Cardiac energetics alteration in chronic hypoxia rat model: a non invasive in vivo 31P magnetic resonance spectroscopy experimental study

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Research Article

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Abstract

**Background:** Energetics alteration plays a key role in the process of myocardial injury in chronic hypoxic diseases (CHD). $^{31}$P magnetic resonance spectroscopy (MRS) can investigate alterations in cardiac energetics *in vivo*. This study was aimed to characterize the potential value of $^{31}$P MRS in evaluating cardiac energetics alteration of chronic hypoxia rats (CHR).

**Methods:** Twenty-four CHRs were induced by SU5416 combined with hypoxia, and six rats were raised as control group. $^{31}$P MRS was performed weekly and the ratio of concentrations of phosphocreatine (PCr) to adenosine triphosphate (ATP) (PCr/ATP) was obtained. The index of cardiac structure and systolic function parameters, including the right ventricular function (RVEF), right ventricular end-diastolic volume index (RVEDVi), right ventricular end-systolic volume index (RVESVi), the left ventricular function parameters were also measured.

**Results:** The declension of resting cardiac PCr/ATP ratio in CHR was observed at the 1$^{st}$ week, compared to control group (2.90±0.35 vs. 3.31±0.45, $p$ =0.045), while the RVEF/RVEDVi and RVESVi decreased at the 2$^{nd}$ week ($p$<0.05). The PCr/ATP ratio displayed a significant correlation with RVEF ($r$ = 0.605, $p$ = 0.001), RVESVi ($r$ = -0.661, $r$ = -0.703; $p$=0.001).

**Conclusions:** $^{31}$P MRS can early detect the cardiac energetics alteration in CHR model before the onset of ventricular dysfunction. The decrease of PCr/ATP ratio likely revealed myocardial injury and cardiac dysfunction.

Background

Chronic hypoxia is a common pathophysiological process of chronic hypoxic diseases (CHD) such as chronic obstructive pulmonary disease, interstitial lung disease, and chronic high altitude disease [1]. Chronic hypoxic can not only lead to myocardial injury by an irregulation of energetics alteration in cardiac myocytes, it can also lead to pulmonary hypertension (PH) under unfavorable conditions, finally, heart failure occurs [2]. However, hypoxia-induced PH progresses slowly, patients often show subclinical symptoms in the early stage [3]. Thus, an earlier detection for myocardial injury and clinically intervention is necessary. A number of data suggest that alterations in cardiac energy balance might be an early feature in the course of cardiac disease before myocardial injury [4, 5].

Currently, there are several non-invasive approaches to evaluate myocardial injury. Serum cardiac biomarkers tend to be sensitive, but not specific enough, and are limited by the time of myocardial injury. Doppler ultrasound cannot indicate myocardial injury directly. Although radionuclide myocardial perfusion imaging can reveal myocardial activity, its application is limited due to its radioactivity and poor tissue resolution.

Cardiac magnetic resonance (CMR) imaging has been used as the gold standard technique for assessing ventricular structure and function. It can also allow evaluation of myocardium energetics by phosphorus magnetic resonance spectroscopy ($^{31}$P MRS) [6]. In the mammalian, cardiac energy balance mainly refers to the dynamic homeostasis of phosphocreatine (PCr) and adenosine triphosphate (ATP) within the mitochondria. However, as a traditional biochemical analysis, invasive tissue biopsy may lead to break down of high-energy phosphates, in particular of PCr [7]. $^{31}$P MRS may be a potential tool to reflect the hypoxic energetic alteration by obtaining the ratio of concentrations of PCr to ATP(PCr/ATP) *in vivo* [6]. Previous studies usually majored in diabetic cardiomyopathy or hypertrophic cardiomyopathy, only a few previous studies investigated hypoxic myocardium abnormalities [8, 9], limited evidence is available on the time course of disruption in myocardial energy balance. The
progress of chronic hypoxic cardiac energetics alteration can be observed by $^{31}$P MRS in this following-up study, and the onset of energetics alteration may be a potential indicator for early assessment of CHD. This study aimed to characterize the potential value of $^{31}$P MRS in evaluating cardiac energetics alteration of chronic hypoxia rats (CHR).

**Methods**

**Animals**

This study was performed in adherence with the National Institutes of Health Guidelines for the Use of Laboratory Animals, and was approved by the Ethical Review Committee of Experimental Animal Welfare of Nanjing Medical University (IACUC-1712011). Thirty male Sprague-Dawley rats weighing 230±10g were obtained and twenty-four rats were given a single subcutaneous injection of SU5416 (20mg/kg, MCE, HY-10374). Then, they were placed into normobaric hypoxia chambers (CYES-II; Shanghai Anting Scientific Instrument Factory) with 10±1% oxygen. The CHRs were divided into four groups (1w/2w/3w/5w, n=6 respectively). The remaining six rats were raised as the control group.

$^{31}$P magnetic resonance spectroscopy

All the MR procedures were performed weekly in 1,2,3,5weeks of modeling separately on a 7.0 Tesla horizontal magnet (Bruker BioSpec 7T). Animals were anesthetized using gaseous isoflurane in oxygen (3% box induction), and maintained at 0.5-2.5% during the entire MR procedures.

In this study, we took a method called “outer volume suppression” as a reference and optimized it [10]. A 20mm diameter dual-tuned ($^{31}$P/$^1$H) surface coil was used for transmission and reception. A standard non localized pulse-acquisition sequence with 6 spatially selective saturation bands surrounding the volume of interest were used to avoid the signal contamination from the structures around the heart. Acquisition parameters were: 50 μsec block pulse, 23°flip angle, 1.5 s repetition time, 10kHz band width (BW), 128 averages.

Cardiac energy status was evaluated by means of PCr/ATP ratio calculated by dividing PCr with $\gamma$-ATP resonances using the spectral analysis software (TopSpin versions 4.0 ) (Fig. 1).

Magnetic resonance imaging for ventricular size and function

The system was equipped with a 72-mm inner diameter volume coil. The heart of was placed at the center of the coil. Prospective-ECG and respiratory-gated cine images were acquired for getting four-chamber and short-axis views of heart, with the following parameters: TE=2.5ms, TR=8ms, flip angle=15°, field of view=4.7*5.0cm, 192×192 matrix, in-plane resolution=244 μm, slice thickness=1.5mm, and 14-23 frames/heart beat. A set of 5 short-axis images was used to cover the heart.

Left and right ventricular volumetric parameters were obtained using the image analysis software (Circle CVI 42, Circle Cardiovascular Imaging Inc.) by outlining the end myocardium of both ventricles during end-systolic and end-diastolic phases. The volumes of ventricles were indexed to body surface area (BSA), which is calculated by the formula of BSA(m$^2$)=0.91*weight(g)$^{2/3}$/1000. The right ventricular ejection fraction (RVEF), right ventricular end-
diastolic volume index (RVEDVi), right ventricular end-systolic volume index (RVESVi), left ventricular ejection fraction (LVEF), left ventricular end-diastolic volume index (LVEDVi), and left ventricular end-systolic volume index (LVESVi) were acquired.

**Hemodynamic measurements**

Every rat was performed with right heart catheter after MR procedures. Rats were weighed and anesthetized with 1.0 g/kg urethane (Sigma-Aldrich), administered intraperitoneally. A polyethylene catheter (PE10, 427400; BD Biosciences,) and heparinsaline (125 U/ml; Changzhou Yinsheng Pharmaceutical Co., Ltd.) was inserted into the right jugular vein and advanced into the right ventricle. The catheter was connected to an MPA Acquisition and Analysis system (MP100; BIOPAC Systems, Inc.) by a pressure transducer (TSD104A; BIOPAC Systems Inc.). The right ventricular systolic pressure (RVSP) was recorded using a multiparameter monitor PM-8000 (Zhuhai Joyful Medical Equipment Co.) [11].

Following measurements of hemodynamic parameters and blood sample collection, the rats were sacrificed by cervical dislocation, and the thorax was opened. The heart was removed and the right ventricle (RV), left ventricle (LV) were separated. The mass ratio of RV to LV (RV/LV) was evaluated.

**Histological analysis**

The cardiomyocyte hypertrophy was observed based on hematoxylin and eosin (H&E) staining. The myocardial fibrosis was assessed using Masson’s trichrome staining. Three 1mm³ pieces were taken from the RV, LV and S separately for transmission electron microscope (TEM) observation. Myocardial mitochondria was observed under the JEOL-1010 TEM.

**Statistical analysis**

Experimental measurement data was expressed as Mean ± SD () and was statistically processed by SPSS 24.0 software (IBM Corporation) using one-Way ANOVA analysis followed by paired or unpaired t-test. Pearson correlation analysis was used for correlation analyses. Results were considered significant at \( p<0.05 \).

**Results**

**Morphometric and hemodynamic in CHRs**

RVSP and RV/LV increased continuously during hypoxia in CHRs, they gained significantly changes in the 1st week of modeling. During this experiment, one healthy rat died of right heart catheter and one CHR died of a surgical operation in the 2nd week of hypoxia (Table 1).

RVEF significantly decreased and RVEDVi and RVESVi increased from the 2nd week. LVEF showed a brief decline in the 1st week, and then recovered in the 2nd and 3rd week. Both LVEF and LVEDVi got a significant reduction in the 5th week for a long hypoxia exposure (Table 2) (Fig. 2).
In vivo $^{31}$P magnetic resonance spectroscopy

$^{31}$P MRS provided the capacity to follow-up the cardiac PCr/ATP ratio in vivo. The PCr/ATP ratio dropped weekly, and it showed significantly decline in the 1st week of hypoxia vs. control group ($p<0.05$) (Table 3) (Fig. 3).

Correlation of hemodynamics with CMR characteristics and cardiac PCr/ATP

RVEF showed a negative correlation with RVSP ($r=-0.798, p<0.001$). RVESVi also positively correlated with RVSP. Cardiac PCr/ATP ratio showed a positive correlation with the RVEF ($r=0.605, P<0.05$), and inverse correlation with RVEDVi ($r=-0.661, p=0.001$) and RVESVi ($r=-0.703, p=0.001$). Cardiac PCr/ATP also showed a negative correlation with RVSP ($r=-0.579, p=0.002$) (Fig. 4).

Discussion

This study reported a follow-up study on the course of CHD development in CHR using $^{31}$P MRS. The decline of myocardial PCr/ATP ratio was observed in the 1st week, while ventricular structure and function index got no modification until the 2nd week of molding. These results indicate that the myocardial energetics alteration occurs before ventricular dysfunction. Furthermore, $^{31}$P MRS is a potential tool for detecting the severity of CHD. As far as the authors are aware, this is the first study in this area.

Hypoxia exposure induced hypoxic myocardial injury and angio-obliterative PH in rats [12-13]. Persistent RV hypertrophy and decreased RV function can be observed from the 2nd week of modeling while the LV appeared insensitive to the decrease in oxygen. This result demonstrates that pathological changes related to hypoxia mainly occur in the RV, numerous studies previously showed the same conclusion [14, 15]. In this study, an interesting decrease in LVEF demonstrated in the 1st week of hypoxia and recovered in the 2nd week, it may due to compensating for decreased oxygen availability, or just individual fluctuations. Karina et al. [16] also pointed out that LV dysfunction can present in any severity of CHD. In contrast to RV dilation, the LV volume decreased in the 5th week, probably due to interventricular septal swing during the late stage of the disease. The strong correlation between RVEF and RVSP indicated that RV function indicators can reflect the course of PH.

However, due to the adaption to chronic hypoxia, RV showed less prone to develop contractile dysfunctions at the beginning. It is necessary to seek a method that can sensitively reflect myocardial damage earlier. $^{31}$P MRS has previously been applied in hypertrophic cardiomyopathy, diabetic cardiomyopathy and ischemic cardiomyopathy for myocardial energetics alteration detection, here we applied it in hypoxic cardiomyopathy.

$^{31}$P MRS provide myocardial metabolism information by detecting high-phosphate energy metabolism (PCr/ATP ratio) in cardiac myocytes. In this study, myocardial PCr/ATP ratio drops significantly in the first week before the modification of ventricular structure and function parameters. As expected, an increasing number of mitochondrial autophagy in cardiomyocytes was observed in the 1st week of hypoxia. Cardiac metabolism principally relies on oxidative metabolism. And adequate supply of oxygen and metabolic substrates is a major prerequisite. Chronic hypoxia can lead to irregular cardiac energetic which can be implicated in the pathophysiology of heart [17, 18]. In response to chronic hypoxic exposure, myocardial mitochondrial function was impaired, and the creatine kinase
system was also affected. The transfer of high-energy phosphate groups of intracellular ATP to PCr was blocked, resulting in a decrease in intracellular PCr levels. At the same time, ATP levels generated via mitochondrial lactate oxidation was decreased [17, 19]. Finally, hypoxic cardiac myocytes necrosis or apoptosis occurred. Thus, the decline of cardiac PCr/ATP ratio that obtaining by $^{31}$P MRS may rather be an early feature in the course of myocardial injury. Therefore, we speculate that $^{31}$P MRS is a reliable tool for detection of myocardial injury earlier, and a more sensitive method compared with traditional cardiac structural and functional parameters in CHD.

PCr/ATP ratio also showed a moderate positive correlation with the RVEF, and inverse correlation with RVEDVi and RVESVi. Therefore, it indicates that $^{31}$P MRS could be a potential tool for detecting the severity of CHD. Nowadays, clinical treatment of CHD is always focused on against inflammatory, hypoxic and PH. Only a few treatments for targeting myocardial injury are involved [15]. With $^{31}$P MRS, an earlier clinically intervention preventing myocardial injury is important to improve the prognosis of CHD.

There are several limitations in our study. First, this study gained a higher cardiac PCr/ATP ratio (2.49-3.31) than some previous studies (1.72-2.70) [10,20-21]. The muscle on the chest wall may resulted in a higher myocardial PCr/ATP ratio [22]. In order to avoid the signal contamination from the chest wall and other structures around the heart, six spatially selective saturation bands were placed around the volume of interest in this study. Second, a standard non localized pulse-acquisition sequence was chosen in this $^{31}$P MRS scanning, a localized scan may help get a more accurate result. Also, cardiac strain analysis may be more sensitive than volume parameter changes, but the relevant strain analysis was not involved in this study.

**Conclusions**

In conclusion, CMR is a reliable tool for observing modification of cardiac size and function during the progression of CHD. $^{31}$P MRS is a potential non invasive tool for analysis of myocardial energy homeostasis in CHD *in vivo*. It can sensitively reveal the cardiac energetics alteration in CHD before the onset of ventricular dysfunction and reflect its severity.

**Abbreviations**

CHD: chronic hypoxic diseases; PH: pulmonary hypertension; CMR: cardiac magnetic resonance; $^{31}$P MRS: phosphorus magnetic resonance spectroscopy; PCr: phosphocreatine; ATP: adenosine triphosphate; CHR: chronic hypoxia rats; BSA: body surface area; RVEF: right ventricular ejection fraction; RVEDVi: right ventricular end-diastolic volume index; RVESVi: right ventricular end-systolic volume index; LVEF: left ventricular ejection fraction; LVEDVi: left ventricular end-diastolic volume index; LVESVi: left ventricular end-systolic volume index; RVSP: right ventricular systolic pressure

**Declarations**

**Ethics approval and consent to participate**

This study was performed in adherence with the National Institutes of Health Guidelines for the Use of Laboratory Animals, and was approved by The Ethical Review Committee of Experimental Animal Welfare of Nanjing Medical University (IACUC-1712011).
Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Authors’ contributions

YSZ and YX designed and directed the study. YSZ, YX, XHY, XMZ, WYL, WQ and YC participated in the acquisition of the data, the literature search, the performance of the statistical analysis and the interpretation of the data. XHY and YSZ drafted and revised the article. YX addressed language issues and took part in revision of the article also. All authors read and approved the final article.

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Competing interests

The authors declare that they have no competing interests.

Acknowledgements

Not applicable.

References


Tables

Table 1 Morphometric and Hemodynamic Characteristics (±s)
<table>
<thead>
<tr>
<th></th>
<th>Control (n=6)</th>
<th>1w (n=6)</th>
<th>2w (n=6)</th>
<th>3w (n=6)</th>
<th>5w (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSA (m²)</td>
<td>0.045±0.01</td>
<td>0.037±0.00</td>
<td>0.038±0.00</td>
<td>0.040±0.00</td>
<td>0.049±0.00</td>
</tr>
<tr>
<td>RVSP (mmHg)</td>
<td>26.11±2.10 (n=5)</td>
<td>35.56±6.22*</td>
<td>40.05±4.19*</td>
<td>45.79±10.17*</td>
<td>57.75±10.31*</td>
</tr>
<tr>
<td>RV/LV</td>
<td>0.31±0.01</td>
<td>0.45±0.04*</td>
<td>0.51±0.07*</td>
<td>0.81±0.14*</td>
<td>0.90±0.12*</td>
</tr>
</tbody>
</table>

BSA body surface area, RVSP Right ventricular systolic pressure, RV/LV the mass of right ventricle (RV) divided by the sum of the mass of left ventricle (LV)

Data are presented as mean ± SD;

* p<0.05 vs. control group

**Table 2** Cardiac magnetic resonance Characteristics (±s)
<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>1w</th>
<th>2w</th>
<th>3w</th>
<th>5w</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Heart Rate</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>310±48</td>
<td>395±31*</td>
<td>360±33*</td>
<td>396±55*</td>
<td>344±16</td>
</tr>
<tr>
<td><strong>Respiratory Rate</strong></td>
<td>52±6</td>
<td>53±12</td>
<td>55±23</td>
<td>49±5</td>
<td>46±7</td>
</tr>
<tr>
<td><strong>RVEF(%)</strong></td>
<td>41.44±0.02</td>
<td>39.92±3.57</td>
<td>34.45±2.42*</td>
<td>31.74±1.94*</td>
<td>23.71±4.77*</td>
</tr>
<tr>
<td><strong>RVEDVi (ml/m²)</strong></td>
<td>4.04±0.89</td>
<td>4.61±0.23</td>
<td>5.30±0.44*</td>
<td>5.88±0.65*</td>
<td>5.33±1.15*</td>
</tr>
<tr>
<td><strong>RVESVi (ml/m²)</strong></td>
<td>2.37±0.54</td>
<td>2.78±0.27</td>
<td>3.48±0.31*</td>
<td>4.02±0.48*</td>
<td>4.08±0.97*</td>
</tr>
<tr>
<td><strong>RVCO (mL/min)</strong></td>
<td>23.06±4.94</td>
<td>26.70±0.91</td>
<td>25.17±3.40</td>
<td>29.22±3.11*</td>
<td>20.55±3.11</td>
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<tr>
<td><strong>RVCI (mL/m²)</strong></td>
<td>524.42±170.89</td>
<td>721.12±23.98</td>
<td>654.75±74.34</td>
<td>732.27±85.16</td>
<td>429.42±101.11</td>
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<tr>
<td><strong>LVEF</strong></td>
<td>57.13±4.83</td>
<td>50.73±5.11*</td>
<td>58.15±2.71</td>
<td>51.32±4.38</td>
<td>47.96±6.67*</td>
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<tr>
<td><strong>LVEDVi (ml/m²)</strong></td>
<td>9.89±1.90</td>
<td>9.25±1.04</td>
<td>9.03±0.65*</td>
<td>8.62±0.49</td>
<td>7.55±1.48*</td>
</tr>
<tr>
<td><strong>LVESVi (ml/m²)</strong></td>
<td>4.27±1.17</td>
<td>4.59±0.97</td>
<td>3.77±0.27</td>
<td>4.18±0.188</td>
<td>4.02±1.00</td>
</tr>
<tr>
<td><strong>LVCO (mL/min)</strong></td>
<td>79.52±22.266</td>
<td>67.88±4.02</td>
<td>72.78±12.08</td>
<td>69.42±7.72</td>
<td>58.68±14.00</td>
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<tr>
<td><strong>LVCI (mL/m²)</strong></td>
<td>1766.54±538.61</td>
<td>1834.97±136.43</td>
<td>1897.98±309.84</td>
<td>1738.27±189.60</td>
<td>1220.31±370.72*</td>
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</table>

*RVEF right ventricular ejection fraction, RVEDV right ventricular end-diastolic volume, RVEDVi right ventricular end-diastolic volume index, RVESV right ventricular end-systolic volume, RVESVi right ventricular end-systolic volume index, RVCO right ventricular cardiac output, RVCI right ventricular cardiac index, LVEF left ventricular ejection fraction, LVEDV left ventricular end-diastolic volume, LVEDVi left ventricular end-diastolic volume index, LVESV left ventricular end-systolic volume, LVESVi left ventricular end-systolic volume index, LVCO left ventricular cardiac output, LVCI left ventricular cardiac index

Data are presented as mean ± SD;

* p<0.05 vs. control group
Table 3  Cardiac PCr/ATP ratio (±s)

<table>
<thead>
<tr>
<th>Time</th>
<th>Control</th>
<th>1w</th>
<th>2w</th>
<th>3w</th>
<th>5w</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCr/ATP</td>
<td>3.31±0.45</td>
<td>2.9±0.35*</td>
<td>2.74±0.35*</td>
<td>2.57±0.23*</td>
<td>2.49±0.16*</td>
</tr>
</tbody>
</table>

PCr phosphor creatine concentration, ATP adenosine triphosphate concentration

Data are presented as mean ± SD;

* p<0.05 vs. control group

Figures

Cardiac energy status was evaluated by means of PCr/ATP ratio calculated by dividing PCr with γ-ATP resonance.

Figure 1
Cardiac magnetic resonance imaging: a-e: Short-axis cardiac images of end diastole in healthy rats and chronic hypoxia rats (1,2,3,5 weeks of modeling); f-j: Short-axis cardiac images of end systole in healthy rats and chronic hypoxia rats (1,2,3,5 weeks of modeling). Right ventricular dilation can be seen during hypoxic exposure. Interventricular septal flat can be observed at the late stage of the disease.
Figure 3

a-b: Myocardial PCr/ATP ratio drops significantly in the 1st week of hypoxia. c-d: An increasing number of mitochondrial autophagy in cardiomyocytes can also be observed at the same time (TEM 25000X).
Figure 4

a-b: Myocardial PCr/ATP ratio drops significantly in the 1st week of hypoxia. c-d: An increasing number of mitochondrial autophagy in cardiomyocytes can also be observed at the same time (TEM 25000X).