High-energy Phosphate Metabolism of the Myocardium in Normal Subjects and Patients with Various Cardiomyopathies

— The study using ECG gated MR spectroscopy with a localization technique —

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Nuclear magnetic resonance spectroscopy (MRS) is a new technique for the evaluation of myocardial metabolism. Recently, localized MRS has been clinically available to measure by a non-invasive method the relative concentrations of the high-energy phosphate metabolites in the myocardium. We performed ECG gated P-31 MRS using ISIS (image-selected in vivo spectroscopy) in 15 normal volunteers, 12 patients with hypertrophic cardiomyopathy, 12 with left ventricular hypertrophy, 6 with dilated cardiomyopathy and 11 with specific heart muscle disease. Myocardial peak height ratios of PCr/ γ -ATP, Pi/ γ -ATP and PCr/Pi were measured.

Myocardial P-31 MRS demonstrated a significant decrease in the ratio PCr/ATP in patients with hypertrophic cardiomyopathy and specific heart muscle disease as compared with normal subjects, indicating myocardial metabolic disturbance in these patients. The ratio of PCr/ATP in patients with dilated cardiomyopathy did not differ significantly from that of normal subjects. However, exercise MRS revealed a marked decrease of PCr peak in an asymptomatic patient with dilated cardiomyopathy, which may indicate a latent metabolic disturbance in the myocardium of dilated cardiomyopathy.

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NUCLEAR magnetic resonance spectroscopy (MRS) is a new non-invasive technique that provides unique information for the evaluation of high-energy phosphate metabolism in vivo. Today, clinical use of P-31 MRS has become a wide-spread method for the investigation of skeletal muscle metabolism. However, clinical research studying

myocardial metabolism using MRS has been limited by technical difficulties, such as localization techniques and cardiac gating^{1–7} ISIS (image-selected in vivo spectroscopy) is a localization technique that allows the selection of a rectilinear volume of interest (VOI) with use of the standard magnetic resonance imaging gradients⁸

Many experimental studies have established that myocardial ischemia results in disturbance of high-energy phosphate metabolism, ie, decreased myocardial phospho-

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Fig.1. Phosphorus-31 spectra of the myocardium obtained from a normal subject (A) and a patient with hypertrophic cardio-myopathy (B). The 3 peaks of ATP (γ , α , and β), each one peak of PCr, PDE and Pi (contaminated by PME and 2, 3 DPG) are seen. The spectrum from the patients has a lower peak of PCr.

creatine (PCr), increased inorganic phosphate (Pi) and, ultimately, depletion of adenosine triphosphate (ATP)⁹⁻¹³. However, the myocardial metabolism of high-energy phosphates in cardiomyopathies is unknown.

The aims of this study are to determine the clinical reliability of phosphorus-31 spectroscopy (³¹P MRS) of the myocardium using ISIS and to evaluate the usefulness of ³¹P MRS for investigating cardiomyopathies.

TABLE 1 METABOLITE RATIOS IN CARDIAC P-31 MRS FOR NORMAL SUBJECTS AND PATIENTS

	No	PCr/ γ -ATP	Pi/ γ -ATP	PCr/Pi
Normal subjects	15	2.09 ± 0.44	1.07 ± 0.29	1.99 ± 0.39
Hypertrophic cardiomyopathy	12	$1.43 \pm 0.36^{***}$	0.82 ± 0.13	1.71 ± 0.50
Dilated cardiomyopathy	6	1.88 ± 0.40	$1.56 \pm 0.49^*$	$1.32 \pm 0.45^{**}$
Left ventricular hypertrophy	12	1.89 ± 0.20	1.11 ± 0.28	1.79 ± 0.38
Specific heart muscle disease	14	$1.30 \pm 0.27^{***}$	$1.46 \pm 0.65^{**}$	$1.06 \pm 0.43^{***}$

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared with normal subjects.

METHODS

Study population: The subjects were 15 normal volunteers; 12 patients with idiopathic hypertrophic cardiomyopathy (HCM) including 3 with obstructive hypertrophy and 9 with nonobstructive asymmetric septal hypertrophy; 12 patients with pressure overloaded left ventricular hypertrophy (LVH) in whom 4 had hypertension and 8 had aortic valvular disease; 6 patients with idiopathic dilated cardiomyopathy (DCM), and 14 patients with specific heart muscle disease (SHMD) including 11 with progressive muscular dystrophy, 2 with cardiac beriberi and 1 with cardiac amyloidosis. The mean ages of these subjects were 33 ± 4 years old in normal volunteers, 53 ± 12 in HCM, 51 ± 13 in LVH, 56 ± 9 in DCM and 26 ± 19 in SHMD. Patients were selected according to their clinical diagnosis by physical examination, echocardiography, and/or cardiac catheterization. Significant coronary artery disease was excluded by the absence of anginal pain and electrocardiographic changes. In addition, patients over 50 years old were required to have either a normal coronary angiogram or a negative exercise test with thallium-201 myocardial imaging.

P-31 MR spectroscopy: All subjects were studied using a 1.5 tesla nuclear magnetic resonance imaging and spectroscopy system (Philips Medical Systems, Gyroscan in National Institute of Radiological Sciences). The subjects were placed in the bore of the magnet in the prone position. ECG gating spectra were recorded by means of a receiving and transmitting surface coil with a diameter of 15 cm which was located outside of the chest and adjacent to the heart. Before selection of VOI, cardiac magnetic reso-

nance imaging was performed, and the X, Y and Z axis coordinates of the volume were determined from the proton MR images. Since preliminary studies with selected volume $4 \times 4 \times 4$ cm and $3 \times 3 \times 3$ cm did not produce adequate spectra for analysis, all data presented were obtained with selected volume $5 \times 5 \times 5$ cm including as much left ventricular myocardium as possible without the skeletal muscle of chest in VOI. No portion of the volume was located laterally beyond the edge of the surface coil. After selection of VOI, the surface coil was tuned and matched to the phosphorus frequency. Spectra were obtained from the subjects with a repetition time greater than 2 sec. Thus, ECG gated ISIS with a repetition time equal to 2 or 3 RR intervals was performed to obtain the P-31 spectra. The signal acquisition was synchronized with the systolic phase gated by ECG, and signal acquisition time for a spectrum was about 20 min.

Analysis of spectra: The P-31 spectra obtained from the myocardium in all subjects consisted of three definite peaks of α , β and γ -ATP and a high peak of PCr. Peak heights of PCr and γ -ATP were measured and the ratio of PCr/ γ -ATP was calculated for each subject. A few peaks produced from phosphodiester (PDE), phosphomonoesters (PME), Pi and diphosphoglycerate (DPG) originations from blood were also present to the left of the PCr peak. PDE peak occurred at the range of 2 to 3 ppm from the PCr peak in most of the subjects, but sometimes the amount of PDE was so small that the peak was not defined. Although the peaks of PME, Pi and DPG were present at the range of 5 to 7 ppm, they were not always differentiated from each other due to overlapping peaks. Despite

Fig.2. P-31 spectra of the myocardium obtained from a normal subject (upper panel) and a patient with idiopathic dilated cardiomyopathy (lower panel) before and during the leg exercise. In a normal subject the contour change of P-31 spectrum by the exercise is small. In the patient whose clinical symptoms of cardiac failure had disappeared, the spectrum before exercise is shown normal pattern. However, a marked decrease of PCr peak was found in the spectrum during the exercise.

difficulty separating these peaks, we thought analysis of the phosphate compounds was very important. Therefore, we tentatively defined the highest peak in the range of 5 to 7 ppm as the peak of Pi and measured the ratios of Pi/ γ -ATP, and PCr/Pi.

Additional studies: In 11 subjects including 6 normal volunteers and 5 patients with HCM, P-31 MRS was performed repeatedly under the same conditions from 1 h to 7 days later to determine the reproducibility of the measurement. We studied exercise MRS

in 3 normal subjects and a patient with DCM for the evaluation of the myocardial metabolism. P-31 MRS was performed before and during exercise (flexing of the legs lifting a 2 kg weight about 20 cm perpendicular distance) were carried out alternately every 1 sec for 20 min in the prone position. This work load was estimated to be about 4 joules with each leg flexion.

Statistical analysis: Data were expressed as mean $\pm$ standard deviation. Comparisons between the patient groups and the normal

group were made by the non-paired Student's t-test. Reproducibility of measurements was assessed by the paired Student's t-test. A p value < 0.05 was considered significant.

RESULTS

Reproducibility: In 11 subjects P-31 MRS were studied on two separate occasions from 1h to 7 days apart. The differences between the first and the second measurements of the ratio PCr/ γ -ATP, Pi/ γ -ATP, PCr/ β , Pi/ β -ATP and PCr/Pi in the myocardium were $-0.2 \pm 12.0\%$, $-0.5 \pm 7.3\%$, $-0.2 \pm 12.4\%$, $0.5 \pm 10.1\%$ and $1.7 \pm 11.8\%$ respectively. There were no significant differences of reproducibility between normal volunteers and patients with HCM. The ratios to γ -ATP had better reproducibility than the ratios to β -ATP because the ISIS experiment had off-resonance effects that altered the intensity of peaks distant from the carrier frequency. Therefore, we used the peak height of γ -ATP for calculation of the peak ratios in our study.

Spectral analysis: All spectra obtained from the normal myocardium had similar patterns (Fig. 1A). The normal ratios of PCr/ γ -ATP, Pi/ γ -ATP, PME/ γ -ATP, PDE/ γ -ATP and PCr/Pi were 2.09 ± 0.44 , 1.07 ± 0.29 , 1.04 ± 0.30 , 1.00 ± 0.17 and 1.99 ± 0.39 , respectively. In some patients with cardiomyopathies, the spectra revealed decrease of the PCr peaks and increase of the Pi peaks compared to normal spectra (Fig. 1B). The results of P-31 MRS myocardial analysis are shown in Table 1. Compared to the normal subjects, the ratios of PCr/ γ -ATP and Pi/ γ -ATP decreased in the patients with septal HCM. The ratio of Pi/ γ -ATP increased, and the ratio of PCr/Pi decreased in the patients with DCM. The ratios of PCr/ γ -ATP and PCr/Pi decreased, and the ratio of Pi/ γ -ATP increased in the patients with SHMD. There were no significant differences between these ratios in patients with LVH compared to normal subjects.

Exercise P-31 MRS: We performed P-31 MRS before and during minimal exercise in 3 normal volunteers and one patient with DCM. The effect of exercise on the ratios PCr/ γ -ATP, Pi/ γ -ATP and PCr/Pi was

small in normal subjects. Although the myocardial spectrum before exercise in the patient with DCM, whose clinical symptoms of cardiac failure had disappeared, was normal, a marked decrease of the PCr peak was found in the spectrum during the exercise (Fig. 2).

DISCUSSION

Measurement of the peaks in P-31 MRS: P-31 spectrum of the myocardium using ISIS contains several peaks; three peaks of ATP, and one peak each from PCr, PDE, PME and Pi. In this study, myocardium, blood, pericardial fat and lung were also contained in the selected volume. Scantiness of the phosphate compounds in the fat and lung indicates that the contribution of these tissues to the spectrum is minimal, while the spectrum of blood has a high peak of 2-3-DPG near the peak of Pi. Therefore the large overlap of DPG signal to Pi signal may disturb measurement of the exact height and position of the Pi peak. However, contributions to PCr and ATP peaks from blood are negligible.

In clinical studies the relative concentrations of the phosphate metabolites were measured from the ratios of the peak heights or the ratios of the peak areas, because the height of each peak or the area under each peak is related to the concentration of the specific phosphate compounds within VOI. Although many studies have chosen the peak area for calculation of the ratios of phosphate metabolites, Schaefer et al found no difference of the ratio PCr/ATP between peak area method and peak height method⁴. They also demonstrated better reproducibility ($< 10\%$) of the PCr to γ -ATP height ratios than the area ratios. Therefore, we used the height method in this study to obtain excellent reproducibility (within 12%) in the ratios of PCr/ γ -ATP, Pi/ γ -ATP and PCr/Pi.

Previous experimental and clinical studies have shown that the ratios of PCr/ATP, Pi/ATP and PCr/Pi in the normal myocardium vary considerably depending on the methods obtained P-31 MRS. For example, the mean values of PCr/ATP have ranged from 2.50 by Rehr et al¹³ to 1.26 by Tamatsu et al¹⁴ comparing to 2.09 in this study. Thus,

we carefully maintained the conditions, such as the selection of volume, selection of site, repetition time and gating phase, as constant as possible in this study.

P-31 MRS in hypertrophic cardiomyopathy: Many animal experimental studies have shown that the PCr level decreases and Pi level increases rapidly after the onset of the ischemia, while ATP levels do not decrease until PCr disappears. These changes of the myocardial P-31 spectrum are also expected in the patient with cardiomyopathy, because metabolic disturbances of the skeletal muscle have been discovered using P-31 MRS in various kinds of myopathy. However, only a few reports have investigated MRS of cardiomyopathy. Whitman et al presented results obtained from two infants who had optimal conditions for the observation of the MR signals by an ordinary surface coil through the thin chest wall!¹⁵ In the elder child with HCM, MRS showed a lower PCr value and a higher Pi value than in the younger sibling who had no sign and symptom of cardiomyopathy. The PCr/ATP ratios measured at different times were 1.3 ± 0.1 in the elder and 2.0 ± 1.0 in the younger. Six patients with HCM were examined by Rajagopalan et al using the rotating frame depth selection technique!¹⁶ A depressed PCr/ATP ratio was observed in a patient with congestive heart failure and severe right ventricular hypertrophy. In our study, decrease of the ratio of PCr/ATP was found in the patients with HCM, but not in the patients with reactive LVH by hypertension or aortic valvular disease. Schaefer et al also reported normal P-31 spectra in 11 patients with pressure overloaded LVH. Thus, our prior studies have indicated that high-energy phosphate metabolism of the myocardium is unchanged in patients with secondary LVH, but is disturbed in some patients with HCM.

P-31 MRS in dilated cardiomyopathy: Abnormalities in high-energy phosphate metabolism have been demonstrated in the Syrian hamster model of DCM!^{17,18} However, disturbance of high-energy phosphate metabolism in the patients with DCM have not been demonstrated clinically. Schaefer et al and Auffermann et al reported that the ratio of PCr/ATP was not changed in patients with DCM!^{6,7} In our study the ratio of PCr/ATP in the patients with DCM was not

significantly decreased compared with normal subjects, but decreased in the patients with SHMD. This difference between DCM and SHMD may be explained from the severity of disease, since the patients with SHMD had larger cardiac silhouettes on chest X-ray films and more hypokinesis of the left ventricle on echocardiogram than the patients with DCM. The fact that the decreased PCr and increased Pi were provoked by minimal exercise in a non-symptomatic patient with DCM also supports the functional change of high energy phosphate metabolism in this myocardial disease. There are two possible explanations for the increase of the ratio of Pi/ATP in patients with DCM and SHMD. One is an increase of Pi concentration in the myocardium of these diseases, and another more probable reason is contamination of peak measurements from blood in the dilated left ventricular chamber. In order to evaluate this, further studies concerning the exact volumes of the myocardium and blood in the VOI will be necessary.

In conclusion, P-31 MRS is a useful method for detecting myocardial metabolic disturbances in symptomatic and asymptomatic cardiomyopathies and for distinguishing these diseases from the reactive left ventricular hypertrophy by hypertension and aortic valvular disease.

REFERENCES

1. ING WALL JS: Phosphorus nuclear magnetic resonance spectroscopy of cardiac and skeletal muscles. *Am J Physiol* 1982; **242**: H729-H744
2. BOTTOMLEY PA: Noninvasive study of high-energy phosphate metabolism in human heart by depth-resolved ³¹P NMR spectroscopy. *Science* 1985; **229**: 769-772
3. BOTTOMLEY PA, HERFKENS RJ, SMITH LS, BASHORE TM: Altered phosphate metabolism in myocardial infarction: P-31 MR spectroscopy. *Radiology* 1987; **165**: 703-707
4. SCHAEFER S, GOBER J, VELENZA M, KARCZMAR GS, MATSON G, CAMACHO A, BOTVINICK EH, MASSIE B, WEINER MW: Nuclear magnetic resonance imaging-guided phosphorus-31 spectroscopy of the human heart. *J Am Col Cardiol* 1988; **12**: 1449-1455
5. GRIST TM, KNEELAND JB, RILLING WR, JESMANOWICZ A, FRONCISZ W, HYDE JS: Gated cardiac MR imaging and P-31 MR spectroscopy in humans at 1.5T. *Radiology* 1989; **170**: 357-361
6. SCHAEFER S, GOBER JR, SCHWARTZ GG,

- TWIEG DB, WEINER MW, MASSIE B: In vivo phosphorus-31 spectroscopic imaging patients with global myocardial disease. *Am J Cardiol* 1990; **65**: 1154–1161
7. AUFFERMANN W, CHEW M, WOLFE I, TAVARES NJ, PARMLEY WW, SEMELKA RC, DONNELLY T, CHATTERJEE K, HIGGINS R: Normal and diffusely abnormal myocardium in humans: Functional and metabolic characterization with P-31 MR spectroscopy and cine MR imaging. *Radiology* 1991; **179**: 253–259
 8. ORDIDGE RJ, CONNELLY A, LOHMAN JAB: Image-selected in vivo spectroscopy (ISIS): a new technique for spatially selective NMR spectroscopy. *J Magn Reson* 1986; **66**: 283–294
 9. KUSUOKA H, INOUE M, TSUNEOKA Y, WATARI H, HORI M, ABE H: Augmented energy consumption during early systole as a mechanism of cyclical changes in high-energy phosphate in myocardium assessed by phosphorus nuclear magnetic resonance. *Jpn Circ J* 1985; **49**: 1099–1107
 10. MATTHEWS PM, TAYLOR DJ, RADDA GK: Biochemical mechanisms of acute contractile failure in the hypoxic rat heart. *Cardiovasc Res* 1986; **20**: 13–19
 11. GROVE TH, ACKEMAN JJH, RADDA GK, BORE PJ: Analysis of rat heart in vivo by phosphorus nuclear magnetic resonance. *Proc Natl Acad Sci USA* 1980; **77**: 299–302
 12. KORETSKY AP, WANG S, MURPHY-BOESCH J, KLEIN MP, JARNES, TL, WEINER MW: P-31 NMR spectroscopy of rat organs in situ, using chronically implanted radiofrequency coils. *Proc Natl Acad Sci USA* 1983; **80**: 7491–7495
 13. REHR RB, TATUM JI, HIRSCLT JI, WETSTEIN L, CLARKE G: Effective separation of normal, acutely ischemic, and reperfused myocardium with P-31 MR spectroscopy. *Radiology* 1988; **168**: 81–89
 14. TAMATSU H, NAKAZAWA M, IMAI S, WATARI H: P-31 topical nuclear magnetic resonance studies of cardiotoxic effects of 5-fluorouracil and 5'-deoxy-5fluorouridine. *Jpn J Pharmacol* 1984; **34**: 375–379
 15. WHITMAN GR, CHANCE B, BODE H: Diagnosis and therapeutic evaluation of pediatric cardiomyopathy using P-31 NMR. *J Am Col Cardiol* 1985; **5**: 745–749
 16. RAJAGOPALAN B, MCKENNA W, BLACKLEDGE M, BOLAS N, OBERHAENSLI R, RADDA GK: Measurement of phosphorus metabolites in hearts of patients with hypertrophic cardiomyopathy by MRS (abstract). Society of Magnetic Resonance in Medicine, 6th Annual Meeting 1987; **1**: 199
 17. MARKIEWICZ W, WU SS, PARMLEY WW, HIGGINS CB, SIEVERS R, JAMES TL, WIKMAN-COFFELT J, JASMIN G: Evaluation of the hereditary Syrian hamster cardiomyopathy by ³¹P nuclear magnetic resonance spectroscopy: improvement after acute verapamil therapy. *Circ Res* 1986; **59**: 597–604
 18. BUSER PT, CAMACHO SA, WU ST, HIGGINS CS, JASMIN G, PARMLEY WW, WIKMAN-COFFELT J: The effect of dobutamine on myocardial performance and high-energy phosphate metabolism at different stages of heart failure in cardiomyopathic hamsters: A P-31 MRS study. *Am Heart J* 1989; **117**: 86–91