrations of [PCr]=24.7±3.4 and [ATP]=5.7±1.3 $\mu\text{mol/g}$ wet wt, with PCr/ATP=4.4±0.7, are in the range of previously published values at lower field strengths using different methods (13,25,26).

Figure 5 shows a typical 1D CSI ^{31}P heart spectrum. The average measured [PCr] and [ATP] for the 18 subjects was [PCr]=10.4±1.5 and [ATP]=6.0±1.1 $\mu\text{mol/g}$ wet wt, respectively, with PCr/ATP=1.77±0.3, again in agreement with previously published values at lower field strengths (Table 2) (7,13,14).

DISCUSSION

This report is the first to quantify skeletal- and cardiac muscle high-energy phosphate concentrations at 3T. The approach is efficient, semi-automated, and does not prolong the spectroscopy exam time of human subjects over that of studies of cardiac PCr/ATP alone. It can be used with non-uniform excitation and detection, common in surface coil studies. The method was validated with studies of phantoms varying in size and phosphate concentration. *In vivo* high-energy phosphate concentration measures in healthy human leg and heart closely agree with published values.

Metabolic MRS cannot match the spatial resolution of MRI, and hence its value must lie in providing physiological information, rather than the morphology provided by images. To be useful, the physiological information obtained from MRS in normal or disease states, or response to interventions or stress protocols, must be quantitative. While metabolite ratios provide convenient measures in many applications, they can only indicate relative changes, and moreover do not allow an assessment of the total metabolite pools which are often compromised, if not depleted, in major diseases that include myocardial (6,7) or cerebral infarction, heart failure (4,5,8), and certain dementias (27). The application of MRS to these diseases requires determination of the absolute concentrations. The prime motivation for the current work is the study of compromised ATP energy supply in the failing heart that requires a measurement of [PCr] in order to determine the absolute flux through CK (4–6).

Unfortunately, transforming peak areas from localized MRS into absolute concentrations is much more difficult than measuring metabolite ratios, as the complexity of Eq. [1] will attest. Much effort was expended at achieving this at lower field strengths, with the presumption that the same methods would apply to the newer higher field systems. This was not the case with our water (^1H)-referenced ^{31}P method at 3T due to differences associated with RF field propagation and detection at the two frequencies (13). The water reference method intrinsically compensated for the variation in sensitivity across the coarsely-resolved 1D CSI voxel/slice (13), and its failure at 3T necessitated an alternative approach that could be accommodated with the rest of our cardiac protocol (11). This had not previously been achieved at 3T. In addition, although comparing metabolite signals with those of a concentration reference and correcting for relaxation, surface coil loading, and voxel-average sensitivity differences are quite standard (12–14), addressing the problem of quantifying metabolites in partial volumes of tissue that intersect coarsely-resolved voxels at varying locations of differing sensitivity is not. Replacing the concentration reference deployed in the present technique by injection of a reference synthetic electrical signal as proposed elsewhere (28), also does not account for spatial variations in coil sensitivity (both amplitude and phase), or partial volume effects within large CSI voxels.

The method described is specifically aimed at quantifying non- ^1H nuclei that have no endogenous concentration reference present in the *in vivo* spectrum, and which thus require an external reference. Quantifying metabolites in ^1H spectra *in vivo* is a simpler task in that the intense water peak in non-water suppressed spectra can serve as a concentration reference, as demonstrated by ^1H MRS measurements of muscle and myocardial creatine (29–31), and intracellular triglyceride (32,33) concentrations. While raw MoI-to-water

signal ratios have also been used (30,34,35), inter-laboratory comparisons of the results cannot be made unless differences in relaxation and/or acquisition protocols are accounted for. For absolute quantification, taking the ratio of MRS signals from creatine, choline, lipid (if the molecular weight is known) or other MoI, to that of unsuppressed water from the same voxel (29–31,36), can eliminate all of the sensitivity corrections and loading factors in Eq. [1] (Step A5; F_L and summation terms in Eq. [1]), assuming the tissue water content is constant across the MRS voxel. However, T_2 decay terms (e^{-TE/T_2}) for water and the MoI will need to be included in the numerator and denominator to account for T_2 decay if spin-echo sequences are used as is typical: $[P]_{ref}$ is replaced by the water proton concentration.

In conclusion, the present work demonstrates quantification of human cardiac ^{31}P metabolite concentrations at 3T by applying MRI-based sensitivity-weighting and segmentation to simultaneously address the effects of non-uniform sensitivity and partial tissue volumes in the MRS voxels or slices. The method is validated in phantoms of different sizes and at various concentrations where the accuracy was shown to be better than 10% on average. Metabolite concentrations measured in human skeletal muscle and heart are consistent with previously published work (Tables 1, 2). As noted in prior formulations (12,13), the complexity of Eq. [1], behooves the user to eliminate as many factors as possible to minimize cumulative errors, including those due to peak area fitting, spatial registration and relative coil loading (F_L). The scanner/coil stability and relative loading over time are important factors and may be checked by repeated phantom measurements. The present protocol used long TRs to minimize the saturation correction. Even so, the acquisition time of ~9 min renders it practical for high-energy phosphate metabolite concentration measurements in conjunction with functional cardiac MRI, and in our case, CK flux measurements at 3T (11). Although demonstrated with 1D CSI (1–3), the approach should be applicable to any method where spatial sensitivity varies significantly across the acquired voxel including ISIS (7,15,16), DRESS (17,18), etc, and where heteronuclear concentration referencing is impractical. It may also be potentially adaptable to water referenced ^1H MRS quantification, as noted above.

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Appendix A: List of abbreviations

1DCSI	One dimensional chemical shift imaging
^{31}P	Phosphorus
AHP	Adiabatic half passage
ATP	Adenosine tri-phosphate
B₁	RF excitation magnetic field
B₀	Static magnetic field
CK	Creatine-kinase
DPG	2,3-diphosphoglycerate
FFE	Fast Field Echo
FOV	Field of view
FSC	Frequency sweep cycling
HF	Heart failure
MI	Myocardial infarction
MRS	Magnetic Resonance Spectroscopy
NA	Number of averages
PCr	Phosphocreatine
rms	Root mean square
SD	Standard deviation
SNR	Signal to noise ratio

Figure 1.

Flow chart of the principal acquisitions (green shading), data analysis (pink shading) and calculations required to measure metabolite concentrations via the described MRS method. Alpha-numeric labels in blue correspond to the acquisition/analysis steps described in the “Methods” section.

Figure 2. Annotated screen photograph of the PC-based user analysis software as applied to the segmentation of cardiac MRIs (top right and below) and computing [PCr] and [ATP] from ^{31}P 1D CSI spectra (list, lower right).

Figure 3.

(a) Calculated concentration for a 5.7cm diameter cylindrical phantom filled with 5 (green filled circles), 10 (black crosses), 20 (red squares) and 30 (blue empty circles) mM of NaH₂PO₄, as a function of depth. (b) Mean±SD phosphate concentrations as a function of true concentration for the 3.7 cm ("small"; black squares), 5.7 cm ("medium"; red crosses) and 9.4 cm ("big"; blue circles) diameter cylinder phantoms. The hashed lines indicate actual concentrations in (a), and identity in (b).

Figure 4. [PCr] and [ATP] (mean $\pm$ SD) measured in the calf muscle of 8 volunteers, as a function of depth. The sample size (n) varies with depth due to different leg sizes.

Figure 5. Axial heart image showing 1DCSI planning (top left) and corresponding cardiac spectrum from slice 7. The spectrum is a 1D projection of the real component of the 3D plot fitted using the "circle fit" software routine without baseline correction (22).

Measured skeletal muscle ³¹P concentration values in μmol/g wet wt for this study compared to other independent work. Concentration values are reported as mean ± SD. Letters M and W are used to indicate Men and Women respectively.

Table 1

	[PCr]	[ATP]	n	MRS method	Muscle	Field
This study	24.7±3.4	5.7±1.3	8	1DCSI, External Ref, Adiabatic	Calf	3T
Ref. (25)	32.0±4.4	6.1 ±0.8	6	NL, Internal ¹ H Ref, Hard pulse	Forearm	1.9 T
Ref. (26)	22.0±1.2	5.7±0.4	M 9	ISIS, External Ref, Adiabatic pulse	Calf	1.5 T
	17.5±1	4.6±0.3	W 7			
Ref. (13)	25.6±4.1	5.6±1.6	---	1DCSI, Internal ¹ H Ref, Adiabatic pulse	Calf	1.5 T
Ref. (37)	23.1±0.7	5.5±0.1	---	Mean published values in 10 studies, External Ref	Calf	---

Measured cardiac ³¹P concentration values from healthy volunteers in μmol/g wet wt for this study and prior work.

Table 2

	[PCr]	[ATP]	n	Time	MRS method	Field
This study	10.4±1.5	6.0±1.1	18	9 min	1DCSL, External Ref, Adiabatic pulse	3 T
Ref. (6)	9.6±1.1	5.5±1.3	15	~10 min	1DCSL, ¹ H Ref, BIR4 pulse	1.5 T
Ref. (38)	8.7±2.6	4.6±1.0	30	30 min	3DCSL, SLOOP, ³¹ P Ref, Hard pulse	1.5 T
Ref. (5)	9.4±1.1	5.5±1.3	14	~10 min	1DCSL, ¹ H Ref, BIR4 pulse	1.5 T
Ref. (4)	10.1±1.3	5.7±1.3	16	~10 min	1DCSL, ¹ H Ref, BIR4 pulse	1.5 T
Ref. (8)	8.8±1.3	5.7±1.0	10	< 60 min	3DCSL, SLOOP, ³¹ P Ref, Hard pulse	1.5 T
Ref. (14)	9.0±1.2	5.3±1.2	7	Up to 40 min	3DCSL, SLOOP, ³¹ P Ref, Hard pulse	1.5 T
Ref. (39)	9.7±2.5	6.4±1.8	15	< 20 min	DRESS, ³¹ P Ref, Slice selective pulse	1.5 T
Ref. (13)	10.0±2.0	5.8±1.6	8	16 min	1DCSL, ¹ H Ref, BIR4 pulse	1.5 T
Ref. (7)	12.1±4.3	7.7 ±3.0	11	Up to 15 min	1DCSL, DRESS, ³¹ P Ref, Slice selective pulse	1.5 T
Ref. (12)	11.0±2.7	6.9±1.6	4	Up to 21 min	2DCSL, DRESS, ³¹ P Ref, Slice selective pulse	1.5 T