JOURNAL OF CARDIOVASCULAR MAGNETIC RESONANCE®
Vol. 6, No. 3, pp. 685–696, 2004

SPECTROSCOPY

Detection of Metabolic Abnormality in Asynergic Regions of Ischemic Human Myocardium Using ^{31}P and ^{1}H Magnetic Resonance Spectroscopy

Ichiro Nakae, M.D.,[1] Kenichi Mitsunami, M.D.,[2,*] Takahiro Yabe, M.D.,[1] Toshiro Inubushi, Ph.D.,[3] Shigehiro Morikawa, M.D.,[3] Shinro Matsuo, M.D.,[1] Terue Koh, M.D.,[1] and Minoru Horie, M.D.[1]

[1]Department of Cardiovascular and Respiratory Medicine,
[2]Department of General Medicine, and
[3]Molecular Neurosciece Research Center, Shiga University of Medical Science,
Seta, Otsu, Japan

ABSTRACT

To assess quantitatively phosphocreatine (PCr), adenosine triphosphate (ATP) and total creatine (CR) in asynergic regions of ischemic human myocardium using phosphorus (^{31}P) and proton magnetic resonance spectroscopy (^{1}H MRS). Patients were divided into two groups: ^{31}P MRS and ^{1}H MRS. In each group, patients were subdivided into three groups using echocardiography: a normokinetic [WNP ($n = 6$) in ^{31}P MRS, WNH ($n = 10$) in ^{1}H MRS], a hypokinetic [WHP ($n = 13$), WHH ($n = 7$)], and a- or dyskinetic wall motion groups [WAP ($n = 14$), WAH ($n = 6$)]. They were compared with normal subjects of each group [CNP ($n = 10$), CNH ($n = 10$)]. ^{31}P MRS spectra were obtained from the anterior and apical regions of the left ventricle by slice-selected 1D CSI. ^{1}H MRS spectra were obtained from the $2 \times 2 \times 2$-cm voxel set in the left ventricular wall by the PRESS method. In the ^{31}P MRS group, myocardial PCr was significantly lower in the WHP and WAP groups than in the CNP group, but myocardial PCr in the WNP group did not differ from that in CNP. A difference in ATP could not be detected among the four groups. In the ^{1}H MRS group, myocardial CR was significantly lower in the WHH and WAH groups than in the CNH group. Myocardial CR in the WNH group did not differ from that in the CNH. The

*Correspondence: Kenichi Mitsunami, M.D., Department of General Medicine, Shiga University of Medical Science, Tsukinowa-cho, Seta, Otsu 520-2192, Japan; Fax: (81)77-548-9701; E-mail: mitunami@belle.shiga-med.ac.jp.

DOI: 10.1081/JCMR-120038529
Copyright © 2004 by Marcel Dekker, Inc.
1097-6647 (Print); 1532-429X (Online)
www.dekker.com

ORDER REPRINTS

noninvasive ^{31}P and ^{1}H MRS approach is useful in the assessment of metabolite reduction associated with wall motion abnormality.

Key Words: Magnetic resonance spectroscopy; High-energy phosphate; Creatine; Myocardial ischemia; Echocardiography.

INTRODUCTION

Using ^{31}P magnetic resonance (MR) spectroscopy (^{31}P MRS), noninvasive quantification of phosphocreatine (PCr) and adenosine triphosphate (ATP) was previously attempted in the human heart (Bottomley et al., 1990; Mitsunami et al., 1992; Yabe et al., 1995). When ^{31}P MRS was obtained by three-dimensional chemical shift imaging (3D CSI), myocardial metabolite content could be quantified with reference to a standard located outside the chest (Bottomley et al., 1990). In the infarcted myocardium, PCr and ATP concentrations were both decreased (Mitsunami et al., 1992; Yabe et al., 1995). Recently, total creatine (CR, total phosphorylated and unphosphorylated creatine) was also quantitatively measured in the human heart by using proton magnetic resonance spectroscopy (^{1}H MRS) (Bottomley and Weiss, 1998). The CR content was decreased in the nonviable infarcted regions of the human heart (Bottomley and Weiss, 1998, 2001). Therefore, PCr and ATP can be assessed noninvasively by ^{31}P MRS, and CR can be measured by ^{1}H MRS.

In the present study, we aimed to assess quantitatively myocardial PCr, ATP, and CR by using ^{31}P MRS and ^{1}H MRS. We examined whether ^{31}P and ^{1}H MRS can detect myocardial metabolic abnormalities associated with left ventricular wall motion abnormality determined by echocardiography in patients with ischemic heart disease. ^{31}P MRS by slice-selected one-dimensional chemical shift imaging (1D CSI) was performed on all patients and normal subjects (Yabe et al., 1995). The PCr and ATP concentrations were quantified with reference to a standard outside the chest. The ^{1}H MRS with point-resolved spectroscopy (PRESS) localization was performed (Bottomley, 1987). The intensity of the tissue water signal from the same voxel was used as the concentration reference (Barker et al., 1993; Bottomley and Weiss, 1998, 2001).

METHODS

The study protocol was approved by the Ethical Committee of Shiga University of Medical Science. The subjects with coronary artery disease were divided into two separate study groups: ^{31}P MRS and ^{1}H MRS.

Subjects

In the ^{31}P MRS study, 33 patients with 50% or greater stenosis of the left anterior descending (LAD) coronary artery and 10 healthy volunteers with no clinical evidence of cardiac disease were evaluated. The patients were further divided into three subgroups by the evaluation of wall motion abnormality using echocardiography. The normokinetic left ventricular wall motion (WNP) group consisted of 4 men and 2 women whose ages ranged from 55 to 72 years (mean ± SD, 61.5 ± 5.9). The hypokinetic wall motion (WHP) group consisted of 11 men and 2 women, 50–77 years old (63.9 ± 8.6). The a- or dyskinetic wall motion (WAP) group consisted of 9 men and 5 women, 44–77 years old (64.3 ± 8.6). In the WNP group, none of the patients had prior myocardial infarction. In the WHP group, 8 patients showed evidence of prior myocardial infarction. In the WAP group, all patients demonstrated prior myocardial infarction and severe hypokinesis, akinesis, or dyskinesis in the anterior, septal and apical regions. The control (CNP) group consisted of 10 normal subjects (6 men and 4 women) whose ages ranged from 27 to 64 years (47.2 ± 12.8). All subjects in the CNP group were free of any previous clinical history of heart disease. There was no significant difference in ages among the WNP, WHP, and WAP groups. However, ages in the CNP group were significantly lower than those in the WNP, WHP, and WAP groups ($p < 0.05$).

In the ^{1}H MRS study, 23 patients with 50% or greater stenosis of the coronary arteries and 10 healthy volunteers comprised the present study. As in the ^{31}P MRS study, the patients were divided into three subgroups by the evaluation of wall motion abnormality using echocardiography: WNH, WHH, and WAH. The WNH group consisted of 8 men and 2 women whose ages ranged from 51 to 68 years (60.0 ± 5.6). The WHH group consisted of 5 men and 2 women, 51–75 years old (64.6 ± 10.0). The WAH group consisted of 6 men, 61–73 years old (68.3 ± 5.0). The control

Copyright © Marcel Dekker, Inc. All rights reserved.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

ORDER REPRINTS

(CNH) group consisted of 10 normal subjects (7 men and 3 women) whose ages ranged from 26 to 66 years (46.0 ± 11.4). There was no significant difference in ages among the WNP, WHP, and WAP groups. However, ages in the CNH group were significantly lower than those in the other three groups ($p < 0.05$).

All patients continued to receive conventional antiischemic medication at the time of the study (nitrates, beta-blockers, calcium-channel blockers and anticoagulants).

Echocardiography

Echocardiography was performed on a day close to the MRS study. There were no significant clinical events such as acute myocardial infarction or unstable angina intervening between the test and the MRS study. Commercially available wide-angle phased-array imaging systems (Toshiba Sonolayer SSH-160A, 2.5 and 3.5 MHz transducers, Tokyo, Japan) were used. In the ^{31}P MRS study, the left ventricle in the region of interest (ROI) was divided into six segments: apical anterior, mid anterior, basal anterior, mid anterior septal, basal anterior septal, and mid septal wall. According to the American Society of Echocardiography, segmental wall motion was graded to analyze each study [normal (score = 1), hypokinesis (score = 2), akinesis (score = 3), and dyskinesis (score = 4)] (Broderick et al., 1988; Marzullo et al., 1993; Sawada et al., 1991; Smart et al., 1993). Global left ventricular wall motion score index (WMSI) in the ROI was derived by the standard formula (Smart et al., 1993): WMSI = sum of the segment scores/6.

Subjects with WMSI of 1.00, which indicated normokinetic wall motion, were defined as the WNP group. Subjects with WMSI of 1.01 to 2.65, which indicated hypokinetic wall motion, were defined as the WHP group. Subjects with WMSI of > 2.65, which indicated almost a- or dyskinetic wall motion, were defined as the WAP group.

In ^{1}H MRS study, wall motion was evaluated by using echocardiography in the region where the MRS study was performed because ^{1}H MRS can acquire signals from smaller region than ^{31}P MRS. The patients were divided into three subgroups: a normokinetic wall motion group (WNH), a hypokinetic wall motion group (WHH), and a- or dyskinetic wall motion group (WAH).

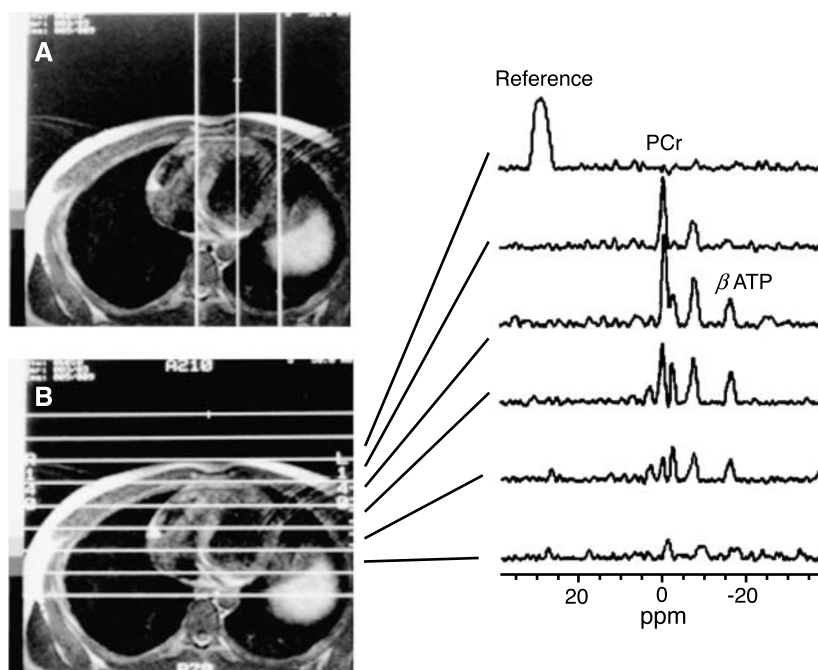

Figure 1. Conventional transaxial ^{1}H magnetic resonance images demonstrating how the region of interest (ROI) was placed into the left ventricle [2 × (6 to 8) × 12.5 cm]. The MR spectra were spatially selected in the sagittal direction (A), and one-dimensional chemical shift imaging was performed (B). *Key*: ATP, adenosine triphosphate; PCr, phosphocreatine.

Copyright © Marcel Dekker, Inc. All rights reserved.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

ORDER REPRINTS

^{31}P MRS Study

^{31}P MR Spectroscopy

^{31}P MRS experiments were performed by using a 1.5-T, 1-m-bore, whole-body MR imaging system (Signa; General Electric Medical Systems, Milwaukee, WI) (Yabe et al., 1995). Circular surface coils of 20-cm diameter transmitter and 12.5-cm diameter receiver were used for both ^{1}H and ^{31}P. Patients were examined in the supine position. Conventional ^{1}H MR imaging was performed prior to ^{31}P MRS to confirm and guide the placement of the surface coil over the anteroapical region of the left ventricle. The images were acquired in the transaxial and coronal planes. Subjects were kept in a constant position on the examination table during the acquisition of ^{31}P MRS data, and the table was maintained at an established center position during the acquisitions.

Figure 1 shows conventional ^{1}H MR images demonstrating how the ROI was placed into the left ventricle and MR spectra were obtained. The MR spectra were spatially localized by 1D CSI with slice selection in the sagittal direction, as previously described (Yabe et al., 1995). Before each measurement, shimming on the proton signal of water in the ROI was performed. The typical line widths were between 0.4 and 0.8 ppm. Phase-encoding gradients were applied in the coronal direction for 500 μsec, with 16-phase encoding views acquired over a 32-cm field of view. Each phase encoding step required an average of 32 free induction decays, which were acquired during every other heart beat at end-systole (350 msec after R wave) on electrocardiogram (ECG). The radio frequency (RF) pulse power was kept constant for all subjects.

^{31}P Slice-Selected 1D CSI Localization Technique

Signal localization to myocardial tissue was achieved by slice-selected 1D CSI with ECG gating to systole. In some cases, PCr resonance is split into two peaks by ordinary one-dimensional approaches for localization, such as depth-resolved surface-coil spectroscopy (DRESS) or 1D CSI. The splitting of the PCr resonance is believed to be caused by the contamination from the left chest wall muscle (Yabe et al., 1995). We added sagittal slice selection for the left ventricle to the 1D CSI sequence, which resulted in a favorable decrease of chest muscle contamination.

The slice thickness in the sagittal direction ranged between 60 and 80 mm, and the slice was positioned so that much of it was filled by the left ventricle. Sixteen-phase encoding steps were applied over a 32-cm field of view. Each resulting spectrum was thus a representative of a 2-cm-thick section in the coronal plane. The surface coil was used both for transmitting and receiving RF signals. The RF excitation was achieved with a selective sinc pulse of 1-msec duration, and pulse power was kept constant between patients. The power of the surface coil was not adjusted between patients because coil loading was almost constant between subjects. Phantom studies demonstrated that at an average depth of 0.0 and 5.5 cm, the coil deposited approximately 145 and 68 flip angles, respectively.

The distance between the surface coil and the center of the slice, which included the anterior myocardium, ranged from 4.6 to 7.2 cm. Phantom studies have shown that less than 15% of ‘‘signal bleed’’ occurs from adjacent sections. Total scanning time ranged from 12 to 15 min per set of spectra. The total MR examination time ranged from 50 to 60 min. The integrated areas of the resonances of PCr and β-ATP were measured after applying a 15-Hz line-broadening exponential filter and fitting the peaks to a Lorentzian line with a custom-built, automatic data processing station using the simplex technique (Lenkinski et al., 1989).

^{31}P MRS Quantitative Processing

For metabolite quantification, a 0.5-cm^3 reference vial of 5.75 M hexamethylphosphoric triamide (HMPT) solution was placed on the axis of the surface detection coil against the chest (Yabe et al., 1995). Our phantom study revealed that the excitation field was comparatively uniform over the ROI for the anterior myocardium in the sensitive volume of the 12.5-cm coil. The concentration of the metabolite was determined by using the formula of Bottomley et al. (1990). The volume of the anterior myocardium in the ROI was estimated by approximate calculations from the transaxial ^{1}H MR image (Yabe et al., 1995). Metabolite concentration in unit tissue volume was converted to concentration per gram wet weight using a specific gravity of 1.05 for adult heart tissue. The ratio of the detection coil sensitivities at the sample and at the reference was derived from phantom studies. Corrections for signal loss due to phase variations and missing data points were not made for our series.

The saturation factors (SF) were determined by the formula of Ernst and Anderson (1966): SF = [1 − exp

Copyright © Marcel Dekker, Inc. All rights reserved.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

ORDER REPRINTS

$(-\mathrm{TR}/\mathrm{T1})] \times \sin\ \alpha/[1 - \exp(-\mathrm{TR}/\mathrm{T1}) \times \cos\ \alpha]$,where TR is a repetition time. The flip angles for the subject were estimated by using a phantom which yielded the same Q factor as the subjects. The T1 values of cardiac PCr and β-ATP were estimated from the spectra of four healthy volunteers acquired at TRs of 1–15 sec with no ECG gating. The T1 values of PCr and β-ATP were 4.2 sec and 1.7 sec, respectively. We applied the same T1 value to all the subjects. This correction assumes that normal and infarcted myocardium have the same spin-lattice relaxation times. The T1 value of HMPT was 11.5 sec.

Levels of ATP were represented by the β-ATP, because the β-phosphate of ATP does not overlap with resonances of other compounds. Contamination of blood ATP from the section intersecting the ventricular cavity was corrected by subtraction of a blood ATP signal corresponding to 15% of the total integrated 2,3-diphosphoglycerate (2,3-DPG) signal from the β-ATP signal (Bottomley et al., 1991).

^{1}H MRS Study

^{1}H MR Spectroscopy

The ^{1}H MRS studies were done with a GE 1.5 T Signa imaging/spectroscopy system (Nakae et al., 2003). Signa 1.5-T General Purpose Flex Coil (GPFLEX, GE Medical Systems) was wrapped around the chest to be centered over the heart. This coil is a receive-only flexible coil designed for irregularly shaped anatomy and consists of two 13 × 17 cm loops that are serially connected to create a corotating ‘‘saddle coil’’ pair. Subjects were examined in the supine position. An ECG-gated spin echo MR image was obtained to determine the location of localized volume elements (voxels) for the MRS investigation. Voxels were localized to $2 \times 2 \times 2\ \mathrm{cm}^3$ in the left ventricular wall by the PRESS method (Bottomley, 1987). This voxel size was chosen to yield useful signal-to-noise (S/N) ratios. The spectral acquisition was gated once per two cardiac cycles using ECG or plethysmography. The acquisition parameters were echo time (TE) of 25 msec and TR of 1.4 to 2.9 sec. At the acquisition, the initial data set of 16 signals was collected without water suppression for the water resonance and then a data set of 128 signals was collected with water suppression for the creatine resonance. Spectral peaks were identified with known chemical shifts: water at 4.75 ppm, cholines at 3.2 ppm, creatine at 3.0 ppm, and lipid at 0.9–1.4 ppm (Bottomley and Weiss, 1998; Bottomley et al., 1996).

^{1}H MRS Quantitative Processing

Quantitation of the creatine peak of the myocardium was performed by using the water signal without suppression as an internal concentration reference. Each peak was fitted with a custom-built, automatic data-processing station. The concentration of total

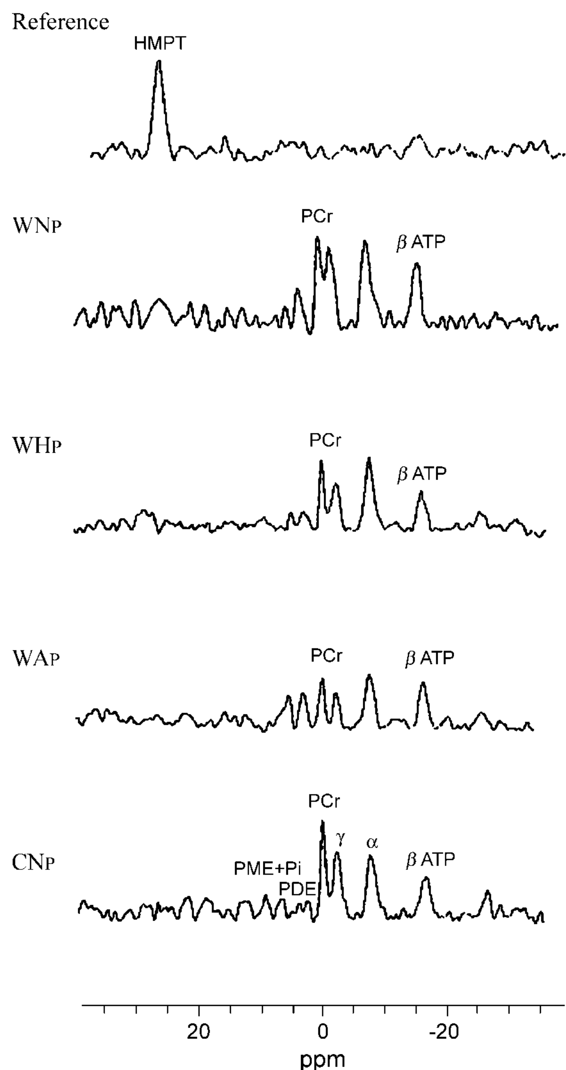

Figure 2. The ^{31}P MR spectra of typical patients in the four groups. The patients in the WHP and WAP groups had reduced PCr peaks compared to patients in the WNP and CNP groups. However, differences in ATP were not clearly observed among the four groups. *Key*: WNP, normokinetic left ventricular wall motion group; WHP, hypokinetic wall motion group; WAP, a- or dyskinetic wall motion group; CNP, normal control group; HMPT, hexamethylphospholic triamide; PCr, phosphocreatine; ATP, adenosine triphosphate; PME, phosphomonoesters; Pi, inorganic phosphate; PDE, phosphodiesters.

Copyright © Marcel Dekker, Inc. All rights reserved.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

ORDER REPRINTS

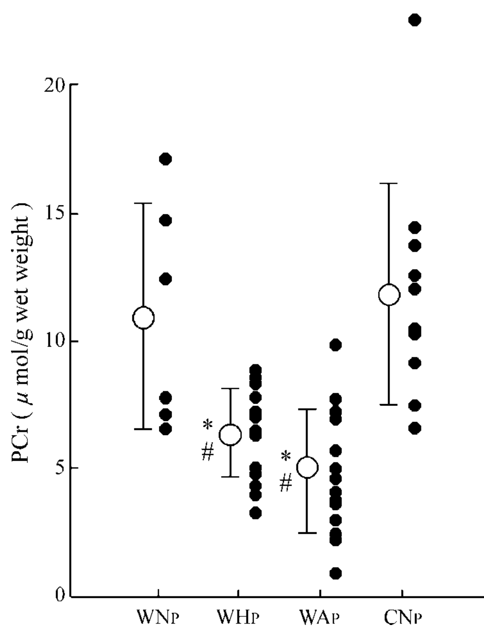

Figure 3. Individual small plots show myocardial phosphocreatine (PCr) concentration measured by phosphorus magnetic resonance spectroscopy (^{31}P MRS). Myocardial PCr in the WHP and WAP groups were significantly lower than those in the WNP and CNP groups. However, there was no significant difference in PCr between the WHP and WAP groups, or between the WNP and CNP groups. $^{*}p < 0.05$ vs. WNP, #$p < 0.05$ vs. CNP. *Key*: Large symbol and bar, mean ± SD; WNP, normokinetic left ventricular wall motion group; WHP, hypokinetic wall motion group; WAP, a- or dyskinetic wall motion group; CNP, normal control group.

creatine [CR] in tissue filling a voxel was calculated from the tissue water concentration [W], according to the following equation (Bottomley and Weiss, 1998; Bottomley et al., 1997):

$$[CR] = [W] \times (2/3) \times (S_{CR}/S_W) \times F_{CR/W} \times E_{CR/W} \quad (1)$$

Numbers 2 and 3 indicate the two protons on water and the three protons of the N-methyl group of creatine, respectively. SCR and SW are the total creatine and water MR signal areas in the tissue, respectively. Myocardial tissue water content in this study was taken as 72.7% by weight (Bottomley et al., 1996; Snyder et al., 1984):

$$[W](\mu mol/g) = 40.3 \times 1000 \quad (2)$$

The T1 values of myocardial CR and W were estimated by using the spectra of five healthy volunteers acquired at TRs of 1.5–6 sec using PRESS without ECG gating. The obtained T1 values were 1.48 and 1.21 sec ($n = 5$) for CR and W, respectively. Spectral acquisition was gated once per two cardiac cycles in this study: TR (second) = 120/HR (HR: heart rate, bpm). The T2 values of myocardial CR and W were obtained from the spectra of five volunteers acquired at TEs of 25–45 msec. The obtained T2 values were 135 and 33.1 msec ($n = 5$) for CR and W, respectively. FCR/W and ECR/W are correction factors for T1 and T2 relaxation effects, respectively.

$$F_{CR/W} = [1 - \exp(-120/HR/1.21)] / [1 - \exp(-120/HR/1.48)] \quad (3)$$

$$E_{CR/W} = [\exp(-25/33.1)]/[\exp(-25/135)] \quad (4)$$

Statistical Analysis

Data are presented as means ± standard deviation (SD). Scheffé's F test for multiple contrasts was applied to detect significant differences as defined by

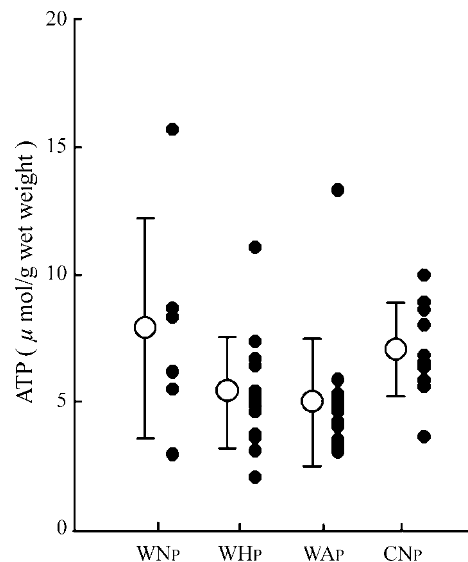

Figure 4. Individual small plots show ATP concentration measured by phosphorus magnetic resonance spectroscopy (^{31}P MRS). No significant differences of ATP concentration existed among the four groups. *Key*: Large symbol and bar, mean ± SD; WNP, normokinetic left ventricular wall motion group; WHP, hypokinetic wall motion group; WAP, a- or dyskinetic wall motion group; CNP, normal control group.

Copyright © Marcel Dekker, Inc. All rights reserved.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

ORDER REPRINTS

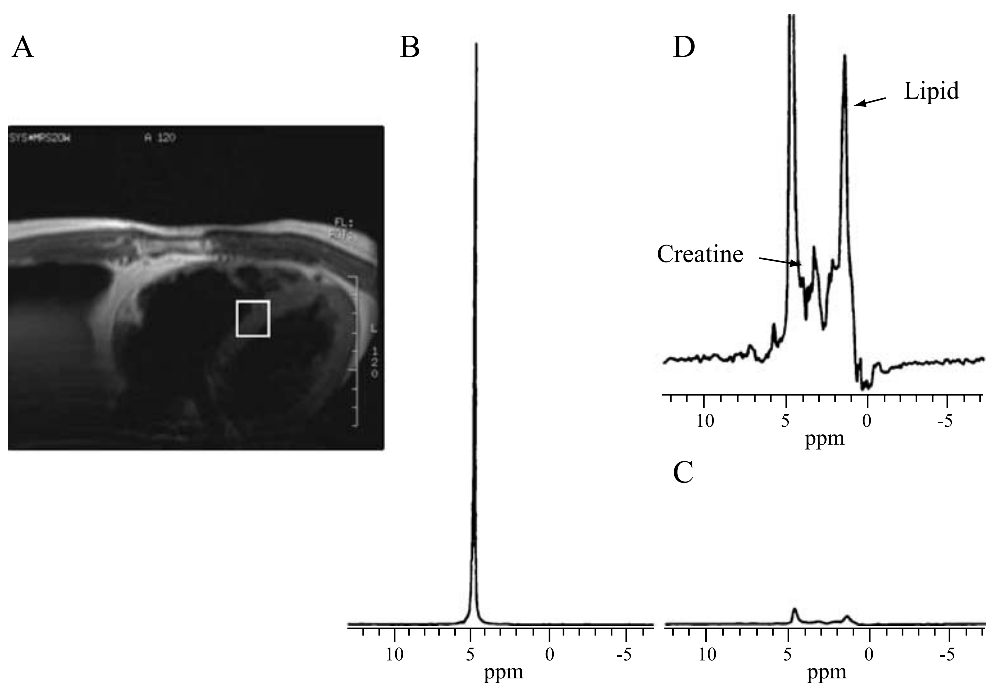

Figure 5. (A) ^{1}H MR spectra acquired from 2 × 2 × 2-cm voxel localized in the intraventricular septum by the PRESS method in a normal volunteer. (B) This spectrum was acquired without water suppression for the water resonance. (C) This spectrum was acquired with water suppression and was shown with the same vertical scale as (B). (D) This spectrum was acquired with water suppression for the creatine resonance and was shown with vertical gain increased by ~30-fold.

the analysis of variance (ANOVA) among the four groups (WNP, WHP, WAP, and CNP in ^{31}P MRS study, and WNH, WHH, WAH, and CNH in ^{1}H MRS study). A probability value of $p < 0.05$ was considered to be significant.

RESULTS

^{31}P MRS Study

There was no significant difference in either heart rate (63.2 ± 5.4, 68.4 ± 17.3, 71.0 ± 12.8, and 66.3 ± 9.7 bpm) for WNP, WHP, WAP, and CNP, respectively, p = NS by ANOVA or left ventricular mass in the ROI (34.0 ± 7.7 g, 41.8 ± 7.4 g, 38.8 ± 7.6 g, and 37.5 ± 7.6 g of wet tissue) for WNP, WHP, WAP, and CNP, respectively, p = NS by ANOVA among the four groups. The ^{31}P MR spectra of typical patients in the four groups are shown in Fig. 2. Myocardial PCr in the WHP and WAP groups were significantly lower than those in the WNP and CNP groups (Fig. 3). However, there was no significant difference in PCr between the WHP and WAP groups, or between the WNP and CNP groups (WNP: 11.0 ± 4.4; WHP: 6.4 ± 1.8*#; WAP:

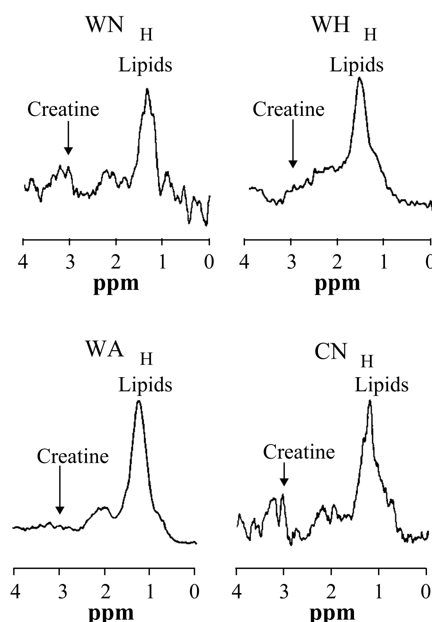

Figure 6. The ^{1}H MR spectra of typical patients in the four groups. The patients in the WHH and WAH groups have reduced creatine peaks (3.0 ppm) compared with patients in the WNH and CNH groups. *Key*: WNH, normokinetic left ventricular wall motion group; WHH, hypokinetic wall motion group; WAH, a- or dyskinetic wall motion group; CNH, normal control group.

Copyright © Marcel Dekker, Inc. All rights reserved.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

ORDER REPRINTS

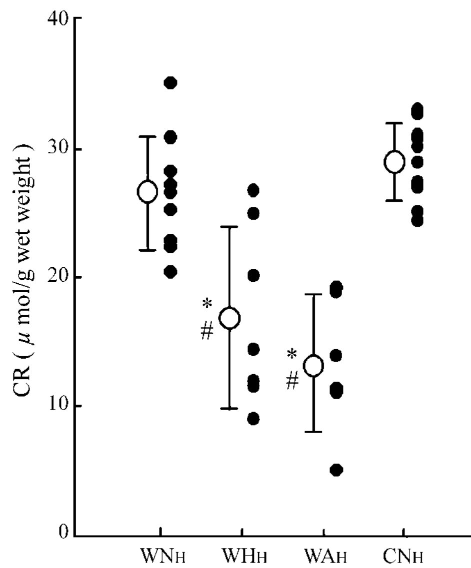

Figure 7. Individual small plots show the total myocardial creatine (CR) value measured by proton magnetic resonance spectroscopy (^{1}H MRS). Myocardial CR in the WHH and WAH groups were significantly lower than those in the WNH and CNH groups. However, there was no significant difference in CR between the WHH and WAH groups, or between the WNH and CNH groups. $^*p < 0.05$ vs. WNH, $\#p < 0.05$ vs. CNH. *Key*: Large symbol and bar, mean ± SD; WNH, normokinetic left ventricular wall motion group; WHH, hypokinetic wall motion group; WAH, a- or dyskinetic wall motion group; CNH, normal control group.

$5.0 \pm 2.4^*\#$; CNP: 11.9 ± 4.4 μmol/g wet weight; $^*p < 0.05$ vs. WNP; $\#p < 0.05$ vs. CNP). As shown in Fig. 4, no significant differences of ATP concentrations existed among the four groups (WNP: 7.9 ± 4.3; WHP: 5.4 ± 2.2; WAP: 4.9 ± 2.5; CNP: 7.0 ± 1.8 μmol/g wet weight).

^{1}H MRS Study

There was no significant difference in heart rate among the four groups (65.7 ± 10.5, 67.7 ± 7.3, 65.2 ± 6.3, and 59.9 ± 8.9 bpm) for WNH, WHH, WAH, and CNH, respectively, by ANOVA. Figure 5 shows cardiac ^{1}H MR spectra from a normal volunteer. Figure 6 shows the ^{1}H MR spectra of typical patients in the four groups. Myocardial CR in the WHH and WAH groups were significantly lower than those in the WNH and CNH groups (Fig. 7). However, there was no significant difference in CR between the WHH and WAH groups, or between the WNH and CNH groups (WNH: 26.5 ± 4.3; WHH: $16.9 \pm 7.1^*\#$; WAH: $13.2 \pm 5.4^*\#$; CNH: 28.8 ± 3.0 μmol/g wet weight; $^*p < 0.05$ vs. WNH; $\#p < 0.05$ vs. CNH).

DISCUSSION

In the present study, we investigated whether ^{31}P and ^{1}H MRS can assess noninvasively metabolic changes in asynergic regions of patients with coronary artery disease. The ^{31}P MRS study demonstrated that PCr concentration was significanly decreased in hearts with wall motion abnormality. However, a difference in ATP concentrations could not be detected. The ^{1}H MRS study demonstrated that CR concentration was significanly decreased in asynergic regions of ischemic myocardium.

The PCr and ATP can be assessed by ^{31}P MRS, and CR can be assessed by ^{1}H MRS, noninvasively. The ^{31}P MR spectra were spatially localized by 1D CSI with slice selection in the sagittal direction (Yabe et al., 1995). The slice thickness of the selection in the sagittal direction ranged between 60 and 80 mm, and the slice was positioned so that much of it was filled by the left ventricle. The distance between the surface coil and the center of the slice ranged from 4.6 to 7.2 cm to largely include the anterior myocardium. This technique enabled us to exclude the contamination of the chest muscle and to acquire favorable MR spectra from the anterior region of the left ventricle. To yield useful S/N ratio, however, selection of a spatially larger region was needed [$2 \times (6$ to $8) \times 12.5$ cm]. Generally, the S/N ratio is proportional to voxel size and to the square root of averaging time. Due to strict time limitation of clinical examinations, larger regions needed to be selected for improving S/N ratio in the ^{31}P MRS study. In contrast, ^{1}H MRS provided favorable S/N ratio for CR spectra of smaller regions ($2 \times 2 \times 2$ cm). This is because ^{1}H MR spectra for the CR contain the three proton signals of the N-methyl group, whereas ^{31}P MR spectra for the PCr or ATP contain only one ptoton signal. Thus, the ^{1}H MRS approach was more useful in the detection of local metabolic change.

Creatine metabolism is essential for normal cardiac function and viability. Creatine is not made in the myocardium but is transported against a concentration gradient from the blood into the myocytes (Cain and Davies, 1962). The PCr was previously shown to be the primary high-energy phosphate reservoir in striated muscle (Neubauer et al., 1995). The ATP is the sole substrate for the myofibrillar ATPase and is absolutely

Copyright © Marcel Dekker, Inc. All rights reserved.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

ORDER REPRINTS

required of muscle contraction. Thus, there are many pathways for ATP synthesis including oxidative phophorylation in the heart for the maintainance of normal ATP levels to continue contraction. The creatine kinase (CK) reaction is important for the rapid resynthesis of ATP. The dephosphorylation of PCr to unphosphorylated creatine catalyzed by CK produces ATP as the essential energy source. The rate of phosphoryl exchange between PCr and ATP is faster than the rate of ATP resynthesis from other pathways (Bittl and Ingwall, 1985; Bittl et al., 1987b; Zimmer et al., 1973). Thus, this system plays an important role in myocardial energetics during hypoxia (Bittl et al., 1987a) and ischemia (Neubauer et al., 1988) and also under the conditions of acute increases in workload. To accurately assess metabolisms in the heart, therefore, quantification of CK metabolites is essential.

In the present study using ^{31}P MRS, myocardial PCr was significantly decreased in groups with wall motion abnormality when compared to controls. Thus, the PCr measurement in the human heart would be clinically useful in the evaluation of myocardial viability. At least in an animal study of acute myocardial ischemia in which PCr reflected the degree of myocardial ischemia, ATP was used as an indicator of myocardial infarct size (Takaoka et al., 1999). However, in this study, a difference in PCr concentrations between the WHP and the WAP groups could not be detected. In ATP measurements, differences in ATP concentrations could not be detected in groups with wall motion abnormality. It is possible that due to selection of a larger region, both necrotic and injured but still viable myocardium were included in WHP and WAP groups. The ^{1}H MRS study could detect the difference in CR concentrations between asynergic and normokinetic segments of human myocardium, because this study enabled us to evaluate the metabolite for smaller region as described above. However, a difference in CR concentrations between WHH and WAH groups could not be detected.

In the present study, myocardial PCr in the WNP group did not significantly differ from those in the CNP group, although the mean age in the WNP group was higher than that in the CNP group (61.5 vs. 47.2 years). A previous study suggested that both PCr and ATP in the myocardium are somewhat decreased with aging (Okada et al., 1998). However, there was no significant difference in either PCr or ATP concentrations between the two groups in the age range studied. Similarly, myocardial CR in the WNH group did not significantly differ from those in the CNH in ^{1}H MRS study (age 60.0 vs. 46.0 years) (Nakae et al., 2003).

In control hearts of our study, PCr and CR were 11.9 and 28.8 μmol/g wet weight, respectively. Thus, the ratio of PCr to total creatine (PCr/CR) was ∼0.41. This ratio was lower than that in a report of Shen et al. (1999). They reported that free creatine and PCr were 70 and 85 nmol/mg protein, respectively, by assay in canine hearts (PCr/CR, ∼0.55 by calculation). However, Bottomley and Weiss (2001) reported that PCr and CR were 10.3 and 24.7 μmol/g, respectively, in canine hearts by ^{1}H and ^{31}P MRS (PCr/CR, ∼0.42). This ratio agreed with ours. Recently, Beer et al. (2002) reported that PCr was 8.82 mmol/kg wet weight in volunteers by ^{31}P MRS. In addition, Kalsi et al. (1999) previously reported that PCr and CR were 0.23 and 10.53 μmol/g wet weight, respectively, in control specimens obtained from unused donor hearts by assay. The difference among the reports suggests technological difficulties of the metabolite quantitation.

Nonischemic region and reversible ischemic region were included in the WNP and WNH groups. The ^{31}P MRS and ^{1}H MRS at rest could not differentiate between the two regions. However, it was previously reported that myocardial ischemia was detected during hand grip exercise by ^{31}P MRS (Yabe et al., 1994). The PCr/ATP ratio was significantly decreased during exercise in the reversible ^{201}Tl defect group on exercise ^{201}Tl scintigraphy, whereas this ratio was not altered in the nonischemic group. It appears that the decrease in PCr/ATP is mostly due to decreased PCr (Takaoka et al., 1999). If exercise test had been added, the MRS study would have been more useful in the assessment of ischemic heart disease.

Recently, the combined ^{31}P and ^{1}H MRS approach was attempted in the animal heart, and it provided detailed information of in vivo myocardial CK metabolism (Bottomley and Weiss, 2001). However, when we attempted to apply this technique to clinical examiations, we encountered some difficulties. Total examination time of ^{1}H MRS in combination with ^{31}P MRS study became over 2 hr in our laboratory. It was difficult to perform both ^{31}P and ^{1}H MRS in patients with heart disease at one time. Therefore, this investigation was performed as a separate study of ^{31}P MRS and of ^{1}H MRS.

Furthermore, there were some technical limitations in the ^{31}P MRS study. One difficulty was the inability to obtain favorable ^{31}P MRS data from regions other than the anterior wall of the left ventricle. Thus, the ^{31}P MRS study was limited in patients with LAD stenosis. Several CSI methods have been proposed since the inception of MR imaging. The CSI with slice selection in the sagittal direction was applied to the

Copyright © Marcel Dekker, Inc. All rights reserved.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

ORDER REPRINTS

patients in our study. We believe that this technique minimized contamination from the surroundings. However, there might have been contributions from surrounding organs, such as signals from ATP in the liver, that occur in the absence of a transaxial slice selection. In contrast, ^{1}H MRS enabled us to gain spectra from smaller regions of the lateral wall, inferior wall, or the other region. However, CR signals were most clearly observed in the septum, probably because they were not influenced by the lipids in the epicardium or the air in the lung. In addition, the voxel was localized to $2 \times 2 \times 2$ cm^3. This size was somewhat larger than the left ventricular wall thickness (< 1.5 cm, if not left ventricular hypertrophy). Thus, this voxel may include the water of the blood in the ventricular cavity, which may underestimate CR values in ^{1}H MRS study (Bottomley and Weiss, 2001; Nakae et al., 2003).

For ^{31}P MRS studies, localized in vivo spectra of metabolites are almost invariably acquired under conditions of partial signal saturation to optimize the S/N ratio per unit time. This technique requires delays of approximately 1.5 msec between the center of the excitation pulse and the commencement of data acquisition to accomplish spatial encoding and rephasing after slice selection (Yabe et al., 1995). This limitation may cause underestimation of ATP concentration rather than PCr, because T2 time of ATP is shorter than that of PCr (Balaban, 1989; Turner and Garlick, 1984). The contamination of blood ATP from the section intersecting the blood-filled ventricular chamber was corrected by subtraction of a blood ATP signal corresponding to 15% of the total integrated 2,3-DPG signal from the β-ATP signal (Bottomley et al., 1991). However, the contribution of a blood ATP to the intensity of the ATP signal may be variable and thus affect the quantitation of the metabolite.

Futhermore, in the present study, the T1 and T2 values for PCr, ATP, and CR, respectively, were obtained from four or five healthy volunteers. The corrections were performed on the assumption that the values of T1 and T2 for all subjects were the same as those in healthy volunteers. However, these values may change with aging or various heart diseases. Thus, these calculations may cause some error in the patients and aging subjects. In addition, the calculation of CR concentrations was performed on the assumption that the values of water concentrations were the same for all hearts. However, water contents in infarcted hearts may differ from that in intact hearts. These may also cause some error in quantification of the metabolites. Despite these limitations, ^{31}P MRS and ^{1}H MRS were very useful in the evaluation of myocardial ischemia. Further investigations into its sensitivity and specificity for disease process, coupled with development of localization techniques, will demonstrate the true role of MRS in the future of cardiac diagnosis.

In summary, noninvasive ^{31}P and ^{1}H MR spectroscopy can evaluate metabolite reduction associated with wall motion abnormality in the ischemic human myocardium. Our results suggest that this approach is useful in the assessment of myocardial viability in ischemic heart disease.

ABBREVIATIONS

ATP	adenosine triphosphate
CNP(H)	normal controls
CR	total creatine
CSI	chemical shift imaging
2,3-DPG	2,3-diphosphoglycerate
HMPT	hexamethylphospholic triamide
^{1}H MRS	proton magnetic resonance spectroscopy
LAD	left anterior descending coronary artery
PCr	phosphocreatine
PDE	phosphodiesters
Pi	inorganic phosphate
PME	phosphomonoesters
^{31}P MRS	phosphorus magnetic resonance spectroscopy
PRESS	point-resolved spectroscopy
RF	radiofrequency
ROI	the region of interest
SCR/SW	ratio of creatine-to-water MR signal area
SF	saturation factor
WAP(H)	a- or dyskinetic left ventricular wall motion group
WHP(H)	hypokinetic left ventricular wall motion group
WNP(H)	normokinetic left ventricular wall motion group

REFERENCES

Balaban, R. S. (1989). NMR spectroscopy of the heart. I. *Concepts Magn. Reson.* 1:15–26.

Barker, P. B., Soher, B. J., Blackband, S. J., Chatham, J. C., Mathews, V. P., Bryan, R. N. (1993). Quantitation of proton NMR spectra of the human brain using tissue water as an internal concentration reference. *NMR Biochem.* 6(1):89–94.

Beer, M., Seyfarth, T., Sandstede, J., Landschutz, W.,

Copyright © Marcel Dekker, Inc. All rights reserved.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

ORDER REPRINTS

Lipke, C., Kostler, H., von Kienlin, M., Harre, K., Hahn, D., Neubauer, S. (2002). Absolute concentrations of high-energy phosphate metabolites in normal, hypertrophied, and failing human myocardium measured noninvasively with ^{31}P-SLOOP magnetic resonance spectroscopy. *J. Am. Coll. Cardiol.* 40(7):1267–1274.

Bittl, J. A., Ingwall, J. S. (1985). Reaction rates of creatine kinase and ATP synthesis in the isolated rat heart. *J. Biol. Chem.* 260:3512–3517.

Bittl, J. A., Balschi, J. A., Ingwall, J. S. (1987a). Contractile failure and high-energy phosphate turnover during hypoxia: ^{31}P-NMR surface coil studies in living rat. *Circ. Res.* 60:871–878.

Bittl, J. A., DeLayre, J., Ingwall, J. S. (1987b). Rate equation for creatine kinase predicts the in vivo reaction velocity: ^{31}P NMR surface coil studies in brain, heart, and skeletal muscle of the living rat. *Biochemistry* 26:6083–6090.

Bottomley, P. A. (1987). Spatial localization in NMR spectroscopy in vivo. *Ann. N.Y. Acad. Sci.* 508: 333–348.

Bottomley, P. A., Weiss, R. G. (1998). Non-invasive magnetic-resonance detection of creatine depletion in non-viable infarcted myocardium. *Lancet* 351:714–718.

Bottomley, P. A., Weiss, R. G. (2001). Noninvasive localized MR quantification of creatine kinase metabolites in normal and infarcted canine myocardium. *Radiology* 219:411–418.

Bottomley, P. A., Hardy, C. J., Roemer, P. B. (1990). Phosphate metabolite imaging and concentration measurements in human heart by nuclear magnetic resonance. *Magn. Reson. Med.* 14:425–434.

Bottomley, P. A., Weiss, R. G., Hardy, C. J., Baumgartner, W. A. (1991). Myocardial high-energy phosphate metabolism and allograft rejection in patients with heart transplants. *Radiology* 181:67–75.

Bottomley, P. A., Atalar, E., Weiss, R. G. (1996). Human cardiac high-energy phosphate metabolite concentrations by 1D-resolved NMR spectrosopy. *Magn. Reson. Med.* 35:664–670.

Bottomley, P. A., Lee, Y. H., Weiss, R. G. (1997). Total creatine in muscle: imaging and quantification with proton MR spectroscopy. *Radiology* 204:403–410.

Broderick, T. M., Bourdillon, P. D., Ryan, T., Feigenbaum, H., Dillon, J. C., Armstrong, W. F. (1988). Comparison of regional and global left ventricular function by serial echocardiograms after reperfusion in acute myocardial infarction. *J. Am. Soc. Echocardiogr.* 2:315–323.

Cain, D. F., Davies, R. E. (1962). Breakdown of ATP during a single contraction of working muscle. *Biochem. Biophys. Res. Commun.* 8:361–366.

Ernst, R. R., Anderson, W. A. (1966). Application of Fourier transform spectroscopy to magnetic resonance. *Rev. Sci. Instrum.* 37:93–102.

Kalsi, K. K., Smolenski, R. T., Pritchard, R. D., Khaghani, A., Seymour, A.-M. L., Yacob, M. H. (1999). Energetics and function of the failing human heart with dilated or hypertrophic cardiomyopathy. *Eur. J. Clin. Investig.* 29:469–477.

Lenkinski, R. E., Allman, T., Scheiner, J. D., Deming, S. N. (1989). An automated iterative algorithm for the quantitative analysis of in vivo spectra based on the simplex optimization method. *Magn. Reson. Med.* 10:338–348.

Marzullo, P., Parodi, O., Reisenhofer, B., Sambuceti, G., Picano, E., Distante, A., Gimelli, A., L'Abbate, A. (1993). Value of rest thallium-201/technetium-99m sestamibi scans and dobutamine echocardiography for detecting myocardial viability. *Am. J. Cardiol.* 71:166–172.

Mitsunami, K., Okada, M., Inoue, T., Hachisuka, M., Kinoshita, M., Inubushi, T. (1992). In vivo ^{31}P nuclear magnetic resonance spectroscopy in patients with old myocardial infarction. *Jpn. Circ. J.* 56:614–619.

Nakae, I., Mitsunami, K., Omura, T., Yabe, T., Tsutamoto, T., Matsuo, S., Takahashi, M., Morikawa, S., Inubushi, T., Nakamura, Y., Kinoshita, M., Horie, M. (2003). ^{1}H MRS can detect creatine depletion associated with the progression of heart failure in cardiomyopathy. *J. Am. Coll. Cardiol.* 42(9):1587–1593.

Neubauer, S., Hamman, B. L., Perry, S. B., Bittl, J. A., Ingwall, J. S. (1988). Velocity of the creatine kinase reaction decreases in postischemic myocardium: a magnetization transfer study of the isolated ferrit heart. *Circ. Res.* 63(1):1–15.

Neubauer, S., Horn, M., Pabst, T., Godde, M., Lubke, D., Jilling, B., Hahn, D., Ertl, G. (1995). Contributions of ^{31}P-magnetic resonance spectroscopy to the understanding of dilated heart muscle disease. *Eur. Heart J.* 16:115–118.

Okada, M., Mitsunami, K., Inubushi, T., Kinoshita, M. (1998). Influence of aging or left ventricular hypertrophy on the human heart: contents of phosphorus metabolites measured by ^{31}P MRS. *Magn. Reson. Med.* 39(5):772–782.

Sawada, S. G., Segar, D. S., Ryan, T., Brown, S. E., Dohan, A. M., Williams, R., Fineberg, N. S., Armstrong, W. F., Feigenbaum, H. (1991). Echocardiographic detection of coronary artery

Copyright © Marcel Dekker, Inc. All rights reserved.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

ORDER REPRINTS

disease during dobutamine infusion. *Circulation* 83(5):1605–1614.

Shen, W., Asai, K., Uechi, M., Mathier, M. A., Shannon, R. P., Vatner, S. F., Ingwall, J. S. (1999). Progressive loss of myocardial ATP due to a loss of total purines during the development of heart failure in dogs. A compensatory role for the parallel loss of creatine. *Circulation* 100(20):2113–2118.

Smart, S. C., Sawada, S., Ryan, T., Segar, D., Atherton, L., Berkovitz, K., Bourdillon, P. D. V., Feigenbaum, H. (1993). Low-dose dobutamine echocardiography detects reversible dysfunction after thrombolytic therapy of acute myocardial infarction. *Circulation* 88:405–415.

Snyder, W. S., Cook, M. J., Nasset, E. S., Karhausen, L. R., Howells, G. P., Tipton, I. H. (1984). *Report of the task group on reference man. International commission on radiological protection*. No. 23. Oxford: Pergamon Press, p. 116.

Takaoka, A., Nakae, I., Mitsunami, K., Yabe, T., Morikawa, S., Inubushi, T., Kinoshita, M. (1999). Renal ischemia/reperfusion remotely improves myocardial energy metabolism during myocardial ischemia via adenosine receptors in rabbits: effects of ''remote preconditioning.'' *J. Am. Coll. Cardiol.* 33:556–564.

Turner, C. J., Garlick, P. (1984). One- and two-dimensional ^{31}P spin-echo studies of myocardial ATP and phosphocreatine. *J. Magn. Res.* 57:221–227.

Yabe, T., Mitsunami, K., Okada, M., Morikawa, S., Inubushi, T., Kinoshita, M. (1994). Detection of myocardial ischemia by ^{31}P magnetic resonance spectroscopy during hand-grip exercise. *Circulation* 89:1709–1716.

Yabe, T., Mitsunami, K., Inubushi, T., Kinoshita, M. (1995). Quantitative measurements of cardiac phosphorus metabolites in coronary artery disease by phosphorus-31 magnetic resonance spectroscopy. *Circulation* 92:15–23.

Zimmer, H.-G., Trendelenburg, C., Kammermeier, H., Gerlach, E. (1973). De novo synthesis of myocardial adenine nucleotides in the rat. Acceleration during recovery from oxygen deficiency. *Circ. Res.* 32(5):635–642.

Copyright © Marcel Dekker, Inc. All rights reserved.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

Request Permission or Order Reprints Instantly!

Interested in copying and sharing this article? In most cases, U.S. Copyright Law requires that you get permission from the article's rightsholder before using copyrighted content.

All information and materials found in this article, including but not limited to text, trademarks, patents, logos, graphics and images (the "Materials"), are the copyrighted works and other forms of intellectual property of Marcel Dekker, Inc., or its licensors. All rights not expressly granted are reserved.

Get permission to lawfully reproduce and distribute the Materials or order reprints quickly and painlessly. Simply click on the "Request Permission/ Order Reprints" link below and follow the instructions. Visit the U.S. Copyright Office for information on Fair Use limitations of U.S. copyright law. Please refer to The Association of American Publishers' (AAP) website for guidelines on Fair Use in the Classroom.

The Materials are for your personal use only and cannot be reformatted, reposted, resold or distributed by electronic means or otherwise without permission from Marcel Dekker, Inc. Marcel Dekker, Inc. grants you the limited right to display the Materials only on your personal computer or personal wireless device, and to copy and download single copies of such Materials provided that any copyright, trademark or other notice appearing on such Materials is also retained by, displayed, copied or downloaded as part of the Materials and is not removed or obscured, and provided you do not edit, modify, alter or enhance the Materials. Please refer to our Website User Agreement for more details.

Request Permission/Order Reprints

Reprints of this article can also be ordered at

http://www.dekker.com/servlet/product/DOI/101081JCMR120038529