Journal of the American College of Cardiology
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Published by Elsevier Inc.

Vol. 41, No. 10, 2003
ISSN 0735-1097/03/$30.00
doi:10.1016/S0735-1097(02)03009-7

Hypertrophic Cardiomyopathy Due to Sarcomeric Gene Mutations Is Characterized by Impaired Energy Metabolism Irrespective of the Degree of Hypertrophy

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OBJECTIVES We investigated cardiac energetics in subjects with mutations in three different familial hypertrophic cardiomyopathy (HCM) disease genes, some of whom were nonpenetrant carriers without hypertrophy, using phosphorus-31 magnetic resonance spectroscopy.

BACKGROUND Familial hypertrophic cardiomyopathy is caused by mutations in sarcomeric protein genes. The mechanism by which these mutant proteins cause disease is uncertain. A defect of myocyte contractility had been proposed, but in vitro studies of force generation have subsequently shown opposing results in different classes of mutation. An alternative hypothesis of "energy compromise" resulting from inefficient utilization of adenosine triphosphate (ATP) has been suggested, but in vivo data in humans with genotyped HCM are lacking.

METHODS The cardiac phosphocreatine (PCr) to ATP ratio was determined at rest in 31 patients harboring mutations in the genes for either beta-myosin heavy chain, cardiac troponin T, or myosin-binding protein C, and in 24 controls. Transthoracic echocardiography was used to measure left ventricular (LV) dimensions and maximal wall thickness.

RESULTS The PCr/ATP was reduced in the HCM subjects by 30% relative to controls (1.70 ± 0.43 vs. 2.44 ± 0.30; $p < 0.001$), and the reduction was of a similar magnitude in all three disease-gene groups. The PCr/ATP was equally reduced in subjects with ($n = 24$) and without ($n = 7$) LV hypertrophy.

CONCLUSIONS Our data provide evidence of a bioenergetic deficit in genotype-confirmed HCM, which is present to a similar degree in three disease-gene groups. The presence of energetic abnormalities, even in those without hypertrophy, supports a proposed link between altered cardiac energetics and development of the disease phenotype. (J Am Coll Cardiol 2003;41: 1776–82) © 2003 by the American College of Cardiology Foundation

Familial hypertrophic cardiomyopathy (HCM) is now known to be a heritable form of cardiac hypertrophy caused by mutations in genes encoding sarcomeric proteins. Affecting 1/500 of the population, HCM is the most common identified cause of sudden death in young people (1). More than 100 mutations have been described, predominantly in the genes for beta-myosin heavy chain (β-MHC) (2), cardiac troponin T (cTnT) (3), and myosin-binding protein C (MyBPC) (4). Features of HCM due to mutations in these genes are well described (5,6), but penetrance is variable and there remain subjects who appear to have a mild phenotype but suffer lethal complications (7).

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The lack of a generally accepted disease-modifying treatment in HCM highlights the importance of finding the pathogenetic mechanisms by which HCM mutations cause disease. Given the qualitatively similar clinical phenotypes despite different genetic etiologies, a common mode of pathogenesis is believed to exist. An abnormality of contractility had been proposed as a possible unifying mechanism (3); however, no consistent changes in contractile properties are shared by the heretofore described mutant proteins (8). Therefore, alternative hypotheses are sought.

One feature that the diverse mutations do appear to have in common is a potential for inefficient utilization of adenosine triphosphate (ATP), resulting in an increase in the energetic cost of force production (8,9). Hence, "energy compromise" has been proposed as a possible stimulus for the development of cardiac hypertrophy in HCM, potentially mediated through failure to maintain normal calcium

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Manuscript received February 21, 2002; revised manuscript received September 25, 2002, accepted October 31, 2002.

Abbreviations and Acronyms

ADP	= adenosine diphosphate
AMP	= adenosine monophosphate
ATP	= adenosine triphosphate
β-MHC	= beta-myosin heavy chain
cTnT	= cardiac troponin T
1D-CSI	= one-dimensional chemical shift-imaging sequence
HCM	= familial hypertrophic cardiomyopathy
LVH	= left ventricular hypertrophy
MWT	= maximal wall thickness
MyBPC	= myosin-binding protein C
PCr	= phosphocreatine
^{31}P-MRS	= phosphorus-31 magnetic resonance spectroscopy
TMHA	= temperature-modulated heteroduplex analysis
TTE	= transthoracic echocardiogram/echocardiography

reuptake (8,9). Energetic abnormalities have been shown in the αMHC403/+ murine model of HCM, in which the calculated free energy of ATP hydrolysis was at a level sufficient to compromise SERCA2a activity (10). A number of phenocopies of HCM have recently been defined at a molecular level and have been shown to be syndromes associated with abnormalities of mitochondrial oxidative phosphorylation (for example, Friedreich's ataxia [11] and MELAS syndrome [12]).

To explore in more detail the potential role of cardiac energetics in the pathogenesis of HCM, we used phosphorus-31 magnetic resonance spectroscopy (^{31}P-MRS) to study cardiac energy metabolism in individuals from families in whom a definitive pathogenic mutation had been identified, thus allowing assessment of individuals with subclinical, as well as established, disease. Cardiac ^{31}P-MRS is a noninvasive technique that allows the in vivo determination of the phosphocreatine (PCr) to ATP ratio (PCr/ATP), which is an indicator of the energetic state of cardiac muscle (13). We recruited subjects harboring mutations in the β-MHC, cTnT, and MyBPC genes to determine whether any observed bioenergetic defects were common to different classes of sarcomeric mutation associated with differing clinical (3,7,14,15) and biophysical (8) characteristics.

We provide evidence that a bioenergetic deficit exists in HCM that is of a similar magnitude in subjects harboring different sarcomeric protein gene mutations and that is present in gene mutation carriers without hypertrophy.

METHODS AND SUBJECTS

Subjects. Thirty-one individuals with HCM from 17 families attending specialist clinics participated in the study. Patients with a pacemaker or implantable defibrillator were excluded. Familial hypertrophic cardiomyopathy was confirmed by genotyping in all subjects, with mutations in the β-MHC (16 patients), cTnT (8 patients), or MyBPC gene (7 patients). Subjects underwent transthoracic echocardiogram (TTE) and a ^{31}P-MRS heart study. Twenty-four age- and gender-matched healthy volunteers on no cardiac treatment (13 males; mean age 40.2 years; range 9 to 73 years) were studied by ^{31}P-MRS. All subjects gave informed consent, and the study was approved by the Central Oxford Research Ethics Committee.

Cardiac ^{31}P-MRS. SPECTRAL ACQUISITION. Patients were studied in a 2-T magnet (Oxford Magnet Technology, Oxford, United Kingdom), which was interfaced to a Bruker Avance spectrometer (Bruker Medical GmbH, Ettlingen, Germany) using a protocol previously described (16). In brief, patients lay prone and were positioned with the heart at the isocenter of the magnet. This was confirmed using standard multislice spin echo proton imaging. Spectra were acquired with an 8-cm diameter phosphorus surface coil using a slice selective, one-dimensional chemical shift-imaging (1D-CSI) sequence including spatial presaturation of lateral skeletal muscle (17). An 8-cm-thick transverse slice was excited followed by one-dimensional phase encoding into the chest to subdivide the signal into 64 coronal layers each of 1-cm thickness (relaxation time = heart rate, 16 averages). All proton and phosphorus data acquisitions were cardiac-gated using a pulse oximeter probe placed on the subject's finger.

DATA PROCESSING. The proton images were used to determine those spectra in the cardiac 1D-CSI data set that arose from the myocardium. After Fourier transformation, the cardiac rows were extracted for analysis, and rows with a similar morphology were added. Spectra were fitted using a purpose-designed interactive frequency domain fitting program (16). After fitting, the ATP signal was corrected for blood contamination, based on the amplitude of the 2,3-diphosphoglycerate signal (18), and the PCr/ATP was calculated. The calculated PCr/ATP was corrected for magnetic saturation effects using previously determined saturation-correction factors (16).

TTE. The TTE was performed using standard equipment and stored on videotape or optical disk for later analysis. Left ventricular dimensions were obtained using M-mode and maximal wall thickness (MWT) recorded from the parasternal long- and short-axis views.

Deoxyribonucleic acid analysis. Mutation detection was performed by screening of polymerase chain reaction-amplified exons of β-MHC, cTnT, and MyBPC genes as previously described (19). In brief, amplifications were performed with "touchdown" polymerase chain reaction using high-fidelity polymerases, and mutation screening was undertaken using temperature-modulated heteroduplex analysis (TMHA) on an automated high performance liquid chromatography instrument (Transgenomic, San Jose, California). Mobile-phase gradients and melting temperatures for TMHA of each amplimer were calculated using the Wavemaker software package. Exons with an abnormal TMHA profile were sequenced using an ABI377 (Applied Biosystems) and

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Table 1. Clinical and Genetic Characteristics of HCM Subjects

Subject	Age/Gender	Therapy	FH of PSD	Disease-Gene (mutation)	Clinical Status
1	42/M	HDBB	Y	β-MHC missense mutation	A
2	12/F	HDBB	N	β-MHC missense mutation	A
3	52/F	—	Y	β-MHC missense mutation	S
4	37/F	HDBB	Y	β-MHC missense mutation	A
5	30/F	HDBB	N	β-MHC missense mutation	S
6	60/M	Amiodarone	N	β-MHC missense mutation	S
7	26/M	—	N	β-MHC missense mutation	A
8	41/F	HDBB	N	β-MHC missense mutation	S
9	51/F	HDBB	N	β-MHC missense mutation	A
10	16/M	HDBB	N	β-MHC missense mutation	A
11	20/F	—	N	β-MHC missense mutation	S
12	72/M	Amiodarone	N	β-MHC missense mutation	S
13	44/M	—	N	β-MHC missense mutation	A
14	37/F	HDBB	N	β-MHC missense mutation	S
15	45/M	HDBB	N	β-MHC missense mutation	S
16	36/M	—	Y	β-MHC missense mutation	A
17	27/M	Amiodarone	Y	cTnT codon deletion	A
18	58/M	—	Y	cTnT codon deletion	A
19	44/F	—	Y	cTnT truncation	S
20	23/F	—	N	cTnT truncation	A
21	46/M	Amiodarone	Y	cTnT missense mutation	A
22	39/M	—	Y	cTnT missense mutation	A
23	29/M	—	Y	cTnT missense mutation	A
24	36/F	—	Y	cTnT missense mutation	A
25	54/F	—	N	MyBPC missense mutation	S
26	62/F	Amiodarone	N	MyBPC missense mutation	S
27	13/F	HDBB	N	MyBPC missense mutation	A
28	58/M	—	N	MyBPC truncation	S
29	42/M	Amiodarone	N	MyBPC truncation	S
30	38/M	—	N	MyBPC truncation	S
31	69/F	—	N	MyBPC truncation	S
Mean	41				
SD	15.7				

A = asymptomatic; β-MHC = beta-myosin heavy chain; cTnT = cardiac troponin T; FH = family history; HCM = familial hypertrophic cardiomyopathy; HDBB = high-dose beta-blocker therapy; MyBPC = myosin-binding protein C; N = no; PSD = premature sudden death in a first-degree relative; S = symptomatic; Y = yes.

compared with published genomic sequences. Mutations were confirmed, and subsequently typed in family members and 100 normal controls, by restriction enzyme digest (where necessary using a modified primer to introduce a new restriction site).

Statistical analysis. Data are presented as mean ± SD. Nonparametric statistical methods (Mann-Whitney and Kruskal-Wallis tests) were used to compare subject and control groups. Spearman log-rank test was used to explore potential correlations of scale variables. A p value of <0.05 was considered statistically significant. Although the subjects were clustered within 17 families, we considered that our results would be principally driven by individual subject variation and, therefore, we did not perform a nested analysis.

RESULTS

The clinical features are summarized in Table 1 and the electrocardiographic (ECG) and echocardiographic features in Table 2. All patients had been established on their respective treatments for >3 months before the cardiac ^{31}P-MRS study (10 on high-dose beta-blocker therapy and 7 on amiodarone). Sixteen patients were asymptomatic and were diagnosed through family screening. No patient had any other significant medical history besides HCM. Twelve patients had a family history of an HCM-related death in a first-degree relative; most of these deaths had been sudden and premature. In most cases (n = 29) the area of MWT was in the septum/anterior wall of the left ventricle; in no case was the hypertrophy predominantly apical. Two patients (subjects 1 and 11) had a residual left ventricular outflow tract gradient despite treatment at the time of the study. All patients had a shortening fraction of >0.25. Seven patients did not have sufficient left ventricle hypertrophy (LVH) to confirm a clinical diagnosis of HCM (defined as an MWT of ≥1.3 cm) (20). Five of these subjects were asymptomatic and lacked diagnostic ECG changes (20) and were included in the study because they were known to be carriers of an abnormal gene.

^{31}P-MRS data. The PCr/ATP data are summarized in Figure 1. The mean PCr/ATP for all the HCM patients was significantly lower than in the controls (1.70 ± 0.43 vs.

Table 2. Echocardiographic and Electrocardiographic Characteristics of HCM Subjects

Subject	Age	ECG	ESD	EDD	IVSd	PWd	MWT	FS
1	42	ND	1.81	3.77	2.10	1.26	2.30	0.52
2	12	Normal	1.81	3.69	0.84	0.92	1.01	0.51
3	52	D	1.94	3.30	2.18	1.66	2.36	0.41
4	37	ND	2.14	4.20	1.16	1.01	1.22	0.49
5	30	Normal	1.81	3.79	1.29	1.14	1.52	0.52
6	60	D	2.40	4.70	2.10	1.00	2.10	0.49
7	26	Normal	2.69	4.62	1.30	1.14	1.44	0.42
8	41	D	1.97	3.93	1.53	1.18	1.60	0.50
9	51	D	3.00	4.39	2.26	1.20	2.26	0.32
10	16	D	2.70	5.20	1.90	0.88	2.29	0.48
11	20	D	2.40	4.20	2.40	1.50	2.40	0.43
12	72	Normal	2.30	4.50	2.00	0.80	2.00	0.49
13	44	Normal	2.90	4.90	1.90	0.70	1.90	0.41
14	37	D	1.70	3.62	1.25	0.98	1.31	0.57
15	45	D	2.62	4.44	1.81	1.16	1.90	0.57
16	36	ND	3.20	5.10	1.30	0.70	1.30	0.37
17	27	D	3.24	5.01	1.60	1.18	1.90	0.35
18	58	D	3.29	4.62	2.08	1.49	2.10	0.29
19	44	Normal	2.93	4.75	0.98	0.76	1.32	0.38
20	23	ND	2.67	4.21	0.66	0.73	0.73	0.37
21	46	D	3.34	5.71	2.30	1.13	2.37	0.42
22	39	D	2.19	3.77	1.12	1.07	1.70	0.42
23	29	ND	3.11	5.01	0.91	0.85	1.09	0.38
24	36	D	2.23	3.99	1.02	1.06	1.13	0.44
25	54	D	3.00	4.70	1.20	0.90	1.20	0.36
26	62	ND	1.56	2.66	2.63	1.73	2.82	0.41
27	13	ND	2.58	4.00	0.91	0.94	1.23	0.36
28	58	ND	2.61	5.09	2.54	1.46	2.54	0.49
29	42	Normal	2.86	4.53	1.76	1.08	1.76	0.37
30	38	ND	2.43	4.41	1.84	0.97	1.97	0.45
31	69	ND	2.49	4.92	1.30	0.96	1.52	0.49
Mean	41		2.51	4.38	1.62	1.08	1.75	0.43
SD	15.7		0.51	0.64	0.56	0.27	0.53	0.07

ECG = electrocardiogram; D = diagnostic of familial hypertrophic cardiomyopathy (20); ND = abnormal but not diagnostic of familial hypertrophic cardiomyopathy (20); EDD = end-diastolic diameter; ESD = end-systolic diameter; FS = fractional shortening; HCM = familial hypertrophic cardiomyopathy; IVSd = interventricular septal thickness in diastole; MWT = maximal wall thickness; PWd = posterior wall thickness in diastole.

2.44 ± 0.30; $p < 0.001$); this difference was preserved in the asymptomatic subjects (1.71 ± 0.47 vs. 2.44 ± 0.30; $p = <0.001$) and in those without LVH (1.57 ± 0.60 vs. 2.44 ± 0.30; $p < 0.001$). The groups of subjects carrying the β-MHC, cTnT, or MyBPC mutations were not significantly different from one another (β-MHC: 1.80 ± 0.34; cTnT: 1.54 ± 0.50; MyBPC: 1.66 ± 0.54; $p = 0.4$). No correlation existed between PCr/ATP and MWT in the entire cohort (Fig. 2) or within disease-gene groups, nor were there any associations with drug therapy or family history of premature sudden death.

DISCUSSION

In this study we have shown that a striking bioenergetic deficit exists in HCM that appears to be of similar magnitude in three groups of patients from families harboring mutations in different sarcomeric protein genes. The severity of the deficit did not correlate with the degree of LVH; indeed, it was as marked in the carriers without hypertrophy. Our data provide in vivo evidence that an abnormality of cardiac energetics may be an early feature in HCM pathogenesis. These findings support the hypothesis that "energy compromise" could be a common mechanism by which mutations in sarcomeric proteins may cause HCM.

Previous studies demonstrating ^{31}P-MRS abnormalities in HCM have only addressed the phenotype of established hypertrophy in clinically diagnosed individuals (21). Our findings provide new insights into the potential role of altered cardiac energetics in HCM pathogenesis because they are based on genotyped subjects. Thus, we were able to demonstrate energetic abnormalities in confirmed carriers with normal echocardiographic findings who, without genetic confirmation, would not have been known to be affected. Demonstration of resting energetic abnormalities in the absence of measurable hypertrophy suggests that such changes may result initially from altered sarcomeric function rather than only being secondary to cardiac hypertrophy.

The time-course of the relationship between alterations in PCr/ATP and the development of cardiac hypertrophy is unclear, particularly in humans, because studies have previ-

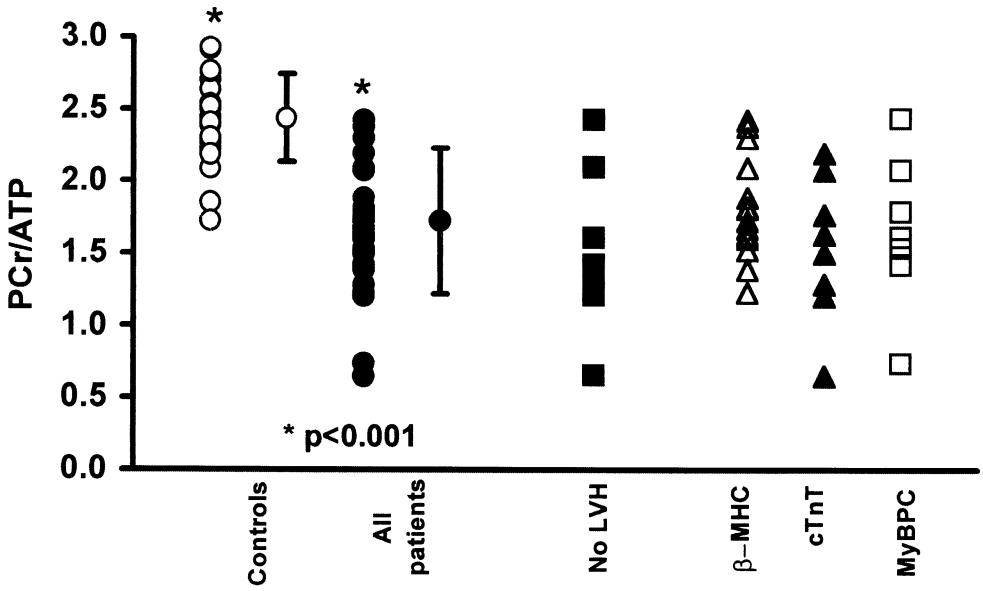

Figure 1. Phosphocreatine/adenosine triphosphate (PCr/ATP) data for all familial hypertrophic cardiomyopathy (HCM) subjects and controls, HCM subjects without left ventricular hypertrophy (LVH), and subjects in each disease-gene group. **Filled circles** = HCM patients; **open circles** = controls; **filled squares** = subjects without LVH; **open triangles** = beta-myosin heavy chain (β-MHC); **filled triangles** = cardiac troponin T (cTnT); **open squares** = myosin-binding protein C (MyBPC). $p < 0.001$ for all HCM patients vs. controls, HCM subjects without LVH vs. controls, and for each disease-gene group vs. controls; p = not significant between each disease-gene group.

ously been done in patients with an established disease process (21,22). More recent work in subjects with other genetically determined cardiomyopathies suggests that alterations in energetics may be a very early as well as a late feature of pathologic cardiac hypertrophy (23).

Phosphocreatine is an important metabolite in the biochemical pathways that supply ATP for muscle contraction; PCr can rapidly phosphorylate adenosine diphosphate (ADP) to ATP through the creatine kinase reaction, which is believed to be at equilibrium. Thus, PCr buffers the concentration of ATP during sudden demands in energy requirements, regulates ADP (a controller of oxidative metabolism), and may also have a role in transferring high-energy phosphates from the mitochondria to the myofibrils (the creatine kinase shuttle) (24). Increased ATP turnover or inefficient communication between mitochondria and sarcomere may result in higher ADP. This would be associated with a fall in PCr/ATP through the creatine kinase equilibrium.

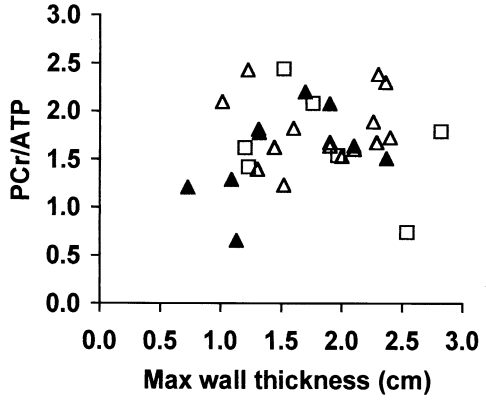

Figure 2. Scatterplot showing lack of correlation between max wall thickness and phosphocreatine/adenosine triphosphate (PCr/ATP). **Open triangles** = beta-myosin heavy chain; **filled triangles** = cardiac troponin T; **open squares** = myosin-binding protein C.

The extent to which the decline in PCr/ATP could also reflect loss of the total creatine pool is unknown. In other cardiomyopathies, for example, Xp-21 muscular dystrophy, the declines in PCr are observed and are attributed to loss of creatine across damaged cell membranes (16). A drop in total creatine in HCM was demonstrated in explanted hearts from HCM subjects with end-stage heart failure (25). However, because of ethical difficulties in obtaining cardiac tissue from subjects with preclinical HCM, no data are available on subjects who are in the early stages of their disease, in whom cell membrane damage would not be expected. Although the PCr/ATP should be interpreted in the context of the total creatine pool, it is noteworthy that total creatine was unchanged in a rat pressure-overload model of cardiac hypertrophy with a similar alteration in PCr/ATP (26). Although reductions in creatine have been found in other models of heart failure, the percentage of creatine that was phosphorylated was unchanged (27). A reduction in the total adenine pool was also found in the same study, although the reduction was much less marked than the reduction in creatine, suggesting therefore that the observed changes in PCr/ATP are principally due to alterations in PCr. Future studies with proton MRS and absolute quantitation of phosphate-containing metabolites will help to address these questions.

We suggest that the underlying problem in HCM due to

sarcomeric mutations is that inefficient utilization of ATP increases the cost of force production, putting excess demands on the myocyte. Most functional studies have shown that β-MHC mutations diminish actin-activated adenosine triphosphatase activity and velocity of actin filament translocation (8,28); because mutant heads are arrayed in series with normal heads they produce internal drag and could reduce efficiency and force production. A more recent study (29) has suggested increased sliding speed and unchanged force generation; such an effect would also increase the energy cost of force production. In vitro studies of the cTnT mutants have demonstrated increased unloaded shortening velocity and diminished maximum force (9), suggesting a shortened power-stroke and so less power generated for each ATP used. The HCM mutations in many of the contractile proteins increase calcium sensitivity, which would be expected to produce a "hypercontractile" phenotype with increased use of ATP; direct experimental evidence of this has recently been obtained for cTnT mutations (30). Thus, energy wastage through inefficient chemomechanical transduction would lead to increased turnover of ATP in certain subcellular compartments, particularly during periods of stress.

Recent data have shown that in oxidative muscle cells mitochondria may be associated into functional units with the sarcomeres (31). This subcellular organization is thought to allow the efficient coupling of energy production to energy utilization. Ultrastructural consequences of mutations in the sarcomere may therefore result in an impaired energy provision in addition to the increased energy demand of the contractile proteins. The global abnormalities of high-energy phosphate stores revealed by the reduced PCr/ATP even at rest are therefore all the more striking in that they might be expected to underestimate the functionally important abnormality in the sarcomeric compartment.

We do not believe that the findings of reduced PCr/ATP can be explained by regional ischemia. Although myocardial ischemia is a potent cause of reduced PCr/ATP, perfusion abnormalities would not be expected at rest in our patient cohort. Only four patients complained of episodes of exertional chest pain (subjects 15, 18, 30, and 31), and in each subject these were infrequent and not sufficient to interfere with daily life. No patients were known to have flow-limiting coronary disease. Nevertheless, it is clear that an abnormality of resting high-energy phosphate metabolism would be exacerbated during stress, especially in the presence of either large- or small-vessel disease.

Genetic support for the hypothesis that alterations in myocardial energetics have a role in HCM pathogenesis may have come from the recent description of mutations in the gamma$_2$ subunit of the adenosine monophosphate (AMP)-dependent protein kinase in families with HCM and Wolff-Parkinson-White syndrome (32–34). This enzyme has been described as the "cellular fuel gauge," and once activated by a rise in AMP/ATP it alters enzyme activities in ATP producing and consuming pathways to maintain essential homeostatic systems (35). The finding of a "nonsarcomeric" disease gene for HCM argues further against an abnormality of force generation as the underlying etiology.

In conclusion, we have demonstrated a bioenergetic deficit in HCM that is of a similar magnitude in patients harboring mutations in three different sarcomeric protein genes and that appears to anticipate development of hypertrophy. This study provides in vivo evidence that supports the hypothesis that an abnormality of cardiac energy metabolism may be the common mechanism by which HCM mutations cause disease.

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