31P NMR Spectroscopy Detects Metabolic Abnormalities in Asymptomatic Patients With Hypertrophic Cardiomyopathy

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Background—Hypertrophic cardiomyopathy (HCM) often causes sudden, unexpected death in adolescents and young adults. Alterations in myocardial metabolism are considered to be causes for contractile dysfunction. We examined the question of whether metabolic abnormalities antedate the manifestation of symptoms in patients with HCM.

Methods and Results—Proton-decoupled 31P NMR spectroscopy of the anterior left ventricular wall of the heart of 14 young, asymptomatic patients with HCM was performed with a 1.5-T whole-body imager. Spectra of the phosphate metabolites were compared with those of normal control subjects. The patients exhibited a significantly reduced ($P<0.02$) ratio of phosphocreatine (PCr) to ATP of 1.98±0.37 (mean±SD), compared with 2.46±0.53 obtained in 11 normal control subjects. In addition, the group of patients with severe hypertrophy of the interventricular septum ($n=8$) showed a significantly increased ($P<0.05$) P$_1$-to-PCr ratio, with a P$_1$×100/PCr of 20.0±8.3 versus 9.7±7.2 in control subjects. Both abnormalities are similar to those found in ischemic myocardium. This view is also supported by a significantly increased ($P<0.01$) phosphomonoester (PME)-to-PCr ratio, with a PME×100/PCr of 20.7±6.7 in control subjects, indicating altered glucose metabolism.

Conclusions—31P NMR spectroscopy detects alterations of myocardial metabolism in asymptomatic patients with HCM. These alterations may contribute to the understanding of the pathophysiology and natural history of the disease. (Circulation. 1998;97:2536-2542.)

Key Words: cardiomyopathy ■ ischemia ■ metabolism

Hypertrophic cardiomyopathy is a primary myocardial disorder characterized by localized hypertrophy of the IVS and the left ventricle that occurs in the absence of aortic stenosis, systemic hypertension, or other obvious causes. Rarely, symmetrical hypertrophy occurs in HCM, involving the right ventricle as well. The myocardium shows zones of disarrayed myocytes and myofibrils with interstitial fibrosis in both hypertrophied and nonhypertrophied regions.

In ≈50% of the patients, a genetically heterogeneous disease is responsible for the defects. Irrespective of the cause, the following abnormalities have been described: abnormal calcium fluxes, abnormal sympathetic stimulation due to increased responsiveness to catecholamines, thickened intramural coronary arteries (occurring in >80% of the patients), abnormal microcirculation leading to increased diastolic stiffness and subendocardial ischemia, and structural abnormalities manifested as cell hypertrophy and disarray. HCM patients, especially adolescents and young adults, have a high risk of sudden death. This risk cannot always be related to the existence of a left ventricular outflow tract obstruction. Myocardial ischemia and altered myocardial metabolism cause contractile dysfunction and ventricular arrhythmias.

Research has focused on symptomatic patients with HCM. Myocardial perfusion abnormalities were detected by positron emission tomography or thallium scintigraphy. A more recent study described heterogeneity in regional myocardial glucose uptake and function, but no impairment in blood flow. Because we know little about how myocardial phosphate metabolites are affected in asymptomatic patients with HCM, we performed the following studies using 31P in vivo NMR spectroscopy. This technique has been used previously to study various heart diseases in humans by determining the myocardial PCr/ATP ratio and, in a few cases, the P$_1$/PCr ratio also. We used these parameters and the PME/PCr ratio as indicators of altered myocardial metabolism. Our results suggest a shift from fatty acid to glucose metabolism, which may be a consequence of metabolic adaptation to hypertrophy and/or chronic ischemia.

Methods

Patients and Control Subjects

We examined 14 young, asymptomatic HCM patients and 11 young, normal control subjects (see Table 1). The protocol was approved by
the institutional Committee for the Protection of Human Subjects, and all subjects gave informed consent.

Five of the patients came to our attention because of their positive family history, 12 because of heart murmurs, and 1 because of signs of left ventricular hypertrophy on the ECG. The diagnosis was confirmed by echocardiography in all patients. Cardiac catheterization was performed in 6. A left ventricular outflow tract obstruction was found in 3 of the 14 patients (see Table 1). All patients were asymptomatic. Holter monitoring for 24 hours revealed no evidence of significant arrhythmias. The blood chemistry was normal in all patients. Five patients were treated with verapamil. Because the characteristic changes of HCM can be found in most cases in the IVS and because in all of our patients the IVS was hypertrophied, the MEIST was used as a criterion for the extent of hypertrophy. MEIST was determined from ECG-gated MRI from images in the long-axis view. The IVS thickness was determined from the end-diastolic image in the thickest region of the IVS. We divided the patients into 2 groups. The group called HCM I (n=6) had a MEIST of <250% and the group called HCM II (n=8) of >250% compared with matched healthy control subjects. Mean MEIST values were 159% for the HCM I group and 387% for the HCM II group, resulting in average end-diastolic septum thicknesses of 14 and 33 mm, respectively.

In control subjects, MEIST was determined from the NMR images in the anteroseptal region, leading to a mean value for MEIST of 111% and a mean septum thickness of 10 mm. The control subjects had normal echocardiograms, and none of them had a known medical disorder.

### In Vivo NMR Spectroscopy
Examinations were carried out on a Magnetom SP 63 Helicon whole-body imager (Siemens) operating at 1.5 T with 31P and 1H. Examinations were carried out on a Magnetom SP 63 Helicon whole-body imager (Siemens) operating at 1.5 T with 31P and 1H. In Vivo NMR Spectroscopy

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LVOTO indicates left ventricular outflow tract obstruction. Mean values±SD are given. See text for details.

Unpaired two-tailed Student’s t test resulted in *P<0.02 vs Control; †P<5×10⁻⁵ vs HCM II; ‡P<10⁻⁷ vs Control; and §P<5×10⁻⁴ vs Control.

The blood contamination in the spectra is a result of the size and shape of the volume element that was necessary to create a satisfying signal-to-noise ratio, but the blood contributions to ATP and Pi, could be corrected by using its 2,3-DPG signals. In 2 examinations of HCM II patients, the volume element could be completely positioned within the myocardium, leading to undetectable 2,3-DPG signals in the spectra (see Figures 1 and 2). In addition to the signals from the myocardium, the spectra also include signals from blood in the left ventricular chamber (see Figure 1): the 2,3-DPG signals and a part of the PDE signals. Furthermore, a small amount of Pi, and a somewhat larger amount of ATP also originate from blood.

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For the evaluation of the signals from the chosen volume element, the time-domain fitting routine VARPRO (VARiable PRojection) was used with gaussian model functions (see Appendix). The metabolite integral ratios of PCr/ATP and Pi/PCr obtained were then corrected for blood contamination with a blood 2,3-DPG/ATP ratio of 3 and a blood 2,3-DPG/Pi, ratio of 15. The correction for saturation

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### Selected Abbreviations and Acronyms
- 2,3-DPG = 2,3-diphosphoglycerate
- FFA = free fatty acid
- HCM = hypertrophic cardiomyopathy
- HCM I = group of patients with HCM and MEIST<250%
- HCM II = group of patients with HCM and MEIST>250%
- IVS = interventricular septum
- MEIST = maximum end-diastolic IVS thickness
- NOE = nuclear Overhauser effect
- PCr = phosphocreatine
- PDE = phosphodiester
- PME = phosphomonoester

### Figure 1. Typical localized 31P NMR spectra of a 25-year-old normal male (left) and a 23-year-old female HCM II patient with a severely hypertrophied myocardium with a septum thickness of 43 mm (right), scaled for equal PCr peak height. The spectrum of the patient showed almost no 2,3-DPG signal of blood and clear signals of Pi, and PME. In the control spectrum, the Pi signal is smaller and a possible PME signal is hidden by the pronounced 2,3-DPG signal. Data processing: the time-domain signal was multiplied with a gaussian function (t1/2=150 ms), zero-filled to 4096 data points, Fourier-transformed, and phase-corrected.
was performed with the average reported longitudinal relaxation times (T1) as given by Bottomley and Ouwerkerk with PCr 4.37 seconds, Pi 4.30 seconds, and g-, a-, and b-ATP 2.52, 2.26, and 2.28 seconds. In addition, the enhancement due to the NOE had to be taken into account. The average enhancements found for our examination protocol were PCr, 61%; Pi, 50%; and g-, a-, and b-ATP, 39%, 34%, and 40%. In addition, pH was calculated according to Petroff et al from the chemical shift of Pi (δPi).

Because of the lack of a suitable model function, the PME signal could not be analyzed with the fitting routine. Therefore, in this case we performed additional data processing by subtracting the fitted time-domain signals of PCr, ATP, and 2,3-DPG from the measured signal (see Figure 2). From the resulting spectra, the peak area of PME was determined with a peak integration routine (see Appendix). Average spectra obtained for each group by adding up all individual spectra scaled to equal PCr were analyzed in the same way. These average spectra (see Figure 3) reflect the mean value of each group for PME, Pi, and PDE. The PME integrals are given in Table 2. T1 and NOE corrections for the PME/PCr ratios were not possible because neither T1 nor the NOE enhancement of PME in human heart is known. Correction for blood contamination was not necessary for PME/PCr (see “Results”).

Statistical Analysis
Statistical evaluation was carried out with the unpaired 2-tailed Student’s t test and the Wilcoxon rank order test (U test). Correlations were analyzed by linear regression. Error probabilities of P<0.05 were considered significant. For the significant correlations, an additional check was carried out by discarding the data point with the maximum values. It was required that the remaining points still resulted in a significant correlation. All data are presented as mean±SD, unless otherwise indicated.

Results
PCr/ATP Ratio, Pi/PCr Ratio, and pH
Figure 1 shows spectra of a 25-year-old normal man (control, left) and a 23-year-old woman with HCM II (patient, right). The septum thickness was 43 mm, and MEIST was 478% in the patient. The spectrum on the right shows almost no 2,3-DPG signal of blood and clear signals of Pi, PME, and PDE. In the control spectrum, the Pi signal was smaller, and a possible PME signal was hidden by the pronounced 2,3-DPG signal. Collective data obtained from fit results of the spectra are given in Table 2 with and without correction for blood contamination, saturation, and NOE enhancement. Compared with control subjects, the spectra of HCM II patients showed a significant (100%) increase in the Pi/PCr ratio and a significant (20%) decrease in the PCr/ATP ratio. The group of all HCM patients also revealed a significant (20%) decrease in the PCr/ATP ratio. No significant difference in the PCr/ATP ratio was obtained between HCM I and either control or HCM II. However, the Pi/PCr ratio in HCM I was significantly smaller than in HCM II. The pH was identical in all groups.

PME/PCr Ratio
Figure 2 shows sections of the individual spectra of all 8 HCM II patients scaled for equal PCr integral. The left column represents the measured spectra; the middle column the Fourier transform of the time-domain fit results for 2,3-DPG, PCr, and ATP; and the right column the difference between the left and the middle columns. Only the part of the spectrum with positive ppm values was plotted to focus on the PME and Pi signals. The Pi, and PME signals were visible in all spectra. The blood contamination decreases from top to bottom and in the 2 bottom spectra was too small for a successful fit of the 2,3-DPG signals. The PME signal observed in all spectra showed no visible dependence on the amount of blood contamination. The 2 bottom spectra showed no consistent difference between the left and the middle columns. Only the part of the spectrum with positive ppm values was plotted to focus on the PME and Pi signals. The Pi, and PME signals were visible in all spectra. The blood contamination decreases from top to bottom and in the 2 bottom spectra was too small for a successful fit of the 2,3-DPG signals. The PME signal observed in all spectra showed no visible dependence on the amount of blood contamination.
HCM II patients showed significant increases of 150% and 100% compared with control subjects and HCM I patients, respectively (Table 2). Even if the 2 HCM II spectra in which the 2,3-DPG signals were too small for a successful fit were discarded, significant differences were still obtained.

Figure 3 shows sections of the average spectra of the 3 groups HCM II, HCM I, and Control scaled for equal PCr integral and with eliminated 2,3-DPG, PCr, and ATP signals. The PME signal decreased progressively from HCM II to HCM I and to Control. The largest Pi signal was visible in the HCM II average spectrum, whereas HCM I and Control exhibited similar Pi signals, which were both smaller. The right signal of the 2 PDE signals observed was predominantly due to blood contamination and was largest in the control group. Thus, although it exhibited the largest amount of blood contamination, the Control group also showed the smallest PME signal, again suggesting that the PME signal must originate from the myocardium. The differences between the HCM II and control groups could be more easily derived from the difference spectrum in the bottom line of Figure 3, which demonstrated the existence of greater PME and Pi signals and a smaller PDE signal from blood in the HCM II group.

The area under the curve of each average spectrum is given in Table 2. All areas of the average spectra agreed very well with the mean values of the areas of the individual spectra. The PME/PCr ratio was threefold greater in the HCM II group than in the control group.

The difference spectrum of the 2 average spectra HCM II and Control [Δ(HCM II−Control)] showed a $\sqrt{2}$ times greater noise level but a superior baseline compared with the 3 group spectra because baseline distortions were subtracted. The flat baseline allowed a quantitative evaluation of the Pi signal in the difference spectrum and revealed a clearly greater Pi, in HCM II, thus confirming the fit results of the individual spectra.

**Extent of Hypertrophy**

Figure 4 shows the correlations of the corrected Pi/PCr ratio with the corrected PCr/ATP ratio (top left) and with the MEIST (top right) and the correlation between the uncorrected PME/PCr and MEIST (bottom). The correlations with

![Figure 4](image-url)
MEIST showed that P/PCr increased and PCr/ATP decreased with the extent of hypertrophy.

Discussion

The results can be summarized as follows. First, in vivo $^{31}$P NMR spectroscopy yielded well-resolved spectra with a good signal-to-noise ratio from small volume elements. Second, we found a significantly decreased PCr/ATP ratio and significantly increased P/PCr and PME/PCr ratios in the myocardium of asymptomatic patients with advanced HCM (HCM II, see Table 2).

Our results on PCr/ATP and P/PCr agree well with findings from de Roos et al., who also found increased P/PCr and decreased PCr/ATP in HCM patients. However, the authors’ patient group consisted of both symptomatic and asymptomatic patients. Only 3 of their patients had a markedly low PCr/ATP ratio, 2 of whom exhibited symptoms of heart failure.

Our findings provide the first evidence that alterations in the myocardial phosphate metabolism are also present in the myocardium of asymptomatic HCM patients. According to Hochachka et al., the 3 most likely causes for such metabolic abnormalities are (1) accelerated work of the heart under resting whole-body conditions, (2) oxygen limitation to cell metabolism severe enough to invoke significant anaerobic contribution to ATP turnover rates, and (3) altered carbon and energy sources fueling the cardiac “engine.” These possible causes will now be discussed in more detail.

1. The large muscle mass itself and the contractile state with increased myocardial shortening and diastolic dysfunction have to be regarded as major determinants of the observed metabolic changes. This will be aggravated even further by the hyperactive action that arises from increased sensitivity to catecholamines. In addition to these changes of the contractile status, the metabolic effects corresponding to the accelerated hormone drive also have to be considered, especially increased rates of glycogenolysis and lipolysis. In this context, it is interesting to note that we found a significant increase in the PME/PCr ratio whereby PME is known to contain glycolytic intermediates such as glucose-6-phosphate and ω-glycerolphosphate, and to a lesser degree, AMP.

2. Energy demand and supply pathways are tightly coupled, and even large changes in the energy expenditure will not lead to gross alterations of the concentrations of PCr and ATP. In normal human heart, the myocardial PCr/ATP ratio does not change with exercise. Thus, in addition to an increased energy demand of the HCM heart, a limited supply of oxygen and substrates in asymptomatic HCM has in fact been suggested by Camici et al., who used pharmacological techniques to estimate the coronary flow reserve. This assumption is supported by O’Gara et al., who used thallium scintigraphy and postulated that silent ischemia occurs transiently in asymptomatic HCM patients after workload.

3. A considerable increase of the utilization of glucose in preference to FFAs is known to occur in myocardial hyper trophy. When the extent of hypertrophy exceeds a critical heart weight, a decrease in the myocardial PCr/ATP ratio can be found that is proportional to the degree of hypertrophy. This decrease is thought to be due to subendocardial ischemia.

Another plausible explanation is the hypothesis advanced by Holden et al., who studied the hearts of Sherpas, who live under hypobaric hypoxia in the Himalaya Mountains. Although their hearts are not hypertrophied, it was suggested that they prefer oxidizing glucose in preference to FFAs. This seems to be indispensable under their circumstances, because the oxidative yield of ATP per mole of oxygen is higher when glucose is oxidized instead of FFAs (P/O ratios, 3.0 for glucose, 2.8 for FFAs). Most interestingly, a reduced myocardial PCr/ATP ratio was also reported by the same group.

Having no evidence for ischemia or increased workload, the authors explained these changes through accelerated aerobic glycolysis. Provided that the creatine kinase reaction functions close to equilibrium, a decrease in PCr/ATP leads to an increase of the ADP concentration, which, in turn, activates the phosphoglycerate kinase and pyruvate kinase enzymes involved in aerobic glycolysis.

Indeed, increased rates of glucose retention were also found in symptomatic patients with HCM by use of $^{18}$F-FDG and positron emission tomography. Keeping in mind that no changes in myocardial pH were found in the asymptomatic HCM patients, the hypothesis of an increase in glucose oxidation offers a possible explanation for the metabolic alterations observed. The accumulation of glycolytic intermediat es represented by the increased PME/PCr ratio have to be regarded under these aspects to arise from increased oxidative glucose metabolism as well as from glycogenolysis and lipolysis of triglycerides. This interpretation is strengthened by the correlations of P/PCr with both PCr/ATP and MEIST. The former connects increased P/PCr ratios with increased ADP concentrations (which are calculated from P/PCr).

These interpretations, however, are correct only if no changes in total creatine occur, because a loss in total creatine could also explain the suggested changes in ADP. The determination of total creatine was not possible in our in vivo
NMR investigations, and this is a limitation of the present method.

The metabolic abnormalities show not only a significant but also an obviously metabolically consistent increase with the extent of hypertrophy. This hypothesis of a true biochemical adaptation to myocardial hypertrophy would support the assumption that the myocardium of patients with advanced HCM (HCM II) suffers from reduced oxygen supply, which is compensated for by an increased amount of glucose oxidation as a mechanism of hypoxia-defense adaptation.

However, the presence of such a mechanism in the HCM heart is questioned by the preliminary results of Zhang et al., who found that changes in the high-energy phosphates in the hypertrophied heart are not a result of impaired oxygen diffusion into the cells but rather reflect alterations in the control of energy metabolism.

Irrespective of their interpretation, the metabolic abnormalities found raise the question of their clinical relevance. Because none of the treatment modalities we have today for HCM, pharmacological or surgical, have thus far been able to correct the basic disorder or to have a favorable effect on the natural history of HCM, Braunwald et al. recently suggested a multicenter clinical trial to evaluate the efficacy of current treatment concepts. However, a great difficulty is that for ethical reasons, symptomatic patients cannot be used as control subjects treated with placebo only. Thus, the findings in asymptomatic patients presented here not only provide tantalizing new insight into the pathophysiology of the disease but also may perhaps help to solve these ethical concerns. We propose that in vivo NMR spectroscopy may be an important tool for the diagnosis and treatment response of asymptomatic patients with HCM.

Appendix

Details on Quantification

After application of the time-domain fitting routine VARPRO (VARiable PROjection) using gaussian model functions, standardization of the PCR signal integral was carried out and PCR was set to 0.00 ppm. The main signals of all spectra could easily be fitted with VARPRO. However, for 1 HCM I patient and 2 control subjects, the fit for PI was not successful as derived from the corresponding Cramer-Rao SDs, which exceeded 0.07 ppm for the chemical shift and 3 Hz for the linewidth (limits taken: ±0.05 ppm, ±2 Hz). Consequently, the reduced number of P values had to be taken into account in the time-domain fit results given in Table 2.

The PME signal contains several different small signals and could therefore not be evaluated with a fitting routine because of the lack of a suitable model function. Thus, the quantification of the PME signal was carried out in the frequency domain after subtraction of the fitted time-domain signals of PCr, ATP, and 2,3-DPG from the measured signal. This leads to spectra that exhibit a flat baseline and that are especially free of the 2,3-DPG signal of blood (see Figure 2). The area under the curve of PME was determined from these edited spectra by use of a peak integration routine and the frequency interval 6.2 to 7.6 ppm. The region 7.6 to 8.6 ppm was used for definition of the baseline. The PME/PCr ratio was obtained from the area of PCr from the fit results.

The identical procedure was also carried out for the average spectra obtained for each group by summation of the individual spectra scaled to equal PCr (Figure 3). Note that the SDs of the spectra obtained for each group by summation of the individual area of PCr from the fit results.

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References


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