Heart failure/cardiomyopathy

Exacerbation of cardiac energetic impairment during exercise in hypertrophic cardiomyopathy: a potential mechanism for diastolic dysfunction

Sairia Dass1, Lowri E. Cochlin2, Joseph J. Suttie1, Cameron J. Holloway1, Oliver J. Rider1, Leah Carden1, Damian J. Tyler2, Theodoros D. Karamitsos1, Kieran Clarke2, Stefan Neubauer1†, and Hugh Watkins1*†

1Division of Cardiovascular Medicine, Anatomy and Genetics, Oxford University, Oxford, UK; and 2Department of Physiology, Anatomy and Genetics, Oxford University, Oxford, UK

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Aims

Hypertrophic cardiomyopathy (HCM) is the commonest cause of sudden cardiac death in the young, with an excess of exercise-related deaths. The HCM sarcomere mutations increase the energy cost of contraction and impaired resting cardiac energetics has been documented by measurement of phosphocreatine/ATP (PCr/ATP) using 31Phosphorus MR Spectroscopy (31P MRS). We hypothesized that cardiac energetics are further impaired acutely during exercise in HCM and that this would have important functional consequences.

Methods and results

31P MRS was performed in 35 HCM patients and 20 age- and gender-matched normal volunteers at rest and during leg exercise with 2.5 kg ankle weights. Peak left-ventricular filling rates (PFRs) and myocardial perfusion reserve (MPRI) were calculated during adenosine stress. Resting PCr/ATP was significantly reduced in HCM (HCM: 1.71 ± 0.35, normal 2.14 ± 0.35; P < 0.0001). During exercise, there was a further reduction in PCr/ATP in HCM (1.56 ± 0.29, P = 0.02 compared with rest) but not in normals (2.16 ± 0.26, P = 0.98 compared with rest). There was no correlation between PCr/ATP reduction and cardiac mass, wall thickness, MPRI, or late-gadolinium enhancement. PFR and PCr/ATP were significantly correlated at rest (r = 0.48, P = 0.02) and stress (r = 0.53, P = 0.01).

Conclusion

During exercise, the pre-existing energetic deficit in HCM is further exacerbated independent of hypertrophy, perfusion reserve, or degree of fibrosis. This is in keeping with the change at the myofilament level. We offer a potential explanation for exercise-related diastolic dysfunction in HCM.

Keywords

Hypertrophic Cardiomyopathy • 31P MRS • Exercise

Introduction

Hypertrophic cardiomyopathy (HCM) affects 1 in 500 individuals and is the commonest cause of sudden cardiac death in the young, including competitive athletes.1,2 Despite being a common genetic cardiomyopathy, determining disease progression and risk stratification remains a clinical dilemma. With the availability of therapeutic measures that prevent sudden death (i.e. implantable cardioverter defibrillators), the identification of high-risk patients is now of even greater importance. Abnormal myocardial energy utilization has been implicated in playing a crucial role in the pathophysiology of HCM.1,3,4

Original predictions based on data from in vitro studies of recombinant proteins, supported by evidence from animal models and patients, including HCM phenocopies (i.e. an HCM-like phenotype)5–7 strongly support the energy depletion hypothesis as a unifying mechanism causing disease in HCM.

Cardiac 31P MRS is the only technique that allows non-invasive measurement of cardiac high-energy phosphate metabolism in vivo. Impaired energetics (decreased PCr/ATP-ratios) have been
documented in genetically modified animal models of HCM; in both symptomatic and asymptomatic patients with clinical features of HCM, and in genotyped HCM patients without overt hypertrophy (mutation carriers). In support of a potentially reversible abnormality in energy metabolism in HCM, Abozguia et al. demonstrated that the metabolic modulator, perhexiline, improves cardiac energetics and diastolic dysfunction in HCM.

While all previous studies of cardiac energetics in HCM have been conducted at rest, an energetic deficit would be expected to worsen, or only become unmasked, when cardiac workload is increased, e.g. during exercise. Furthermore, it is conceivable that the high incidence of exercise-related death in HCM may be explained by a possible further acute impairment of myocardial energetic resulting in ion-pump dysfunction, calcium overload, and ventricular arrhythmias. However, cardiac energetics during exercise conditions have not been previously examined in patients with HCM. The measurement of 31P MRS during exercise is technically challenging due to increased susceptibility for contamination from surrounding structures coupled with an acceptable scan time needed to acquire sufficient data while exercising. We developed a 31P MRS acquisition which was short enough to facilitate scanning during exercise and which effectively addresses issues of contamination and signal distortion. Using this, we were able to test whether cardiac energetics are further impaired acutely during exercise in HCM, and evaluate the impact on cardiac function.

**Methods**

**Ethics and study population**

The study was approved by the institutional ethics committee and informed written consent was obtained from subjects. Thirty-five patients with HCM were recruited from the University of Oxford Cardiomyopathy clinic. The diagnosis of HCM was based on genetic determination of a pathogenic mutation. All HCM subjects underwent a 13 gene screen (MYBPC3: myosin binding protein C; MYH7: myosin heavy chain; TNNI3: cardiac troponin I; TNNT2: cardiac troponin T; MYL2: regulatory myosin light chain; MYL3: essential myosin light chain; TPM1: alpha tropomyosin; ACTC1: cardiac actin; CSRP3: MLP; PKA2G: AMPK gamma 2; PLN: phospholamban; GLA: alpha galactosidase; FH-L1: four and a half LIM domains 1). There were 18 MYBPC3 and eight MYH7 subjects. In the absence of an identified mutation (nine subjects), HCM was defined as the presence of asymmetrical septal left-ventricular hypertrophy (LVH, ≥12 mm) not originating from other causes and family history of HCM. Patients had a Bruce protocol exercise test and were excluded if there was coronary artery disease or more than 20 mmHg systolic pressure drop with exercise. Patients were also excluded if there was resting left-ventricular outflow tract gradient >30 mmHg.

Normal controls were of similar age and gender, non-smokers, had no history of cardiac disease or family history of cardiomyopathy or sudden death and had a normal 12-lead ECG. Enrolled subjects had no contraindications for MR scanning.

**CMR protocol**

All CMR examinations were performed on a Siemens 3T Trio MR system (Siemens Healthcare Erlangen, Germany). Cardiac volumes, ejection fraction, and peak filling rates (PFRs) were calculated based on a series of single breath-hold balanced SSFP cine images as previously described. Figure 1 describes the scan protocol. 31P MRS was acquired using acquisition-weighted chemical shift imaging (AW-CSI), with 10 averages in the centre of K-space. Subjects lay prone, with the left ventricle at magnet iso-centre. A 16 × 8 × 8 CSI grid with a 240 × 240 × 200 mm field of view was centred in the mid-ventricular septum on the first short-axis image showing the papillary muscles. In order to minimize contamination from surrounding tissue, three saturation bands, each 25 mm thick, were placed over chest wall muscle and liver (Figure 2). Magnetic resonance spectroscopy data were acquired with 720 ms repetition time, using an optimized radiofrequency pulse and ultrashort echo time. Each acquisition took 8 min to acquire.

MRS measurements were acquired while subjects were resting and again during alternating flexion of both legs with 2.5 kg weights attached at the ankles. Subjects exercised for the duration of the spectral acquisition. A short-axis image was taken before and after exercise to check for any significant chest-wall displacement during exercise (Figure 2). Exercise was performed at a rate sufficient to raise the rate–pressure-product (RPP) by at least 25%.

Perfusion imaging was performed every heartbeat during the first pass of the contrast bolus with the use of a T1-weighted gradient echo sequence with saturation-recovery magnetization preparation. Stress perfusion and stress PFR data were acquired using adenosine vasodilator stress rather than leg exercise as these sequences were more susceptible to respiratory variations and motion artefacts. Adenosine was used as the...
pharmacological vasodilator and was administered at a rate of 140 μg/kg per minute. In order to calculate stress PFR, the short-axis stack was repeated after 3–4 min of intravenous adenosine. For measurement of perfusion reserve, the mid-ventricular short axis was chosen to match the slice used for voxel placement for 31P MRS and 0.03 mmol/kg of Gadolinium was injected with stress and the same dose of Gadolinium was repeated for rest acquisition. Heart rate and blood pressure were measured before, at 2 min intervals during and after adenosine stress.

For late-gadolinium enhancement CMR, a top-up bolus of 0.06 mmol/kg of Gadodiamide followed by a 15 mL saline flush was administered. After a 5 min delay, ECG-gated images were acquired as previously described.18

Image analysis
Using commercially available software (Argus version VA60C, Siemens Healthcare, Erlangen, Germany), LV volumes, ejection fraction, and LV mass were as previously described.18 End-diastolic wall thickness was determined using an in-house software MC-ROI (programmed in IDL v.6.1, www.itvis.com).

Peak filling rate was used as an index of diastolic function.19,20 The endocardial borders of the LV short-axis images were manually contoured from base to apex and across the cardiac cycle end-diastole to end-diastole to yield a volume–time curve. This was done with SA stacks acquired at both stress and rest. From the volume–time curves, a first-order derivative (dV/dT) is determined, and the relative change in volume of the ventricle per second is plotted from end systole giving a filling rate curve in diastole.

As a result, PFR and the time to PFR (TFR) can be derived, both of which are global indices of diastolic function.19,20 Using a similar method, on a group basis, we have shown significant changes in diastolic filling rates in the obese group pre- and post-weight loss.22 Similar methods were also used by Abozguia et al.12 to measure PFR in a HCM population pre- and post-metabolic modulation with perhexiline.

Only data sets where a clear PFR was observed were included in the analysis. Due to the combination of the variable heart rate, prospective acquisition and temporal resolution (60.3 ms), not all of the diastolic filling curves could be included in the final analysis (final data set: HCM n = 12; normal n = 11).

Analysis of spectra was performed in Java Magnetic Resonance User Interface23 and fitted using the AMARES algorithm (Advanced Method of Accurate, Robust, and Efficient Spectroscopic fitting24). Spectral peak areas were corrected for nuclear overhauser effect25 and for radiofrequency saturation.25 The resulting peak areas of the three ATP signals were averaged and corrected for blood contamination.26 For perfusion analysis, signal-intensity curves were generated in order to measure myocardial perfusion reserve index (MPRI) as previously described18 (QMass software, version 6.2.3, Medis, Leiden, The Netherlands).

Late-gadolinium enhanced (LGE) images were quantified in MC-ROI. Hyperenhanced pixels were defined as those with signal intensities >2 standard deviations (SD) above the mean signal intensity in remote normal myocardium in the same slice.27 The extent of fibrosis was expressed as the affected LGE volume fraction of the mid-ventricular septum segment, area corresponding to voxel for measurement of PCr/ATP.

Statistical analysis
All data are expressed as mean ± standard deviation and checked for normality using Kolmogorov–Smirnov test. Comparisons between the groups were performed using Student t-test; paired t-tests were used to compare values during rest and exercise. Bivariate correlations were performed using Pearson’s correlation coefficient. Statistical tests were two-tailed, and a P-value of <0.05 was considered to indicate a statistically significant difference. Statistical analysis was performed with commercially available software packages (IBM SPSS Statistics, version 19.0 and MedCalc version 12).

An a priori sample size calculation was performed to detect a 12% drop in PCr/ATP ratio in the HCM cohort during stress. Based on pilot data (PCr/ATP rest 1.73 ± 0.40, stress 1.53 ± 0.50) assuming one-tailed paired t-test analysis (α = 0.05 and β = 0.8), calculations suggested that 17 HCM participants would be needed. A second a priori sample size calculation was also performed to detect a 10% difference in PCr/ATP ratio in HCM when compared with normal. Assuming two-tailed independent t-test analysis (α = 0.05 and β = 0.8) pilot data (PCr/ATP HCM 1.80 ± 0.33, normal populations 2.22 ± 0.28) suggested that 10 HCM and 10 normal subjects would be needed to detect a 19% difference in PCr/ATP ratio at rest. Due to excellent recruitment, these targets were achieved in our study.

Results

Study population and haemodynamic measurements
Subject characteristics are described in Table 1. There were no differences in age and gender between HCM and normals. The HCM
cohort showed significantly increased LV mass, increased ejection fraction and reduced end-diastolic and end-systolic volumes compared with normal subjects. Overall the HCM group was considered low risk according to clinical risk stratification (Table 2). In the HCM cohort, nine individuals did not have significant hypertrophy but were gene carriers; two individuals had predominantly apical hypertrophy; and the remaining 24 had asymmetrical septal hypertrophy.

With exercise the rises in RPP were similar (HCM 72 ± 35%, normal 72 ± 26%, \(P = 0.90\)). During adenosine stress there was also an equivalent rise in RPP (HCM 72 ± 23%, normal 71 ± 22%, \(P = 0.99\)). The rise in RPPs achieved with exercise and adenosine stress were comparable in both groups (HCM \(P = 0.96\), normal \(P = 0.85\)), Table 3.

### Cardiac energetics at rest and during exercise

Resting PCr/ATP was significantly reduced in HCM (1.71 ± 0.35) compared with normal controls (2.14 ± 0.35, \(P < 0.001\)). During exercise, there was a further reduction in PCr/ATP in HCM (1.56 ± 0.29, \(P = 0.02\)) but no change in normal controls (2.16 ± 0.26, \(P = 0.98\), Figure 3). Furthermore, the percentage change of PCr/ATP during exercise in HCM (\(-8 \pm 17\%\)) was significantly different (\(P = 0.03\)) from the change in normal controls (+1 ± 13%). There was no significant movement of the chest wall with exercise in the studied population hence all rest and exercise 31P MRS data were presented. Figure 4 shows examples of acquired spectra.

### Cardiac energetics and hypertrophy, perfusion, patchy fibrosis

In HCM, there was no correlation between rest PCr/ATP and measures of hypertrophy such as LV mass index (\(R = 0.01, P = 0.95\)) or LV mass normalized to end-diastolic volume (\(R = 0.21, P = 0.22\)). The change in energetics with exercise also did not correlate with left-ventricular mass index (\(R = 0.02, P = 0.91\)) or mass normalized to end-diastolic volume (\(R = 0.20, P = 0.25\)). In HCM, the average wall thickness at the site of voxel placement for PCr/ATP measurement was 17.3 ± 5.9 (range 7.8–28.8). There was no correlation between wall thickness and resting PCr/ATP and in the studied population hence all rest and exercise 31P MRS data were presented. Figure 4 shows examples of acquired spectra.

### Cardiac energetics and diastolic function

There were significant reductions in PFR in HCM both at rest (HCM 572 ± 175 mL/ms; normal 745 ± 135 mL/ms, \(P = 0.01\)) and during adenosine stress (HCM 648 ± 187 mL/ms, normal 845 ± 157 mL/ms, \(P = 0.02\)). PFR and PCr/ATP correlated significantly (\(R = 0.48, P = 0.02\), Figure 5).

### Discussion

The main finding of the present study was that cardiac energetics show further impairment during exercise in HCM but not in normal volunteers. This impairment correlates with diastolic dysfunction and is not influenced by degree of hypertrophy, perfusion reserve, or patchy fibrosis. To our knowledge, this is the first report of cardiac energetics during exercise in HCM.
Rest and exercise energetics in normal controls

ATP is used for almost all energy consuming reactions in the heart, including contraction of cardiac myofilaments (70%) and for active ion-pump function and calcium re-uptake via sarco/endoplasmic reticulum Ca2+ ATPase (SERCA). Myocardial ATP levels are kept relatively constant over a wide range of cardiac loads in the normal heart, buffered by the transfer of energy from PCr. As the equilibrium constant of the creatine kinase reaction favours the synthesis of ATP over PCr by a factor of \( \approx 100 \), in disease states with inefficient energy production or energy utilization, the ATP levels would initially remain constant at the expense of PCr; the ratio of PCr/ATP is therefore a sensitive indicator of the underlying energetic state of the heart. Our data support previous reports for normal volunteers where there was no change in PCr/ATP ratios during physiological exercise either via hand grip or leg movement. In normal controls, small reductions in PCr/ATP only occur during maximal pharmacological stress using high-dose dobutamine and atropine, which lead to rises in RPP of >300%.

Rest and exercise energetics in HCM

Depressed resting PCr/ATP in HCM, and the further reduction during moderate exercise observed in the HCM cohort only, indicate an impaired resting metabolic profile and an inability to adapt to the increased energy demand of exercise.
Impairment of resting energy profile in HCM as measured by PCr/ATP is a consistent finding in the literature and has been demonstrated in asymptomatic patients with clinical features of HCM and even in mutation carriers without hypertrophy. Hence, in HCM, an imbalance must exist between energy demand and supply at rest. Our results indicate that this imbalance is further exacerbated during exercise.

In principle, the possible mechanisms which may underlie the further decreased cardiac energetics during stress in HCM include an increased energy demand coupled with decreased energy supply. The primary sarcomeric mutations in HCM increase the energy cost of contraction. This has been found to be a unifying feature of the diverse myofilament mutations, first documented in biochemical and biophysical analyses but later confirmed in ex vivo studies, including from human myectomy samples. Hence increased energy demand at rest and during exercise in HCM may be explained by the intrinsic energy wastage caused by the sarcomeric mutations.

Decreased supply of substrate and oxygen may result from microvascular dysfunction, a recognized feature of HCM. However, the lack of correlation in this study between MPRI and PCr/ATP change with exercise would suggest that impaired perfusion does not significantly contribute to the observed energetic abnormalities. Ineffective oxidative phosphorylation will also affect energy supply and mitochondrial morphological disorganization with impaired function has been demonstrated in HCM. Defects in PCr formation and transfer are yet another factor potentially affecting energy supply in HCM. However, further studies are needed to demonstrate the relative contribution of these defects in energy supply during stress in HCM.

Cardiac energetics, mass, and patchy fibrosis

Our findings showed that resting and exercise energetic profiles were not related to cardiac mass or wall thickness in HCM. This is concordant with published data which showed similarly reduced resting PCr/ATP ratios in HCM patients with and without hypertrophy.

Patchy fibrosis as assessed by late-gadolinium burden has also been thought to be related to energetics. Esposito et al. observed a correlation between PCr/ATP and fibrosis in HCM. This report also demonstrates a weak negative correlation of LGE burden (in the myocardium matched to the area where PCr/ATP was measured) and resting PCr/ATP. This may arise because both the presence of LGE and reduction in PCr/ATP reflect disease severity rather than by a causal relationship. Importantly, we also demonstrate that LGE is not related to the change in energetics with exercise. Therefore, the presence of LGE, per se, should not affect changes of PCr/ATP with exercise.

In aggregate, the lack of association of energetic impairment with cardiac morphology, or with secondary tissue changes in the myocardium, is in keeping with this being an intrinsic property of the mutant myofilaments themselves.

Cardiac energetics and diastolic function

As PCr levels are reduced and fall further acutely during exercise in HCM, free ADP levels must also increase acutely. Elevated ADP levels reduce the free energy change of ATP hydrolysis. As a result, less free energy is available to drive ATP. SERCA function is...
particularly sensitive to reductions in free energy release as it has one of the highest minimum energy requirements.46  

Studies have shown that a limited exercise capacity is common in patients with HCM and is related to the development of diastolic dysfunction during peak exercise.47,48 Our finding of a strong correlation between PFR and PCr/ATP at rest and more so during exercise supports the role of abnormal cardiac energetics as a key factor in the diastolic dysfunction in HCM. It is tempting to speculate that further impairment of SERCA function during more strenuous exercise could potentially cause Ca2+ overload with subsequent VT and VF. This may be a possible explanation for the relatively high incidence of sudden cardiac death during exercise in HCM. Studies are needed to further clarify this potential mechanism.

Limitations
Exercising in the magnet bore to a degree that is comfortable and does not produce chest wall movement significantly limits the achievable intensity of physical stress. We report modest increases in RPP during exercise. Additionally, as both calcium channel blockers and beta blockers are expected to lower energy consumption and 22 of the 35 HCM patients were on these medications, it is possible that the measured change in PCr/ATP with exercise was blunted due to medication effect. It is reasonable to speculate that the change with exercise might have been more pronounced if these medications had been discontinued prior to testing.

We were unable to determine PFRs for the entire population. Although assessment of diastolic function with CMR has significant advantages, allowing reproducible imaging irrespective of body habitus and degree of chest wall fat, the temporal resolution is significantly slower than echo Doppler techniques.

Myocardial perfusion reserve and PFR were measured during adenosine stress as these sequences are particularly susceptible to movement artefacts. However, physiological exercise was used for measurement of exercise PCr/ATP in order to minimise the length of the adenosine infusion. None the less, the RPP increase with both forms of stress was comparable.

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References