Assessment of Myocardial Creatine Concentration in Dysfunctional Human Heart by Proton Magnetic Resonance Spectroscopy

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Creatine depletion in the non-viable infarcted human heart was previously demonstrated with proton magnetic resonance (MR) spectroscopy (1H MRS). In the present study, we assessed total creatine (CR) in human hearts with non-ischemic dysfunctions such as cardiomyopathy. Using cardiac-gated 1H MRS with MR image-guided PRESS localization, we measured septal CR in healthy and diseased human hearts. Fifteen patients with chronic heart failure (CHF, left ventricular ejection fraction \(<45\%\)) and 14 age-matched normal subjects were examined. Myocardial CR was significantly (p < 0.001) lower in failing hearts (15.1 ± 5.0 μmol/g wet weight, range 8.0–22.9) than in normal hearts (27.6 ± 4.1 μmol/g wet weight, range 20.8–36.2). Myocardial CR concentrations in six heart failure patients with plasma B-type natriuretic peptide (BNP) levels of >200 pg/ml (11.5 ± 0.9 μmol/g wet weight, range 9.9–12.3) were significantly lower than those in four heart failure patients with plasma BNP levels of <200 pg/ml (19.8 ± 2.5 μmol/g wet weight, range 17.7–22.9, p < 0.001). Thus, our study showed that myocardial CR was decreased in non-ischemic dysfunctional hearts. Noninvasive measurements of myocardial CR by 1H MRS may be useful in the assessment of the severity of heart failure.

Keywords: 1H magnetic resonance spectroscopy, heart failure, myocardium, creatine

Introduction

Recently, total phosphorylated and unphosphorylated creatine (CR) in the human myocardium was noninvasively measured with proton magnetic resonance spectroscopy (1H MRS). 1H MRS with stimulated-echo acquisition mode (STEAM) localization was used on patients with ischemic heart disease to demonstrate that CR in non-viable infarcted regions was decreased compared with that in non-infarcted regions. The decrease in creatine content measured with 1H MRS was confirmed by biochemical assay at biopsy. These measurements were useful for the assessment of myocardial viability. However, 1H MRS has not yet been used to fully investigate creatine metabolism in non-ischemic dysfunctional hearts.

In the present study, we measured the CR concentration in human myocardium using cardiac-gated 1H MRS with point-resolved spectroscopy (PRESS) localization. Using 1H MRS, we compared the myocardial CR concentration in hearts with non-ischemic dysfunctions such as cardiomyopathy with that in healthy controls. We also examined the relationship between the myocardial CR concentration and plasma brain or B-type natriuretic peptide (BNP) level as an indicator of the severity of heart dysfunction.

Methods

MRS study

The Ethical Committee of the Shiga University of Medical Science approved the study protocol. MRS studies were conducted with a GE 1.5T Signa imaging/spectroscopy system (General Electric Medical Systems, Milwaukee, WI, USA). Subjects were in the supine position when examined. A Signa 1.5T General Purpose Flex Coil (GPFLEX,
GE Medical Systems) was wrapped around the chest and centered over the heart. This coil is a receive-only flexible coil designed for irregular shapes. It comprises two 13 × 17 cm loops serially connected to create a corotating “saddle coil” pair. Cardiac-gated (once per cardiac cycle) spin-echo MR imaging (MRI) was conducted to determine the location of localized volume elements (voxels) for the MRS investigation [echo time (TE), 15 ms; repetition time (TR), 700–1450 ms; field of view (FOV), 24 × 24 cm; axial view]. Voxels were localized to 8 cm³ (2 × 2 × 2 cm) in the interventricular septum by the PRESS method.³ This voxel size was chosen to yield useful signal-to-noise (S/N) ratios. Automatic shimming was performed. The spectral acquisition was gated once per two cardiac cycles with an electrocardiogram or plethysmography. The acquisition parameters were a TE of 25 ms and a TR of 1.4–2.9 s. At the acquisition, the initial data set of 16 signals was collected without water suppression for the water resonance and a data set of 128 signals was collected with water suppression for creatine resonance. The intensity of the tissue water signal from the same voxel was used as an internal concentration reference.¹⁶ A three-pulse chemical-shift-selective sequence was used to suppress the water signal. The chemical shift of water resonance was taken as 4.75 ppm, as indicated in the Signa Horizon LX™ PSD manual. Spectral peaks were identified with the following chemical shifts: cholines at 3.2 ppm, as indicated in the Signa Horizon LX™ PSD manual. Spectral peaks were identified with the following chemical shifts: cholines at 3.2 ppm, creatines at 3.0 ppm, and lipids at 0.9–1.4 ppm.¹³ ³ To confirm the accuracy of the localization of selected voxels, we collected spectra from the voxel set within the left ventricular (LV) chamber in some studies, which did not yield a creatine signal.

**Preliminary study**

Phantom studies were performed prior to the human study. MR spectra were acquired from a bottle containing 10, 20, 30 or 40 μmol/g of creatine placed in a 19-mmol/L NaCl solution.⁸ After spin-echo MRI was performed, a voxel (2 × 2 × 2 cm) was located within the bottle. The MR spectra were acquired with a TE of 25 ms and a TR of 1500 ms.

**Quantitative processing**

Quantitation of creatine spectra of the myocardium was performed with water signals without suppression as an internal concentration reference. The concentration of total creatine [CR] in the tissue filling a voxel was calculated from the tissue water concentration [W] according to the following equation¹⁷:

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

Numbers 2 and 3 indicate the two protons of water and the three protons of the N-methyl group of creatine, respectively. S_{CR} and S_W are the total creatine and water MR signal areas in the tissue, respectively. Analysis was performed with the simplex technique on a custom-built automatic data processing station. Each peak was fitted to an 80% Gaussian and a 20% Lorentzian line. In this study, myocardial tissue water content (55.5 M) was taken as 72.7% by weight.⁹ ¹⁰

\[
[W] (\mu\text{mol/g}) = 55.5 \times 0.727 \times 10^0 \tag{2}
\]

F_{CR/W} and E_{CR/W} are correction factors for T₁ and T₂ relaxation effects, respectively. The T₁ values of myocardial CR and W were estimated from the spectra of five healthy volunteers acquired at TRs of 1.5 to 6 s using PRESS without cardiac gating. The T₂ values of myocardial CR and W were obtained from the spectra of five volunteers acquired with TE of 25–45 ms.

**Study group**

Fifteen patients, 12 males and 3 females with CHF (LV ejection fraction, EF < 45%) in the New York Heart Association (NYHA) functional class II or III (Table 1) and 14 age-matched healthy volunteers, 10 males and 4 females, were included in this study. The clinical characteristics are summarized in Table 1. The averaged period between the onset of the disease and examination by MRS was 75 ± 89 months in 15 patients. There were no significant differences between the CHF and control groups in terms of age, sex, body weight or heart rate. The NYHA functional class was determined when MRS was performed. Thirteen patients (87%) had a clinical history of severe heart failure (NYHA IV). All patients underwent an MRS study when they were clinically stable. The causes of heart failure were idiopathic dilated cardiomyopathy (DCM) in 11 patients, hypertrophic cardiomyopathy (HCM, dilated phase) in one patient, cardiac amyloidosis in 2 patients, and valvular disease in one patient. Of the patients with CHF, 8 patients (53%) were treated with digitalis, 13 (87%) with diuretics, 13 (87%) with a beta-blocker, 13 (87%) with angiotensin-converting enzyme (ACE) inhibitor, and 2 (13%) with angiotensin II antagonist. All patients gave their written informed consent before the study.

**BNP measurements in patients with CHF**

In 10 inpatients with CHF, the plasma BNP levels, which could reflect the severity of heart failure, were assessed. The BNP concentrations were measured with a commercially available specific immunoradiometric assay kit for human BNP (Shionoria BNP Kit, Osaka, Japan). Nine of
Table 1. Characteristics of normal subjects and patients with chronic heart failure (CHF)

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<tr>
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<th>CHF</th>
<th>Control</th>
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<tr>
<td>n</td>
<td>15</td>
<td>14</td>
<td></td>
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<tr>
<td>Age (years)</td>
<td>52.1 ± 12.9</td>
<td>51.5 ± 11.5</td>
<td>NS</td>
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<tr>
<td>Sex (M/W)</td>
<td>12/3</td>
<td>10/4</td>
<td></td>
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<tr>
<td>Body weight (kg)</td>
<td>58.6 ± 14.1</td>
<td>62.2 ± 9.5</td>
<td>NS</td>
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<tr>
<td>Heart rate (bpm)</td>
<td>68.2 ± 15.2</td>
<td>60.6 ± 9.8</td>
<td>NS</td>
</tr>
<tr>
<td>NYHA class (n)</td>
<td>II (10)</td>
<td>III (5)</td>
<td></td>
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<tr>
<td>History of NYHA IV</td>
<td>13 (87%)</td>
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<td>Etiology of CHF</td>
<td></td>
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<tr>
<td>Dilated cardiomyopathy</td>
<td>11 (73%)</td>
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<tr>
<td>Hypertrophic cardiomyopathy</td>
<td>1 (7%)</td>
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<tr>
<td>Cardiac amyloidosis</td>
<td>2 (13%)</td>
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<tr>
<td>Valvular heart disease</td>
<td>1 (7%)</td>
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<tr>
<td>LV diastolic dimension (mm)</td>
<td>63.5 ± 13.7</td>
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<tr>
<td>LV systolic dimension (mm)</td>
<td>54.2 ± 13.6</td>
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<tr>
<td>LV ejection fraction (%)</td>
<td>31.1 ± 11.2</td>
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MRS study in normal volunteers

In five normal volunteers, the T1 values obtained were 1.48 and 1.21 s (n = 5) for CR and W, respectively, and the T2 values obtained were 135 and 33.1 ms (n = 5) for CR and W, respectively. In the present study, echo time (TE) was set to 25 ms. Therefore,

\[
F_{CR/W} = \left[ 1 - \exp \left( - \frac{TR}{1.21} \right) \right] / \left[ 1 - \exp \left( - \frac{TR}{1.48} \right) \right] \quad [3]
\]

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

Spectral acquisition was gated once every two cardiac cycles in this study: TR (s) = 120/HR (HR: heart rate, bpm). When this gating was difficult, as in the case of tachycardia, spectral acquisition was gated once every three cardiac cycles. CR concentrations in eight healthy volunteers were measured with equations [1] to [4] twice on the same day to confirm reproducibility. The repeated CR measurements yielded a coefficient of variation of 7.4%.

CR measurements in CHF patients compared with normals

Myocardial CR concentrations measured by 1H MRS in 15 patients with CHF (LVEF < 45%) were compared with those in 14 age-matched healthy volunteers. Figure 2 shows the representative cardiac-gated 1H MRS obtained from a normal volunteer, and Fig. 3 shows that from a patient with CHF. The acquisition time was approximately 5 min per single examination. A reduced CR peak was observed at 3.00 ± 0.06 ppm in CHF. As shown in Fig. 4, the myocardial CR concentrations in CHF (15.1 ± 5.0 μmol/g wet weight, range 8.0-
Fig. 1.
A: Spin-echo magnetic resonance image (MRI) of a bottle (5 cm in dia. × 9 cm in height) containing creatine solution placed in a 19 mmol/L NaCl solution. The white box shows the location of the voxel (2 × 2 × 2 cm) in the MRI.
B: MR spectrum (MRS) acquired from the voxel in a creatine solution (30 μmol/g). Two peaks are derived from creatine resonances at 3.0 (N-CH₃) and 3.9 ppm (N-CH₂).
C: Correlation between the creatine concentration (10–40 μmol/g) and creatine-to-water signal area ratio (S_Cr/S_W) measured by MRS.

Fig. 2. Spin-echo magnetic resonance image (A) and PRESS spectrum (B) from a 2 × 2 × 2 cm voxel in an interventricular septum (white box) of a 34-year-old man as a normal (NML) subject. Total creatine resonance at 3.0 ppm (arrow). Lipid resonances at 0.9–1.4 ppm.
Fig. 3. Spin-echo magnetic resonance image (A) and proton magnetic resonance spectrum (B) from a 2 × 2 × 2 cm voxel in an interventricular septum (white box) of a 79-year-old woman with hypertrophic cardiomyopathy as a chronic heart failure (CHF) patient. The creatine peak (3.0 ppm, arrow) is reduced in CHF compared with that in NML.

Fig. 4. Individual plots show the total myocardial creatine (CR) value measured by proton magnetic resonance spectroscopy in normal controls (NML, open symbols) and patients with chronic heart failure (CHF, closed symbols). Circle and bar: Mean ± SD. Myocardial CR was significantly lower (p < 0.001) in CHF than in NML.

Fig. 5. Myocardial total creatine (CR) concentrations and plasma BNP levels in 10 patients who had been hospitalized because of heart failure. Myocardial CR concentrations in heart failure patients with plasma BNP levels of > 200 pg/ml were significantly lower than those in heart failure patients with plasma BNP levels of < 200 pg/ml (p < 0.001). Individual plots show total myocardial CR value measured by proton magnetic resonance spectroscopy. Circle and bar: Mean ± SD.
concentration. Myocardial CR concentrations in heart failure patients who had plasma BNP levels of $>200$ pg/ml ($11.5 \pm 0.9 \, \mu$mol/g wet weight, range 9.9–12.3) were significantly lower than those in heart failure patients who had plasma BNP levels of $<200$ pg/ml ($19.8 \pm 2.5 \, \mu$mol/g wet weight, range 17.7–22.9, $p<0.001$).

Discussion

Cardiac-gated $^1$H MRS with MRI-guided PRESS localization enabled us to measure myocardial CR quantitatively in humans. Myocardial CR was lower in non-ischemic dysfunctional hearts than in normal hearts. Reduced myocardial CR concentrations were related to the severity of heart failure estimated from the plasma BNP levels.

Metabolic abnormalities in various organs of CHF patients have been reported with MRS. Abnormalities in phosphocreatine (PCr), inorganic phosphate (Pi) and pH in skeletal muscle were previously detected with $^{31}$P MRS. In a recent study using $^1$H MRS, creatine depletion in the non-viable infarcted human heart was shown. More recently, metabolic abnormalities of the cerebrum were also reported with $^1$H MRS in CHF patients. Thus, previous studies showed the MRS approach was useful in the detection of metabolic changes in various organs in CHF.

In the present study, we used cardiac-gated $^1$H MRS with MRI-guided PRESS localization to measure myocardial CR in patients with non-ischemic CHF. The myocardial CR in CHF patients was significantly lower than that in the control subjects. Myocardial CR concentrations in patients who had higher BNP levels were significantly lower than those in patients who had lower BNP levels. Our findings suggest that reduced CR concentrations are associated with the severity of heart failure.

Myocardial energy metabolism is extremely important for the maintenance of normal cardiac function. The dephosphorylation of PCr to unphosphorylated creatine catalyzed by creatine kinase (CK) produces ATP as an essential energy source. ATP is essential to muscle contraction. PCr was previously shown to be the primary high-energy phosphate reservoir in striated muscle. Creatine is not made in the myocardium but is transported from the blood into the myocytes. The CK system plays an important role especially in myocardial energetics during hypoxia or ischemia, since the rate of ATP supply by this system is faster than that from other pathways. It was reported that a decreased energy reserve is closely related to reduced cardiac contractile function in heart failure.

In the present study, the voxel was localized to 8 cm$^3$ ($2 \times 2 \times 2$ cm) in the interventricular septum with the PRESS method. This size was selected to yield a suitable S/N ratio, but may be somewhat larger than the wall thickness in some cases. The larger space may include the blood of the ventricular cavity. However, spin-echo MRI showed no signal intensity in the chamber. The integrated signal intensity in the larger portion was small owing to the “black blood” properties of the sequence. Even if the voxel includes a blood space of 30% in total volume, the CR value of 30 $\mu$mol/g is underestimated as being 27.6 $\mu$mol/g at the most. This difference did not critically affect our results.

Our MRS study suggests that myocardial CR is reduced in a variety of failing hearts. As shown in Fig. 3, reduced CR was observed in DCM patients who constituted the majority of the CHF group in this study. It was previously reported that the myocardial PCr-to-ATP ratio (PCr/ATP) in DCM patients was found to be low with $^{31}$P MRS and that this ratio was related to the severity of heart failure as estimated from the NYHA class and LV systolic function. Thus, the reduced PCr/ATP ratio in DCM may be caused by the reduction of myocardial CR levels, and reduced CR may be attributable to altered kinetics of the membrane-associated specific creatine transport protein.

Myocardial CR was markedly reduced in the two cases of cardiac amyloidosis in the present study, whereas myocardial CR was relatively preserved in one case of valvular disease. Because the number of patients studied was very small, however, it was unclear whether this difference is related to a difference in pathology or a difference in the stage of heart disease. Further studies would be needed to elucidate this.

In summary, our study suggests that noninvasive MRS study is useful in the assessment of the severity of disease in dysfunctional hearts.

References

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Myocardial Creatine by \(^1\)H MRS