

³¹P cardiac magnetic resonance spectroscopy during leg exercise at 3 Tesla

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Abstract Investigation of phosphorus (³¹P) magnetic resonance spectroscopy under stress conditions provides a non-invasive tool to examine alterations in cardiac high-energy phosphate metabolism that may not be evident at rest. Our aim was to establish cardiac ³¹P MR spectroscopy during leg exercise at 3T. The increased field strength should provide a higher signal to noise ratio than at lower field strengths. Furthermore, relatively high temporal resolution at a sufficiently fine spatial resolution should be feasible. ³¹P MR spectra were obtained with a 3D acquisition weighted chemical shift imaging sequence in 20 healthy volunteers at rest, during

dynamic physiological leg exercise and after recovery at 3T. Haemodynamic measurements were made throughout and the rate pressure product calculated. With exercise, the mean heart rate increased by 73%, achieving a mean increase in rate pressure product of 115%. The corrected PCr/ATP ratio for subjects at rest was 2.02 ± 0.43 , exercise 2.14 ± 0.67 ($P = 0.54$ vs. rest) and at recovery 2.03 ± 0.52 ($P = 0.91$ vs. rest, $P = 0.62$ vs. exercise). A cardiac ³¹P MR spectroscopy physiological exercise-recovery protocol is feasible at 3T. There was no significant change in high-energy cardiac phosphate metabolite concentrations in healthy volunteers at rest, during physiological leg exercise or during recovery. When applied to patients with heart disease, this protocol should provide insights into physiological and pathological cardiac metabolism.

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Introduction

The use of phosphorus (³¹P) magnetic resonance spectroscopy (MRS) provides a unique non-invasive tool to investigate myocardial high-energy phosphate metabolism. Impaired resting energetics have been demonstrated in a number of disease states including diabetes, hypertension and cardiomyopathies [1–4]. Reduced levels of high-energy phosphate metabolites

have correlated with prognosis in disease states [5] and changes in ^{31}P MRS have been used to monitor metabolic responses to therapy and surgery in patients [1, 6–8].

In many situations, alterations in cardiac energetics will only be unmasked, or in some cases exacerbated, when the heart is stressed [9, 10], and the true extent of altered energetics and the functional role of high-energy phosphate metabolism can only be fully examined under stress conditions. The potential of ^{31}P -MR spectroscopy to assess cardiac metabolism during stress has previously been utilised in a small number of disease groups using different stress methods [10–13]. Firstly, using isometric exercise with a hand grip, several groups have demonstrated impaired cardiac energetics in selected patients with severe ischaemic heart disease [10, 12, 14]. This technique has also been applied to heart transplant patients and women with chest pain and no significant coronary stenoses [12, 15]. However, the increase of cardiac workload possible with this form of exercise is small.

The use of pharmacologically induced stress with dobutamine and atropine to achieve a target heart rate has been investigated in animal models, healthy volunteers, dilated cardiomyopathy patients and elite athletes [9, 13, 16, 17]. Although pharmacological stress is particularly practical in the MRI environment and has been found to provide a predictable response with a resulting substantial increase in cardiac workload, it is not necessarily a good reflection of physiological exercise and can occasionally have some potentially unpleasant side-effects [18].

To date, exercise ^{31}P MRS spectral acquisition at field strengths of <2 Tesla has been limited by a number of factors. These have included low spatial and temporal resolution requiring the achievement and maintenance of a modest level of exercise for long scan durations (up to 30 min) [11, 19, 20], uncomfortable repetitive hand grip exercise within a relatively confined space, motional artefacts and variable heart rates.

Higher magnetic field strengths (up to 4.1 Tesla) have already demonstrated higher signal to noise ratio (SNR) and this has been used successfully to image at higher spatial resolution [21–24]. Limited cardiac phosphorus spectroscopy exercise protocols with a single subject have also been demonstrated at field strengths above 2 Tesla [25].

In this work we have achieved exercise levels higher than those previously reported using non-pharmaceutical methods, whilst keeping spectral acquisition times short (less than 9 min). With the SNR advantages of cardiovascular magnetic resonance imaging at 3 Tesla, we have established a dynamic exercise model on a standard clinical scanner, which could have widespread applications in patients with heart disease.

Methods

Study population

Twenty healthy sedentary volunteers (10 male and 10 female, mean age 28 ± 3 years, mean height 175 ± 7 cm, mean weight 72 ± 11 kg, mean heart rate 59 ± 9 bpm and mean resting systolic blood pressure of 112 ± 9 and diastolic blood pressure of 67 ± 5 mmHg) with no history of cardiac disease, hypertension or cardiac risk factors and a normal baseline electrocardiogram (ECG) were recruited. Subjects with contraindications to CMR were not enrolled. The study was carried out according to the principles of the Declaration of Helsinki and was approved by our institutional ethics committee. Each subject gave informed written consent.

The ^{31}P MRS spectroscopy exercise protocol

All CMR scans were performed using a 3T MR system (Trio, Siemens Medical Solutions, Erlangen, Germany). All data were acquired with a commercially available 1.5T heart/liver $^{31}\text{P}/^1\text{H}$ coil (Siemens Medical Solutions) that was modified for use at 3T. This coil contained a large single loop for excitation at both phosphorus (^{31}P) and proton (^1H) frequencies and for detection of ^1H signals, and included a quadrature coil for detection of ^{31}P signals, which comprised a figure-of-eight shaped loop and a circular coil. Subjects were placed in the prone position with their chest on the centre of the ^{31}P coil. The subject's upper body was locked in position using Velcro straps and a blood pressure cuff (In vivo Instruments, Orlando, USA) attached to the right upper arm. A pulse oximeter (In vivo Instruments, Orlando, USA) was attached to the left index finger. To provide additional resistance to the exercise, non-magnetic weights (0.5 kg) were

applied to each ankle and an elastic cord was connected between the subject's ankles and the wall of the scanner room, as shown in Fig. 1.

After localisers, piloting was performed in the vertical long axis (VLA), horizontal long axis (HLA) and short-axis planes (FLASH images, slice thickness 10 mm, TR/TE 7/3.37 ms, FOV 400×340 mm). ^{31}P MRS data were acquired at baseline (rest), during exercise and on recovery from exercise. All spectra were acquired with a 3D acquisition weighted chemical shift imaging sequence (AW-CSI), TR/TE 1000/2.3 ms, Acquisition matrix size $8 \times 8 \times 12$ (interpolated to $8 \times 16 \times 16$), 10 averages at centre of k -space, FOV $240 \times 240 \times 200$ mm, voxel size 15 ml (the use of acquisition weighting means that, although the nominal resolution is 15 ml, the extent of the spectral pointspread function can cause that each spectrum to include signals from some 230 ml of tissue [24]), and a local flip angle in the interventricular septum of 37° (the Ernst angle for PCr). The CSI matrix was aligned using a mid-ventricular short-axis slice, positioning the voxels along the interventricular septum. To prevent confounding saturation issues with changing heart rates during exercise, the repetition time was fixed at 1.0 s, resulting in a total acquisition time of 8 min 29 s.

The target heart rate is a frequently used parameter in clinical cardiac stress investigations and is defined as a heart rate of 85% of maximum predicted heart rate. Target heart rate is calculated using



Fig. 1 The experimental set-up for prone dynamic exercise ^{31}P spectroscopy at 3 Tesla. Subjects were requested to perform alternate knee flexion with 0.5 kg leg weights attached to each ankle. Haemodynamic measurements of pulse, blood pressure, oxygen saturations were made continuously

$0.85 \times (220 - \text{age})$ for males and $0.85 \times (200 - \text{age})$ for females [26, 27]. In our study, we aimed for subjects to reach over 50% of their target heart rate in order to produce a clinically relevant physiological exercise protocol. The rate pressure product (RPP) was calculated using the product of the heart rate and systolic blood pressure, and this parameter provides a measure of the work performed by the heart [28]. The starting RPP was calculated during the baseline resting spectral acquisition. On completion of the resting ^{31}P -MRS, volunteers were asked to initiate exercise with repeated and alternate knee flexion, aiming to double the baseline RPP. When maintained at a steady level of exercise, reached after a mean of 3.2 ± 1.4 min, the exercise spectra were acquired. Haemodynamic measurements were taken and recorded every 90 s and the mean exercise RPP calculated. Subjects were encouraged to maintain a steady exercise level during the 8 min acquisition of spectra. At the end of the exercise spectral acquisition, the volunteers stopped exercising. Recovery time for normalization of the haemodynamic parameters to the starting level was noted (mean 3.3 ± 0.9 min).

Re-piloting using FLASH images in the VLA, HLA and short-axis views were performed and any subject movement noted. The recovery spectra were then planned and acquired in the same way as the resting and exercise spectra.

MR data processing

Spectral data were processed in the time domain using the AMARES algorithm contained in jMRUI [29] (fitting for the peaks of phosphocreatine (PCr), adenosine-triphosphate (ATP), phosphodiester (PDE) and 2,3-diphosphoglycerate (2,3-DPG)). A voxel was selected from the anterior septum by the consensus of two experienced observers using FLASH images in the standard way [24, 30]. No voxels were chosen or rejected on the basis of the spectrum from that voxel. The same voxel was then analyzed for the exercise and recovery spectra. If the volunteer had moved after the exercise, a new voxel was selected by consensus of two experienced observers using the new FLASH images. The intensities of PCr and γ -ATP were corrected for partial saturation [31] and blood contamination [1] and quoted as the ratio of their concentrations.

Statistics

All data are presented as mean \pm SD unless stated otherwise. For all comparisons, a paired student's *t*-test was used to compare PCr/ATP and subject haemodynamics at rest, during exercise and on recovery. Statistical significance was taken at $P < 0.05$.

Results

Cardiovascular magnetic resonance exercise scanning was well tolerated by all participants. All datasets were of sufficient quality to be analysed and included in the study. Haemodynamic parameters of all subjects at rest, during exercise and on recovery are shown in Table 1.

Physiological parameters

With prone leg exercise, the mean heart rate increased by 73% (range 34–125%, mean resting heart rate of 59 ± 9 bpm to mean exercise heart rate of 102 ± 9 bpm) and the systolic blood pressure increased by 18% (range 2–30%, mean resting systolic blood pressure 112 ± 9 mmHg to mean exercise systolic blood pressure 132 ± 13 mmHg). This led to a mean RPP during exercise of 215% that of the rest condition (an increase of 115%) (Table 1). Heart rate during exercise was $70 \pm 9\%$ (range 52–90%) of the calculated target heart rate for the healthy volunteers.

Cardiac high-energy phosphate metabolism during rest, stress and recovery

Typical examples of ^{31}P MR spectra at rest, during exercise and at recovery in a healthy male volunteer are shown in Fig. 2. With a RPP of 215%, there was no significant difference in the PCr/ATP ratio with exercise or on recovery in the healthy volunteers. The mean corrected PCr/ATP for the subjects at rest was 2.02 ± 0.43 , during exercise 2.14 ± 0.67 ($P = 0.54$ vs. rest) and at recovery 2.03 ± 0.52 ($P = 0.91$ vs. rest and $P = 0.62$ vs. exercise). Individual values for each subject at rest, during exercise and on recovery are shown in Fig. 3. Thus, myocardial PCr/ATP ratios did not change during exercise with an increase of the cardiac rate pressure product to 215%.

Discussion

This is the first report on cardiac ^{31}P -MRS during exercise at 3T. The shorter acquisition duration with physiological exercise has allowed us to exceed the previously reported RPP increases without the need for pharmacological intervention. We have developed a method using leg exercise in healthy volunteers at this increased field strength, enabling non-invasive measurement of myocardial high-energy phosphate metabolism during stress. This method was well tolerated by all subjects and resulted in a mean increase in RPP of 215% during the exercise protocol, a level considerably higher than that

Table 1 Mean haemodynamic parameters of 20 healthy volunteers at rest, during exercise and on recovery

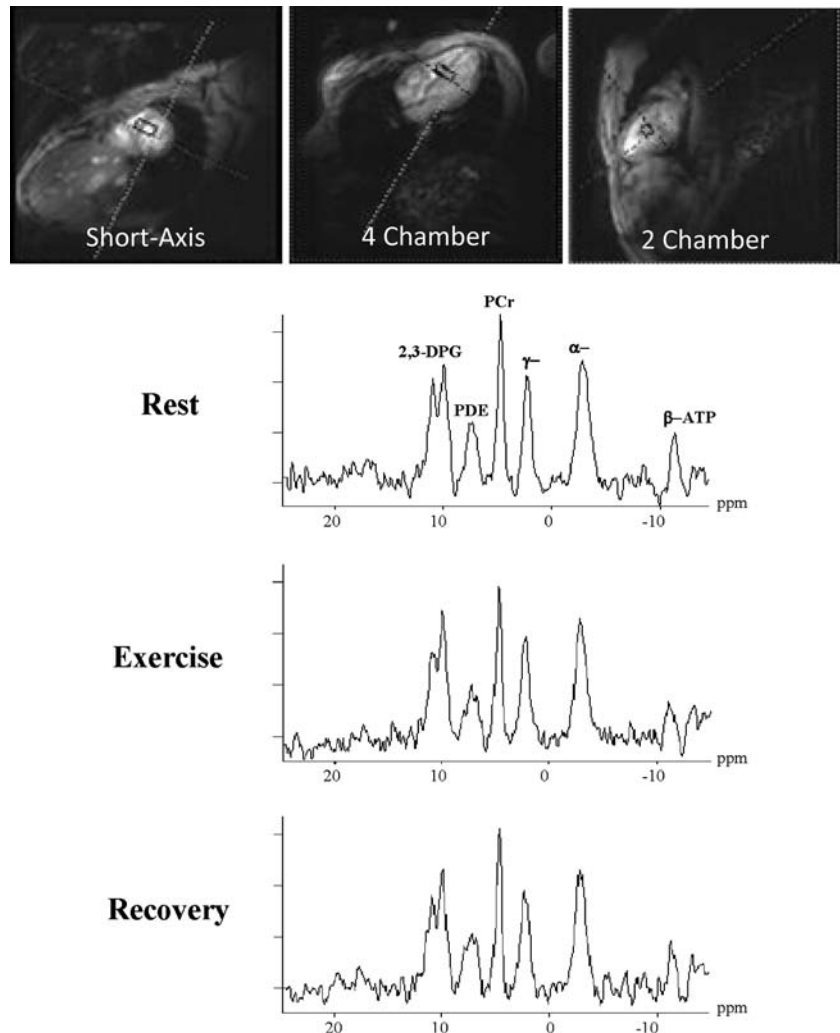
	Rest	Mean during exercise	Recovery
Heart rate (bpm)	59 ± 9 (47–79)	102 ± 9 (87–119)*	66 ± 10 (49–85)*.#
Systolic blood pressure (mmHg)	112 ± 9 (93–129)	132 ± 13 (113–157)*	108 ± 9 (91–126)#
Diastolic blood pressure (mmHg)	67 ± 5 (60–76)	77 ± 11 (50–92)*	65 ± 8 (51–88)#
Rate pressure product (mmHg/min)	$6,586 \pm 1,032$ (5,088–9,322)	$13,349 \pm 1,605$ (11,178–17,365)*	$7,099 \pm 1,135$ (5,390–9,559)*.#
Rate pressure product (RPP) (%)	100	215 ± 40 (136–310)	109 ± 14 (89–135)

Mean \pm SD (range)

* $P = <0.05$ vs. rest

$P = <0.05$ vs. exercise

Fig. 2 Anatomical location of the selected spectra are shown in the cardiac short-axis, four chamber and two chamber views along with typical cardiac ^{31}P spectra from a male healthy volunteer at rest, during exercise and on recovery



achieved with hand grip exercise and higher than previously reported for leg exercise in the magnet [20], but still below that achieved using pharmacological stress [16, 17]. Previous studies on this subject are summarized in Table 2 showing the changes in RPP and PCr/ATP ratio in control subjects.

The difference in the heart rate between rest and exercise affects the repetition time (TR) of ECG gated acquisitions and results in variable levels of saturation of PCr and ATP between acquisitions. This complicates the calculation of correction factors that are used to adjust the PCr/ATP concentration ratio for the different levels of saturation of PCr and ATP due to their different longitudinal relaxation times (T_1). In order to prevent this effect, we acquired our phosphorus spectra ungated, using a constant TR of 1,000 ms. The acquisition of ungated spectra will

inevitably increase the effects of cardiac motion, but controlling the effects of a variable TR was considered to be a more significant factor in the acquisition of quantifiable spectra. In preliminary studies, we compared gated and ungated acquisitions in resting subjects using these exact acquisition parameters found the spectra to show no appreciable differences (data not shown). Previous in vivo animal work comparing systolic and diastolic phosphorus metabolites also showed no differences between the different phases of the cardiac cycle [32].

At this level of exercise, there was no significant change in the PCr/ATP ratio at rest, during exercise and on recovery in healthy volunteers, demonstrating that the energy requirements of the myocardium can be met by upregulation of oxidative phosphorylation. Our data are in agreement with leg exercise cardiac

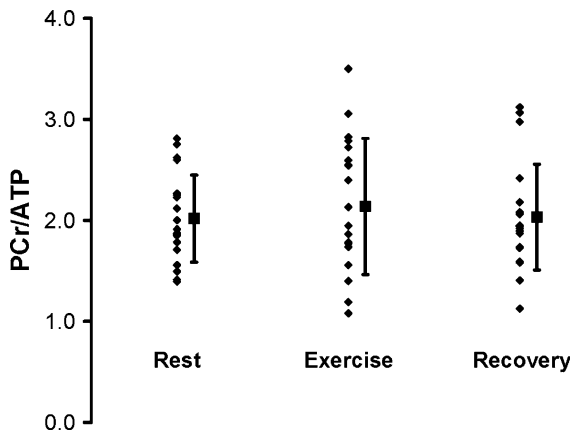


Fig. 3 The ratio of PCr/ATP in the 20 healthy volunteers at rest (2.02 ± 0.43), during exercise (2.14 ± 0.67) and on recovery (2.03 ± 0.52). There was no significant difference in the mean values at rest, during exercise or on recovery. The open diamonds represent individual data points and the closed squares indicate group means

MRS studies at 2T which showed no change in PCr/ATP in five healthy male volunteers during a similar increase in RPP to 170% [19]. However, subjects were required to maintain a significant exercise level for a prolonged period of time (over 30 min) whilst the cardiac spectra were obtained and hence this protocol could never be applied to patients. Lamb et al. have previously demonstrated a 14% reduction in PCr/ATP with high dose dobutamine/atropine in 20 healthy volunteers (18 male) with an extreme RPP increase to 310% [16]. This shows that at these extreme increases, reductions in PCr/ATP occur which are not apparent at more modest increases in workload.

The variability of our data, particularly during exercise, as shown by the standard deviation reflects a number of factors. These include the potential skeletal chest muscle contamination from movement during the exercise protocol. However, we re-piloted subjects using FLASH images and identified that 12 (60%) of subjects had not moved after the exercise and the same voxel was selected for all three spectral measurements. Movement during the exercise spectral acquisition with resultant skeletal muscle and liver contamination cannot be excluded, but with experimental real-time images during our exercise protocol, there were only small body movements. Although the average PCr/ATP values reported here are the highest of those reported in Table 2, they are in line with many previous studies by ourselves and

Table 2 Published effects of exercise on the PCr/ATP ratio in normal volunteers

Publication	Group (N)	Field strength	Source of stress	RPP% of baseline	PCr/ATP (rest)	PCr/ATP (stress)	Fractional change PCr/ATP
Conway et al. [20]	Control (5)	2T	Prone leg exercise	173%	1.56 ± 0.20	1.58 ± 0.14	1% NS
Weiss et al. [10]	Control (11)	1.5T	Hand grip exercise	133%	1.72 ± 0.15	1.74 ± 0.17	1% NS
Yabe et al. [14]	Control (11)	1.5T	Hand grip exercise	128%	1.85 ± 0.28	1.90 ± 0.23	3% NS
Kuno et al. [11]	Control (6)		Cycling exercise	+20% heart rate	1.51 ± 0.01	1.51 ± 0.04	0% NS
Kuno et al. [11]	Runners (6)		Cycling exercise	+13% heart rate	1.51 ± 0.03	1.51 ± 0.03	0% NS
Pluim et al. [13]	Control (12)	1.5T	Atropine-dobutamine	319%	1.42 ± 0.18	1.21 ± 0.20	-15% $P < 0.001$
Pluim et al. [13]	Elite cyclists (21)	1.5T	Atropine-dobutamine	398%	1.41 ± 0.20	1.16 ± 0.13	-18% $P < 0.001$
Buchthal et al. [12]	Women (12)	1.5T	Hand grip exercise	130% ^a	1.63 ^a	1.57	-3% NS
Lamb et al. [16]	Control (13)	1.5T	Atropine-dobutamine	300%	1.42 ± 0.18	1.22 ± 0.20	-14% $P < 0.01$
Evanochko et al. [15]	Control (11)	1.5T	Hand grip	124%	1.25 ± 0.30	1.27	1.50% NS
This study	Control (20)	3T	Prone leg exercise	215%	2.02 ± 0.43	2.14 ± 0.67	6% NS

^a Patient group RPP increase, single male subject for baseline PCr/ATP

many others and indicate the inherent variability in the measurement of this important biological parameter [23, 24].

Technical developments at 3T are still evolving, including the application of smaller voxels and the addition of saturation bands, which should further minimise contamination and help improve reproducibility. Despite these limitations, the standard deviation produced in this study would suggest that we had an 87% power for detecting a 20% difference in PCr/ATP ratios between rest and exercise in these subjects.

At the increased field strength of 3 Tesla, we can acquire spectra with a high SNR in 8 min 29 s, where previous studies at lower field strengths required considerably longer. This length of time will be much more suitable for the investigation of cardiac stress in patient groups who are only capable of maintaining a steady level of exercise for a limited period of time [11, 19]. We plan to apply this new protocol, which is a more appropriate reflection of exercise physiology than pharmacological stress, to patients with ischaemic heart disease, cardiomyopathies and diabetes. Ours is also the largest study of ^{31}P spectroscopy during physiological exercise in healthy volunteers, and the only one to include an equal representation of both males and females.

The increase in RPP with our dynamic prone leg exercise was predominantly mediated by the increase in heart rate, in keeping with the reduced total peripheral resistance in supine exercise physiology [33]. The healthy volunteers achieved and maintained a mean of 70% of target heart rate during the exercise acquisition, a level approaching the desired target heart rate for clinical stress studies.

Limitations

MR Spectroscopy during a dynamic stress protocol remains a noisy method with relatively high measurement variability, even with the improved SNR 3T affords. Thus, this method, as the current state-of-the-art, remains unsuitable for the reliable assessment of stress energetics in *individual* subjects or patients. The method is, however, well suited for the investigation of statistical differences in the energetic response of the myocardium to stress between groups of patients or volunteers. As such, this method should have a number of interesting research applications.

Conclusion

We report on the first application of cardiac ^{31}P MRS at 3T and have developed a novel dynamic physiological exercise protocol at 3 Tesla. This protocol allowed rapid, non-invasive measurement of high-energy phosphate metabolism in the myocardium in response to substantial changes in cardiac workload. We have shown that there was no significant difference in high-energy phosphate concentration ratios in healthy volunteers at rest, during exercise and on recovery. This protocol can now be applied to patients with heart disease and should provide new insights into physiological and pathological cardiac metabolism during exercise.

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