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# $^{31}$P MR Spectroscopic Measurement of Intracellular pH in Normal Human Hearts[1]

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**Purpose:** To assess the usefulness of intracellular pH (pHi), calculated by determining the shift of a high-energy metabolite such as inorganic phosphate (Pi) or γATP after performing MRS with ECG-gated two-dimensional $^{31}$P CSI (chemical shift imaging), as a parameter for the overall state of the intracellular milieu.

**Materials and Methods:** Proton decoupled $^{31}$P CSI was performed on a 1.5-T scanner using a $^{1}$H-$^{31}$P dual-tuned surface coil. Cardiac MRS data were obtained from eight normal volunteers aged 24 - 32 years with no history of heart disease. From the spectra obtained from several regions of the heart, peak position and peak area were estimated. The metabolic ratios of α, β, γATP, PCr, Pi, phosphodiester and diphosphoglycerate were calculated, and pHi was estimated from the chemical shift of Pi and γATP resonance. We then compared the data for the anterior myocardium with those previously published.

**Results:** The major phosphorous metabolites identified in these human hearts were as follows: PCr, at - 0.1 to + 0.1 ppm; three phosphate peaks from ATP, with a chemical shift centered at about - 2.7 ppm (γATP), - 7.8 ppm (αATP), and - 16.3 ppm (βATP); and phosphodiester (PDE) at 2 - 3 ppm, inorganic phosphate (Pi) at 4.5 - 5.4 ppm, and diphosphoglycerate (DPG) at 5.4 - 6.3 ppm. The PCr/βATP ratio was 2.20±0.17 and the PDE/βATP ratio, 1.04±0.09. pHi readings were 7.31±0.23 (calculated by the shift of Pi) and 6.81±0.20 (calculated by the shift of γATP). Pi/PCR was 0.539, a ratio higher than that mentioned in previously published reports.

**Conclusion:** The measurement of intracelluar metabolism was affected by various kinds of factors. We believe, however, that pHi readings indicate the overall state of the cardiac intracelluar milieu. An unexpected pHi readings, seen at MRS, may reflect errors in the MR procedure itself and, or in the alanytical method.

**Index words :** Heart, MR spectroscopy
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The use of MR imaging (MRI) has focused on the depiction of normal and abnormal cardiac anatomy and function. ECG-gated spin-echo imaging provides high-quality static images that clearly demonstrate both cardiac anatomy and a variety of anatomic abnormalities (1 - 2). With the use of fast imaging techniques, cardiac function can be quantified: can now be measured non-invasively by means of image-guided, localized nuclear magnetic resonance spectroscopy (MRS) (3 - 11). Moreover, the use of proton and $^{31}$P MR spectroscopy provides additional information, and the sequential monitoring of both cardiac function and metabolism is thus possible.

Human cardiac spectrocsopy, a technique used extensively since the 1970 's, has so far been confined to the $^{31}$P nucleus; by means of $^{31}$P MRS, however, cardiac high-energy phosphate metabolism can be registered, thus permitting non-invasive determination of the heart 's energy state. ATP, phosphocreatine, inorganic phosphate, monophosphate esters and intracellular pH can all be quantified (12 - 15). Thus we aimed to assess the accuracy of our MRS examination and the possibility of using pHi as a parameter for the overall state of the intracellular milieu by performing MRS with ECG-gated two-dimensional $^{31}$P chemical shift imaging (CSI) and quantitatively analysing high-energy metabolite and intracellular pH (pHi).

## Materials and Methods

Proton-decoupled $^{31}$P CSI was performed on a 1.5 T scanner (Magnetom Vision Plus, Siemens, Erlangen, Germany) using a $^{1}$H-$^{31}$P dual-tuned surface coil. Scout images, on the other hand, were obtained using a body coil. By means of ECG gating, cardiac MRS data were obtained from eight normal volunteers aged 24 - 32 years with no history of heart disease. Imaging sequence parameters for $^{31}$P CSI were as follows: TR/TE = 323 msec/2.3 msec, FOV = 350 mm, slice thickeness = 40 mm. For faster MRS measurement, an 8×8 phase-encoding step was adopted during data aquisition and 16×16 or 32×32 was interpolated during law data processing. From the spectra obtained from several regions of the heart, peak position and peak area were estimated. The MR spectrum of phosphate metabolism in the lumen of the right atrium was used to accurately determine diphosphoglycerate levels (DPG); for the measurement of high-energy phosphate metabolism in the anterior myocardium, interventricular septum and right atrium of heart patients, image-guided, localized nuclear magnetic resonance (MR) spectroscopy was used, and to estimate the DPG peak from blood, measurements were obtained within the right atrium.

The metabolic ratios of α, β, γATP, PCr, Pi, PDE

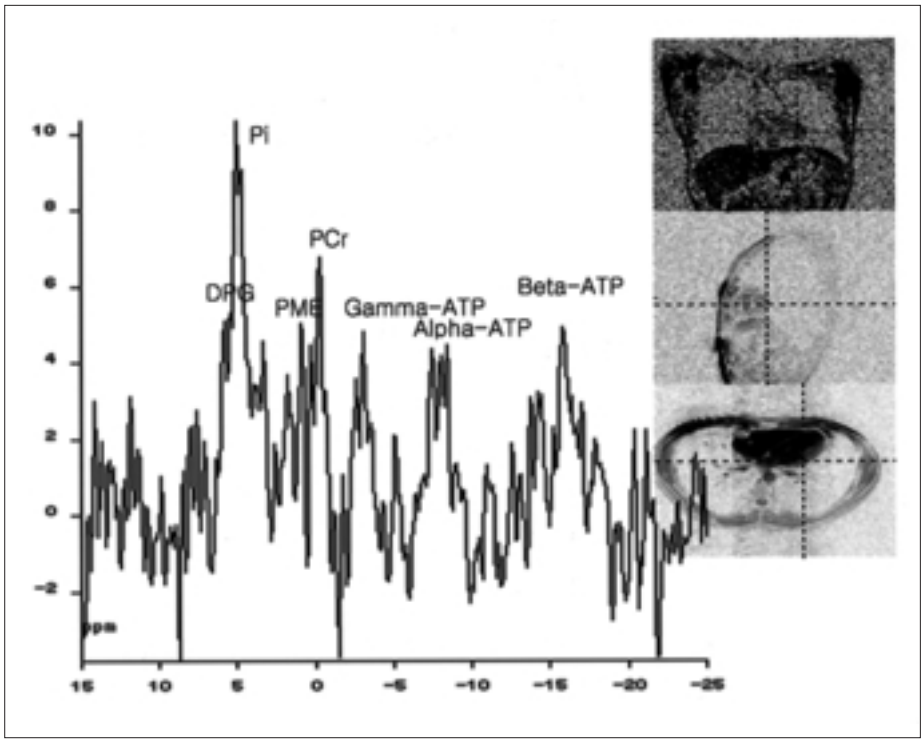


**Fig. 1.** Spectrum from chamber of right atrium of a 28-year-old healthy man. Because there is no respiration oxidation system in blood, spectrum shows low peak of PCr, contaminated from adjacent muscle. The Pi peak originating from the myocardium is obscured by the resonance of DPG in the blood, making pH difficult to be determined.

(phosphodiester) and DPG (2, 3-diphosphoglycerate) were calculated, and following the procedure adopted by Neubauer et al. (15), pHi was estimated from the chemical shift in Pi and γATP resonance.

Intracellular pH was calculated as follows:

$$pH(pi) = 6.75 + \log\left[\frac{\delta - 3.27}{5.69 - \delta}\right]$$

pKa(pi) = 6.75, δ= chemical shift (Pi)

To calculate pH from the γATP peak, the following formula, introduced by Houston et al. (20), was used:

$$pHi\ (\gamma ATP) = 0.59\ \delta^2 - 5.0\ \delta + 15.9$$

δ= chemical shift (γATP)

We then compared our for the anterior myocardium with that previously published.

## Results

In MR spectra of phosphorus metabolites in the lumen of the right atrium, relatively high DPG peaks were identified at 5.4 - 6.3 ppm (Fig. 1). All anterior myocardia in all subjects were successfully evaluated (Fig. 2). The major phosphorous metabolites idenfied were PCr at - 0.1 to + 0.1ppm; three ATP peaks occuring during chemical shift and centered at about - 2.7 ppm (γATP), - 7.8 ppm (αATP) and - 16.3 ppm (βATP); PDE at 2 - 3 ppm, and Pi at 4.5 - 5.4 ppm. The metabolic ratios were as follows: PCr/βATP = 2.20±0.17, PDE/βATP = 1.04±0.09, Pi/βATP = 1.26±0.36, DPG/βATP = 0.29±0.10, PCr/ATP = 0.69±0.05, PDE/ATP = 0.31±0.03, Pi/ATP = 0.35±0.10, DPG/ATP = 0.10±0.03, and DPG/PDE = 0.31±0.11.

**Table 1.** Results of Chemical Shift in Comparison with Published Data

| Functional group and/molecule | Results | Data from Reference (8) |
|---|---|---|
| αATP | - 7.8 | - 7.8 |
| βATP | - 16.3 | - 16.3 |
| γATP | - 2.7 | - 2.7 |
| PCr | 0.0 | 0.0 |
| Pi | 4.5 - 5.4 | 3.7 - 5.2 |

**Table 2.** Pi/PCr Ratio and Intracellular pH

| Ratio of molecule | Result | Data from Reference |
|---|---|---|
| Pi/PCr | 0.539±0.02 | |
| | | < 0.25* |
| | | 0.14±0.06† |
| Intracellular pH | 7.31±0.23(Pi) | |
| | 6.81±0.20 (γATP) | |
| | | 7.15±0.2* |
| | | 7.15±0.03† |

*: Data from reference (16), †: Data from reference (11)

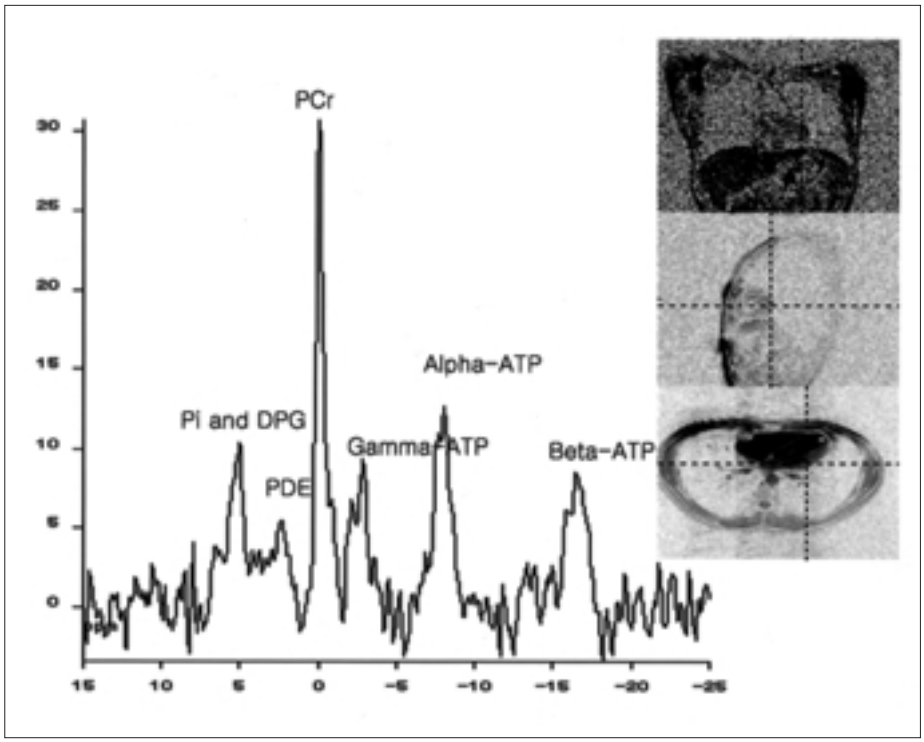


**Fig. 2.** Spectrum from left ventricular wall of a 28-year-old healthy man. It is difficult to differentiate the peak of Pi from that of DPG. But the high energy metabolite peaks such as PCr and ATP are readily readable.

pHi were 7.31±0.23, calculated with the shift of Pi; and 6.81±0.20, calculated from the shift of γATP. We compared our results with those previously published (Tables 1 and 2): Pi/PCR was 0.539, which was higher than previously determined.

## Discussion

$^{31}$P MRS is an attractive method for the non-invasive measurement of myocardial levels of high-energy phosphate and pHi, and cardiac $^{31}$P MRS has research applications in at least three clinical areas. In addition, a biochemical stress test for non-invasive ischemia detection and viability assessment using MRS may become feasible (17 - 20). Patients with non-Q wave myocardial infarction exhibit moderate thinning of the myocardial wall, persistent contractile dysfunction and significant reductions in regional MIBI uptake and myocardial energy reserves, as reflected by a reduced PCr/ATP ratio (21). The phosphocreatine/ATP ratio may provide an independent index for the grading of heart failure, permit monitoring of the long-term effects of different forms of drug therapy on cardiac energy metabolism in heart failure, and be able to provide prognostic information on survival (22 - 23). However, if the findings of MRS are obscure, this may hinder their clinical application; in that it provides guidelines, a baseline study is therefore important. We performed high-SNR multivoxel 31-phosphorus cardiac MRS, using ECG-gating, obtaining data sets of quantitative measurements of phosphorus metabolites and calculating pHi. It was uncertain, however, whether the data obtined represented the intracellular milieu of cardiac muscle cells; initially they were distorted, and we modified the analytical method. The most difficult task was to differentiate between the Pi peak and the DPG peak: to clear these, we obtained a blood-void spectrum of the PCr peak. DPG has two phosphate and doublet peak near Pi and PME. The Pi/PCr ratio was higher than those of references, probably because of contamination of the Pi peak by that of DPG, which was apparent when pHi was calculated. In isolated buffer-perfused hearts, phosphorus MRS can measure myocardial tissue pH from the chemical shift of Pi, but in vivo, the Pi peak originating from the myocardium is obscured by the resonance of DPG in the blood, making it difficult to determine pH.

Magnetic resonance imaging and localized $^{31}$P magnetic resonance spectroscopy can be used to assess, respectively, myocardial perfusion and energy metabolism. In an experimental animal model, the attenuation of intracellular acidosis by propranolol during myocardial ischemia was evident at MR spectroscopy (18). During tissue hypoxia induced by arterial hpoxia, as well as during ischemia, a fall in intracellular pH can be problematic, particularly in tissue with limited resistance to hypoxia or limited capacity for anaerobic metabolism. pHi is regarded as a cornerstone in the evaluation of ischemic or injured cells (25 - 28), and although the precise mechanism of cell death remains unknown, theoretical hypotheses such as coagulation necrosis, including infarction, suggest that following the depletion of high energy metabolites, pHi decreases (24). The measurement of pHi is useful for evaluating the state of muscles in ischemic conditions. Moreover, because cardiac muscle cells use fatty acid rather than glucose as a source of energy, they seldom produce lactic acid, and cause reductions in pHi levels. To date, a number of experimental studies have demonstrated that for several organic phosphates, pH depends on chemical shift as a functioning of the state of inonization (12 - 14). The chemical shifts of phosphorus metabolites are very complex, with no linear changes, and correspond to change in pH. Ideally, the δ(chemical shift) parameter shows linear change between pH 6.8 and pH 8.0, and is either relatively unaffected or predictably affected by cations (other than proton) such as magnesium. It is derived from readily observable 31-phosphrus nuclear magnetic resonance images whose deltas can be accurately assesed under all physical conditions. Under pH 6.0 - 8.0, chemical shifts of DPG, Pi, and γATP are more sensitive than those of other phosphorus metabolites. DPG in red blood cells combines with hemoglobin, causing a decrease in its affinity for oxygen; displacement of the oxyhemoglobin dissociation curve to the right reflects the tissue oxidation state. Unfortunately, however, regions of interest of MRS are too large to separate cardiac tissue from blood. In order to accurately determine the DPG position which descriminated between the Pi peak and DPG, Pi and γATP (usually used to indicate pHi, and with a cellular concentration ten times higher than that of ECF), we measured MR spectra in the lumen of right atrium.

Over a pH range of 6.0 - 7.3, the chemical shift of Pi varies from 3.7 to 5.2 ppm relative to PCr: our results showed a figure for this of 4.5 - 5.4 (average, 5.0) ppm, and average pHi was 7.31±0.23. According to the published data, pHi was lower than pH of ECF and changes in pHi were smaller than those occuring in the pH of

ECF. There was, in other words, a response to changes in arterial pH, such as the hypoxic state, which demonstrated an effective myocardial intracelluar proton-buffering mechanism. In experimental study (29) in which a linear function was applied to the results, the relationship between arterial pH and pHi was as follows:

$$pHi = 0.23 \text{ arterial pH} + 5.43$$

The normal pHi of cardiac muscle is thus expected to be around 7.1, a level from which our data deviated slightly. In normal heart, Pi is small and difficult to resolve unambiguously from blood DPG. The method of calculating the chemical shift between Pi and PCr becomes problematic if Pi drops to a very low level, or if its peak overlaps another. Indeed, in our data, Pi peak were obscured by DPG blood peaks from time to time.

An alternative way of calculating pHi is to use shift of γATP. The average shift between γATP and βATP for stable working heart has been shown to be 13.69 ppm: our figure was 13.60 ppm and a pHi (ATP) 6.81 was lower than expected. Figure 1 shows that ATP peaks, which represent the blood concentration of ATP, are relatively distinct compared with PCr peaks. To more accurately estimate ATP levels, contaminated blood-ATP peaks should be eliminated (11).

We believe that pHi measurements can be distorted by various factors, including both the scanner itself and the software employed, and also by the application methods used. Our date showed that blood and skeletal muscle signals can contaminate MRS data. We conclude that the pHi can be an overall parameter for the cardiac intracellular milieu, but that unexpected pHi levels revealed by MRS in healthy human hearts may be due to errors in the MR protocol applied or the analytical methods. Nonetheless, the measurement of pHi may be useful for assessing the accuracy of MRS, and pHi may be a good parameter for determining the overall state of the intracellular milieu.

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# 정상 인체 심장에서 인자기공명분광법을 이용한 세포내 pH의 측정[1]

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윤성원 · 김혜정 · 김성훈 · 김용주 · 강덕식

**목적:** 심전도 게이트 2차원적인 화학적 전환 영상 (ECG - gated two - dimensional $^{31}$P chemical shift imaging)를 이용한 인간 심근의 자기공명분광을 시행하여, inorganic phosphate (Pi)나 γ- ATP 등의 고에너지 대사물의 위상전위로부터 세포내 pH (pHi)를 측정한후, 세포내 pH가 세포내 환경을 총괄적으로 반영하는 지표로 사용할 수 있는지 알아보려 하였다.

**대상과 방법:** 수소원자 - 31인원자 이중의 화학적 위상 전환 영상 (chemical shift imaging:CSI)을 1.5 T 자기공명영상기기와 $^{1}$H - $^{31}$P 쌍 방향 표면 코일을 이용하여, 이전에 심장질환이 없는 8명 (나이: 24 - 32 세)의 정상인으로부터 심근 자기공명분광을 얻었다. 심장의 여러 영역으로부터 얻은 스펙트럼으로부터, 봉오리 위치와 봉오리 영역을 측정하였다. Alpha - , β- , γ- ATP, PCr, Pi, PDE and DPG 의 대사비를 측정하였다. Pi와 γ- ATP의 위상전위로부터 pHi를 계산하였으며 결과를 이전에 보고된 결론과 비교하였다.

**결과:** 확인된 주요 인간 심장의 인대사물은 - 0.1에서 +0.1 ppm에서 PCr이, ATP로부터 각각 약 - 2.7 ppm ( γ- ATP), - 7.8 ppm ( α- ATP) 그리고 - 16.3 ppm ( β- ATP)의 화학적 전위 중심에 인산의 봉우리를 보였으며, phosphodiester (PDE)는 2 - 3 ppm, inorganic phosphate(Pi)는 4.5 - 5.4 ppm 그리고 diphosphoglycerate (DPG)는 5.4 - 6.3 ppm에서 보였다. 대사물의 비는: PCr/β- ATP=2.20±0.17, PDE/β- ATP=1.04±0.09, Pi/β- ATP=1.26±0.36, DPG/β- ATP=0.29±0.10이었으며, pHi 는 Pi의 전위로부터 계산된 것은 7.31±0.23이었으며, γ- ATP로부터 계산된 값은 6.81±0.20이었으며, 예측치는 7.1이었다. Pi/PCr 는 0.539 였으며, 이는 기존의 발표된 결과보다 높았으며, Pi의 위상전위로부터 얻은 pHi는 예상되는 pHi와 비교시에도 높았다.

**결론:** pHi는 MRS 시행에 영향을 미치는 다양한 요인들에 영향받으나 세포내 환경을 반영하는 지표로 생각되며, 정상환자에서 기대되지 않는 pHi가 계산 될 경우, MR 시행 자체나 뿐 아니라, 분석 방법에 대한 재고가 있어야 할 것으로 생각된다.