Abnormalities in lysine degradation are involved in the regulation of early stage compensated cardiac hypertrophy in pressure-overloaded rats

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Research article

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Abstract

Background

Cardiomyocyte metabolism changes before cardiac remodeling. However, its role in early detection of cardiac hypertrophy remains unclear. This study investigated the early changes in serum metabolomic in a pressure overload cardiac hypertrophy model induced by transverse aortic constriction (TAC).

Methods

The TAC model was constructed by partly ligating the aortic arch. Twelve Sprague-Dawley rats were randomly divided into the TAC group (n = 6) and sham group (n = 6). Three weeks after the surgery, cardiac echocardiography was performed to assess cardiac remodeling and function. HE and Masson staining were used to observe the pathologic changes. The plasma metabolites were detected by UPLC-QTOFMS and Q-TOFMS. The specific metabolites of the model were screened by orthogonal partial least squares discriminant analysis (OPLS-DA). The metabolic pathways were characterized by KEGG analysis, and the predictive value of the screened metabolites was analyzed by receiver operating characteristic (ROC) curve analysis.

Results

Three weeks after the surgery, the TAC and sham groups had similar left heart function and thickness of the interventricular septum and diastolic left ventricular posterior wall. However, in pathologic examination, the cross-sectional area of cardiac myocytes and the severity of myocardial fibrosis were significantly elevated in TAC rats. OPLS-DA analysis showed different metabolic patterns between TAC and sham groups. Based on the criterion of VIP > 1 and p < 0.05, 13 metabolites were screened out. KEGG analysis identified the disruption of the lysine degradation through the related metabolites 5-aminopentanoic acid, N6-acetyl-L-lysine, and L-lysine, with the AUC of 0.917, 0.889, and 0.806 in predicting compensated cardiac hypertrophy, respectively.

Conclusion

The disruption of lysine degradation might be involved in the early pathology of cardiac hypertrophy, and the related metabolites might be potential predictive and interventional targets for subclinical cardiac hypertrophy.

Background

Cardiac hypertrophy is a common pathological change in the pathogenesis and progression of multiple cardiovascular diseases, including hypertension, coronary artery disease, and valvular heart disease. It is
a pathological process the heart goes through to respond and adapt to stimuli such as ischemia, hypoxia, and pressure or volume overload [1, 2]. Continuous progression of cardiac hypertrophy will eventually result in a decompensated heart and subsequent heart failure [3, 4]. Early reversal of cardiac hypertrophy is an important strategy to postpone heart failure [5].

The progression of heart failure is accompanied by changes in the metabolism of sugar and fatty acids [6, 7]. Lopaschuk GD et al [8] reported that increased rates of fatty acid oxidation immediately after an ischemic event have been implicated in exacerbation of reperfusion injury. In the case of diabetes, the heart presents an independence on fatty acids for oxidative energy production, and the increase in lipid metabolism has been proposed to contribute to the etiology of impaired cardiac function [9]. Moreover, more evidence showed that improving myocardial metabolism usually ameliorates the clinical manifestation and prognosis of heart failure patients [10]. Therefore, the chronic shift of energy metabolism had been considered both a cause and consequence in the pathogenesis of heart dysfunction [11, 12]. However, it is not clear whether nonenergetic small-molecule metabolites exert regulatory functions in the early pathogenesis of cardiac hypertrophy.

To further elucidate the early metabolic changes and regulatory mechanisms of cardiac hypertrophy, we established a rat model of early-stage left ventricular cardiac hypertrophy via transverse aortic constriction (TAC) and explored the serum metabolomic changes and possible metabolic pathways at the early stage of pressure overload-induced compensated left ventricular hypertrophy.

Methods

Experimental animals and the establishment of the TAC model

Male Sprague-Dawley (SD) rats (6 weeks old, body weight 200g±20 g) were provided by the Experimental Animal Center of Chongqing Medical University and housed in the individually ventilated cage (IVC) facility at the Experimental Animal Center of Chongqing Medical University. The animal study protocol was approved by the Ethics Committee of Animal Welfare at the Medical Centers of Chongqing Medical University (Chongqing, China) and Shenzhen University General Hospital (Guangdong, China). All animal experiments comply with the ARRIVE guidelines and were carried out in accordance with the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978).

Establishment of the model [13]: After anesthesia through intraperitoneal injection of pentobarbital (60 mg/kg), the rat was immobilized in the supine position atop a heating pad maintained at 37°C. Endotracheal intubation was performed, tidal volume: 4-6 ml/200 g; respiratory rate: 70 breaths/min; inspiratory-to-expiratory time ratio: 1:1. Disinfection and skin preparation were performed in the surgical field. After the skin of the left chest was scissored open, the pectoralis major and the pectoralis minor were separated via blunt dissection to expose the ribs. A horizontal incision of 1.0 cm was scissored between the 2nd and 3rd ribs close to the left side of the sternum under sterilization, the blood vessels and
fasciae were separated and the thymus was gently moved to expose the aortic arch. The aortic arch was lifted with custom-made curved forceps and a 2-0 silk suture was placed between the innominate and left carotid arteries. A blunt curved 16-G needle (1.6 mm in diameter) was placed next to the aortic arch, and after ligation of the aorta, the needle was promptly removed, and the thymus was replaced in the thoracic cavity. After confirmation of the absence of bleeding during ligation, the chest was closed and the skin incision was sutured. After approximately 10 more min of ventilation, when spontaneous breathing was restored, the rat was extubated and returned to the housing facility for maintenance.

Fourteen SD rats were randomly conducted the TAC or sham surgery. The sham group underwent the same surgical procedures as the TAC group but without ligation of the aortic arch. One animal in the sham group died of bleeding during the surgery. One animal in the TAC group died of pneumothorax during the surgery. Finally, 6 animals in the sham group (n=6) and 6 animals in the TAC group (n=6) survived and were used for the experiment. Heart rate and blood pressure were assessed 3 weeks post-surgery by measuring parameters such as blood flow, blood pressure and pulse at the base of the tail using a rat tail-cuff blood pressure system.

**Echocardiography**

Echocardiography was performed 3 weeks post-surgery, by technicians from the Department of Sonography who were experienced with sonography of small animals, using the VeVo2100 high-resolution ultrasound imaging system for small animals (Visual Sonics, Canada). After anesthesia through intraperitoneal injection of 2% pentobarbital, all limbs of the rat were immobilized on a Styrofoam board, furs on the chest were shaved to fully expose the positions of sternum and left thoracic cage, and M-mode echocardiography was carried out to measure parameters such as diastolic interventricular septal thickness, systolic interventricular septal thickness, end-diastolic left ventricular internal dimension, end-systolic left ventricular internal dimension, diastolic left ventricular posterior wall thickness (LVPWd), systolic left ventricular posterior wall thickness (LVPWs), left ventricular ejection fraction (LVEF), left ventricular fractional shortening (LVFS), left ventricular mass (LV Mass), and left ventricular end-diastolic volume (LVEDV).

**Pathology**

All specimens were anesthetized using an intraperitoneal injection of 30 mg/kg sodium pentobarbital, then euthanized by thoracotomy and heart removal 3 weeks after surgery. Cardiac tissues were excised and placed in glass receptacles containing phosphate-buffered saline (PBS). Residual blood in the heart was extruded with gentle pressure. After the cardiac tissues were washed with PBS, the residual tissues of the aortic arch and pericardium were carefully scissored off. With ophthalmology scissors, the atriums were removed along the atrioventricular groove, and the right ventricular free wall was cut from the lower right side of the interventricular septum. What remained were the left ventricle and the interventricular septum. The tissues were fixed in 4% paraformaldehyde for 24 h and submitted to histopathological analysis.
Untargeted metabolomics analysis

Sample collection: A blood-drawing needle was used to collect 5 ml of blood from the abdominal aorta of the rat, and the blood was placed in a heparin anticoagulant tube. After sitting at room temperature for 30 min, the blood was centrifuged at 2000 rpm for 10 min and the supernatant was collected, flash-frozen in liquid nitrogen, and stored at -80°C for future use.

Sample pretreatment: We added 400 µl prechilled methanol-acetonitrile solution (1:1, v/v) into 100 µl of each sample, and the solution was vortexed for 60 s. The sample was placed at -20°C for 1 h to precipitate the proteins and centrifuged at 14,000 rcf for 20 min at 4°C, and the supernatant was collected and lyophilized.

Chromatography conditions: The Agilent 1290 Infinity LC ultrahigh-performance liquid chromatography (UHPLC) system and the hydrophilic interaction liquid chromatography (HILIC) column were used for sample separation, with column temperature 25°C and flow rate 0.3 mL/min. Mobile phase component A was water + 25 mM ammonium acetate + 25 mM ammonia, and B was acetonitrile. Gradient elution procedures were as follows: 0–0.5 min, 95% B; 0.5–7 min, linear transition of B from 95% to 65%; 7–8 min, linear transition of B to 40%; 8–9 min, B maintained at 40%; 9–9.1 min, linear transition of B from 40% to 95%; 9.1–12 min, B maintained at 95%.

Quadrupole time-of-flight (Q-TOF) mass spectrometry (MS) conditions: Testing was carried out using electrospray ionization (ESI) in positive- and negative-ion modes. After UHPLC separation, the sample was subjected to mass spectrometry analysis in the Agilent 6550 mass spectrometer. ESI source parameters were as follows: gas temperature: 250°C; drying gas: 16 L/min; nebulizer: 20 psig; sheath gas temperature: 400°C; sheath gas flow: 12 L/min; Vcap: 3000 V; nozzle voltage: 0 V; fragment: 175 V; mass range: 50-1200; acquisition rate: 4 Hz; cycle time: 250 ms.

After testing, the AB Triple TOF 6600 mass spectrometer was used to identify metabolites and collect primary and secondary spectra of QC samples. ESI source parameters were as follows: ion source gas 1 (Gas1): 40; ion source gas 2 (Gas2): 80; curtain gas (CUR): 30; source temperature: 650°C; ion spray voltage floating: ±5000 V (positive- and negative-ion modes). Secondary spectra were obtained using information-dependent acquisition (IDA) (settings: exclude isotopes within 4 Da; candidate ions per cycle: 10); high-sensitivity mode; declustering potential: ±60 V (positive- and negative-ion modes); and collision energy: 35±15 eV.

Statistical analysis

To expand the collection rate of secondary spectra, Q-TOF data collection was segmented according to mass range: 50-300, 290-600, 590-900, and 890-1200. Four replicates were collected for each method in each segment. The original data collected were transformed into ProteoWizard into .mzXML format, and then peak alignment, retention time correction, and peak area extraction were performed with the XCMS
program. Metabolite structures were identified by matching the exact mass (<25 ppm) and the secondary spectrum against molecules in the database established in our laboratory.

After pretreatment of data obtained from Q-TOFMS and UHPLC-QTOFMS via Pareto-scaling, multidimensional statistical analysis was performed, including unsupervised principal component analysis (PCA), supervised partial least squares discriminant analysis (PLS-DA), and orthogonal partial least squares discriminant analysis (OPLS-DA). Differential metabolites with variable importance in projection (VIP) values >1.0 in multidimensional statistical analysis were further screened with the unpaired t test (P<0.05). Finally, metabolic pathway enrichment analysis was performed on the differential metabolites using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (https://www.genome.jp/kegg/), and receiver operating characteristic (ROC) curve analysis was performed on positive metabolites from the screen to calculate the area under the ROC curve (AUC) and the sensitivity and specificity of the model.

Data were expressed as mean±SE, and statistically analyzed with SPSS software (IBM Corp., version 19.0). Pairwise comparisons were carried out using the unpaired t test. Differences with P<0.05 were considered to be statistically significant.

Results

Establishment of the cardiac hypertrophy model

Compared with the sham group, rats in the TAC group had higher tail arterial pressure (158±16.30 mmHg vs 132±5.85 mmHg, n=6, P<0.01) 3 weeks after aortic constriction, while no significant difference was observed in heart rate or body weight. Echocardiography suggested trends of greater left ventricular mass, LVPW, and LVEF in the TAC group, but the differences were not significant. Pathological analysis indicated greater cardiomyocyte cross-sectional area (348.60±9.70 µm² vs 283.20±6.80 µm², n=6, P<0.05) and level of fibrosis (4.84±0.21% vs 3.11±0.15, n=6, P<0.05) in the TAC group than the sham group. The combined echocardiography and pathology results revealed marked increases in systemic arterial pressure and hypertrophy of cardiomyocytes in rats from the TAC group 3 weeks after surgery, without detectable abnormalities in cardiac structure or function on sonography, indicating early-stage subclinical compensated cardiac hypertrophy in rats (Table 1, Figure 1).

Metabolic pattern analysis

Comparison of the total ion chromatogram between samples from the TAC and sham groups in positive- and negative-ion modes revealed that the intensities and retention times of the peaks mostly overlapped, indicating that the variations caused by instrument errors were relatively small throughout the experimental process. OPLS-DA analysis of the two-dimensional distribution patterns of the metabolites showed that in both positive- and negative-ion modes, there was significant separation of metabolite patterns when the TAC group was compared with the sham group. The R² and Q² were 0.977 and 0.324, respectively, in positive mode and 0.993 and 0.435 in negative mode. The results indicated good data
fitting and reproducibility, a stable and effective model, and metabolite patterns that distinguished the sham group from the group with early-stage compensated cardiac hypertrophy (Figure 2).

**Differential metabolites**

We analyzed in depth the differential metabolites between the sham group and TAC group 3 weeks after surgery. Based on the VIP values of characteristic variables obtained from the OPLS-DA model, the differential metabolites with VIP>1 and p<0.05 were identified. There were 13 metabolites that showed significant differences between groups at 3 weeks postsurgery (Table 2, Figure 3), including amino acids and polypeptides (5-aminopentanoic acid, N6-acetyl-L-lysine, L-lysine, N6-methyl-L-lysine, N2-acetyle-L-ornithine, L-phenylalanine), fatty acids (pentadecanoic acid, nervonic acid), and pyrimidine (5-methylcytosine).

**KEGG pathway analysis**

We analyzed the metabolic pathway changes in the TAC group 3 weeks postsurgery compared with the sham group, and found that the metabolic pathways involved in early-stage cardiac hypertrophy included lysine degradation, pyrimidine metabolism, aminoacyl-tRNA biosynthesis, arginine and proline metabolism, linoleic acid metabolism, central carbon metabolism, and glycerophospholipid metabolism. The lysine degradation pathway exhibiting the most marked changes. The metabolites related to lysine degradation were 5-aminopentanoic acid, N6-acetyl-L-lysine, and L-lysine (Table 3, Figure 4).

**ROC analysis**

We next calculated the predictive value of the three metabolites related to the lysine degradation pathway for early-stage cardiac hypertrophy. The AUC values of 5-aminopentanoic acid, N6-acetyl-L-lysine, and L-lysine for predicting compensated cardiac hypertrophy were 0.917, 0.889, and 0.806 respectively (Figure 5).

**Discussion**

Cardiac hypertrophy is a common pathological characteristic during the pathogenesis and development of multiple cardiovascular diseases, such as hypertension, coronary artery disease, and valvular heart disease, and early reversal of cardiac hypertrophy has great value in maintaining heart function and delaying heart failure \[13, 14\]. In this study, we established a cardiac hypertrophy model through TAC and found pathological evidence of cardiomyocyte hypertrophy and fibrosis 3 weeks after surgery, though no significant increase in ventricular wall thickness or decrease in cardiac function was detectable through echocardiography. This indicated that there are possibly pathological changes during early-stage subclinical cardiac hypertrophy, when there are clinical indications of risk factors for cardiac remodeling but no detectable thickening of the ventricular wall on sonography. In fact, pathological cardiac hypertrophy is difficult to reverse once formed \[15\]. In addition, for patients with risk factors for cardiac hypertrophy, besides actively controlling the risk factors, early detection and intervention of cardiac...
hypertrophy are of great importance to the protection of the target organ and the prevention and treatment of chronic heart failure.

Heart failure usually is accompanied by changes in the energy metabolism of the cardiomyocytes. Cardiomyocyte metabolism often changes before cardiac structure\textsuperscript{[16,17]}. However, it is still unclear whether metabolic changes could allow early detection of subclinical cardiac hypertrophy. We studied early-stage cardiac hypertrophy 3 weeks after TAC surgery, analyzed the plasma metabolomic changes through UHPLC-QTOFMS, and uncovered significant separation of metabolic patterns between the sham and TAC groups, indicating that the two-dimensional distribution patterns of serum metabolites can be used to identify early-stage cardiac hypertrophy. Furthermore, we screened for differential metabolites by setting the thresholds of VIP > 1 and p < 0.05, according to the VIP values of characteristic variables obtained from the OPLS-DA model, and found 13 metabolites that showed significant differences between groups at 3 weeks postsurgery, including amino acids and polypeptides such as 5-aminopentanoic acid, N6-acetyl-L-lysine, L-lysine, N6-methyl-L-lysine, N2-acetyl-L-ornithine, and L-phenylalanine; fatty acids such as pentadecanoic acid and nervonic acid; and pyrimidines such as 5-methylcytosine. Among these metabolites, the levels of 5-aminopentanoic acid, N6-acetyl-L-lysine, L-lysine, L-phenylalanine, and thymidine were increased, while levels of N2-acetyl-L-ornithine, 5-methylcytosine, and pentadecanoic acid were decreased. Consistent with Sansbury et al\textsuperscript{[18]}, we found that the amino acid changes caused by cardiac hypertrophy were the most pronounced. However, Sansbury et al\textsuperscript{[18]} reported more significant changes in branched-chain amino acids during cardiac hypertrophy and heart failure. Branched-chain amino acids are usually related to metabolic factors such as insulin resistance. We found that changes in amino acid metabolism, mainly of lysine, were more pronounced during early cardiac hypertrophy. Lysine is an essential amino acid for humans and mammals. Because its concentration in cereals and foods is very low, and it is prone to destruction during processing, lysine is also called the first-limiting amino acid. Lysine has important functions in the promotion of human physiologic development and oxidation of fatty acids. Fust et al\textsuperscript{[19]} found that addition of lysine to the diet could treat osteoporosis. Shimomura et al\textsuperscript{[20]} found that moderate dietary supplementation of lysine could relieve vascular calcification in uremic rats, while the plasma lysine content was not increased by the addition of lysine to the diet. Our study found that, at the early stage of subclinical cardiac hypertrophy caused by pressure overload, plasma N6-acetyl-L-lysine, L-lysine, and N6-methyl-L-lysine were all elevated, suggesting that changes in metabolites of amino acids such as lysine may become early predictive serum markers for subclinical cardiac hypertrophy caused by pressure overload.

KEGG pathway enrichment analysis uncovered the metabolic pathways involved in cardiac hypertrophy at 3 weeks, including lysine degradation, pyrimidine metabolism, aminoacyl-tRNA biosynthesis, arginine and proline metabolism, linoleic acid metabolism, central carbon metabolism, and glycerophospholipid metabolism et al. The lysine degradation pathway exhibited the most pronounced changes. Posttranslational modifications of proteins have important functions in the growth, differentiation, and metabolic regulation of cells. Thanks to breakthroughs in detection techniques, the research on protein
phosphorylation has progressed very quickly, and there are many reports the involvement of signal molecule phosphorylation in cardiac hypertrophy \cite{21, 22}. With the advancements in the techniques of high-resolution mass spectrometry–based omics, the understanding of the epigenetic modifications of histones has progressed far in recent years \cite{23, 3}. Among the proteins expressed by mammals, over 50% can have various posttranslational modifications at certain times and in certain subcellular locations, which is a way the body precisely regulates pathological and physiological processes. Such posttranslational modifications are mainly reversible modifications of certain amino acid residues, and lysine is one of the most frequently modified \cite{24}.

The effects of methylation, acetylation, ubiquitination, and glycosylation of lysine on cardiovascular diseases have garnered much attention \cite{25}. With continuous improvements of the sensitivity, scan speed, and resolution of biological mass spectrometry, more and more acylation modifications of lysine have been discovered, such as succinylation and malonylation. The lysine modifications of histones undergo dynamic changes under the effects of regulatory enzymes and exert regulatory functions on gene transcription by altering the interaction between histone and DNA, and recruitment of binding proteins \cite{26}.

Metabolomics can provide new clues on the pathogenic mechanisms, severity, progression, and potential treatment methods of disease through the measurement of target metabolites \cite{27}. In this study, we found that at the early stage of pressure overload–induced cardiac hypertrophy, the levels of metabolites related to lysine degradation, such as 5-aminopentanoic acid, N6-acetyl-L-lysine, and L-lysine, were all elevated in the TAC group compared with the sham group, indicating that acetylation of lysine may be involved in the pathogenesis of pressure overload–induced early-stage cardiac hypertrophy. The ROC AUC values of 5-aminopentanoic acid, N6-acetyl-L-lysine, and L-lysine for predicting compensated cardiac hypertrophy were 0.917, 0.889, and 0.806, respectively, indicating that 5-aminopentanoic acid, N6-acetyl-L-lysine, and L-lysine may become serum markers for prediction of pressure overload–induced early-stage subclinical cardiac hypertrophy. However, the results of this study mainly came from animals, with the limitations inherent to them. Therefore, the relationships between lysine modifications and the clinical importance of such relationships await further verification.

Conclusion

Cardiac hypertrophy accompanies nonenergetic metabolism. The disruption of lysine degradation might be involved in the early pathology of cardiac hypertrophy, and the related metabolites including 5-aminopentanoic acid, N6-acetyl-L-lysine, and L-lysine might be potential predictive and interventional targets for subclinical cardiac hypertrophy.

Abbreviations

HILIC: hydrophilic interaction liquid chromatography; IVSd: diastolic thickness of intraventricular septum; LVPWd: left ventricular diastolic posterior wall thickness; LVEDV: left ventricular end-diastolic volume; LVEF: left ventricular ejection fraction; LV mass: left ventricular mass; MS: mass spectrometry; OPLS-DA:

**Declarations**

**Ethics approval and consent to participate**

The study protocol was approved by the Ethics Committee of Animal Welfare at the Medical Centers of Chongqing Medical University (Chongqing, China) and Shenzhen University General Hospital (Guangdong, China). All animal experiments comply with the ARRIVE guidelines and were carried out in accordance with the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978).

**Consent for publication**

Not applicable.

**Availability of data and materials**

The datasets generated and/or analysed during the current study are not publicly available due to privacy or ethical restrictions, but are available from the corresponding author on reasonable request.

**Competing interest**

The authors declare that there is no conflict of interest.

**Funding**

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**Authors’ Contributions**

JLL and JHH contributed equally to this work and share the first authorship. QZ and XW contributed equally to this work and share the corresponding authorship. QZ and XW contributed to the study conception design and design. Material preparation and data collection were performed by JHH. JLL and LLT performed some experiment and statistical analysis. The draft of the manuscript was written by XW. All authors read and approved the final manuscript.
Acknowledgments

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Tables

Table 1. Changes in blood pressure and cardiac function at 3 weeks after TAC surgery.

<table>
<thead>
<tr>
<th></th>
<th>Sham (n=6)</th>
<th>TAC (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>258±18</td>
<td>274±5</td>
</tr>
<tr>
<td>Heart beat (BPM)</td>
<td>385±8</td>
<td>407±3</td>
</tr>
<tr>
<td>Blood pressure (mmHg)</td>
<td>132±5.85</td>
<td>158±16.30**</td>
</tr>
<tr>
<td>IVSd (mm)</td>
<td>1.41±0.11</td>
<td>1.71±0.07</td>
</tr>
<tr>
<td>LVPWd (mm)</td>
<td>1.54±0.28</td>
<td>1.77±0.41</td>
</tr>
<tr>
<td>LVEDV(ul)</td>
<td>161.38±28.78</td>
<td>203.04±14.94</td>
</tr>
<tr>
<td>LVEF(%)</td>
<td>82.91±7.79</td>
<td>86.57±4.31</td>
</tr>
<tr>
<td>LV mass (mg)</td>
<td>502.81±38.78</td>
<td>679.44±29.28</td>
</tr>
</tbody>
</table>

Values are mean±SE. TAC: transverse aortic constriction; IVSd: diastolic thickness of intraventricular septum; LVPWd: left ventricular diastolic posterior wall thickness; LVEDV: left ventricular end-diastolic volume; LVEF: left ventricular ejection fraction; LV mass: left ventricular mass. **P<0.01

Table 2. Significantly different metabolites identified 3 weeks after TAC surgery.
<table>
<thead>
<tr>
<th>Name of metabolites</th>
<th>Category</th>
<th>VIP</th>
<th>FC</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-Aminopentanoic acid</td>
<td>Amino acids, peptides</td>
<td>1.13</td>
<td>1.45</td>
<td>0.004</td>
</tr>
<tr>
<td>N6-Acetyl-L-lysine</td>
<td>Amino acids, peptides</td>
<td>1.21</td>
<td>1.46</td>
<td>0.023</td>
</tr>
<tr>
<td>L-Lysine</td>
<td>Amino acids, peptides</td>
<td>9.94</td>
<td>1.30</td>
<td>0.028</td>
</tr>
<tr>
<td>ε-Caprolactam</td>
<td>Others</td>
<td>2.91</td>
<td>1.40</td>
<td>0.029</td>
</tr>
<tr>
<td>γ-L-Glu-ε-L-lysine</td>
<td>Others</td>
<td>2.96</td>
<td>1.45</td>
<td>0.030</td>
</tr>
<tr>
<td>N6-Methyl-L-lysine</td>
<td>Amino acids, peptides</td>
<td>2.17</td>
<td>1.34</td>
<td>0.033</td>
</tr>
<tr>
<td>N2-Acetyl-L-ornithine</td>
<td>Amino acids, peptides</td>
<td>1.12</td>
<td>0.78</td>
<td>0.034</td>
</tr>
<tr>
<td>Thioetheramide-PC</td>
<td>Others</td>
<td>1.12</td>
<td>1.40</td>
<td>0.036</td>
</tr>
<tr>
<td>5-Methylcytosine</td>
<td>Pyrimidines</td>
<td>2.72</td>
<td>0.82</td>
<td>0.037</td>
</tr>
<tr>
<td>Pentadecanoic Acid</td>
<td>Fatty acids</td>
<td>3.08</td>
<td>0.50</td>
<td>0.003</td>
</tr>
<tr>
<td>d(-)-β-Hydroxybutyric acid</td>
<td>Others</td>
<td>2.11</td>
<td>2.31</td>
<td>0.008</td>
</tr>
<tr>
<td>Nervonic acid</td>
<td>Fatty acids</td>
<td>2.05</td>
<td>0.44</td>
<td>0.018</td>
</tr>
<tr>
<td>L-Phenylalanine</td>
<td>Amino acids, peptides</td>
<td>2.47</td>
<td>1.41</td>
<td>0.030</td>
</tr>
</tbody>
</table>

TAC: transverse aortic constriction; VIP: variable importance in projection; FC: fold change

**Table 3.** The significantly altered pathways between TAC and sham rats
<table>
<thead>
<tr>
<th>Pathway</th>
<th>Metabolites</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysine degradation</td>
<td>5-Aminopentanoic acid, N6-acetyl-L-lysine, L-lysine</td>
<td>0.0002</td>
</tr>
<tr>
<td>Pyrimidine metabolism</td>
<td>Thymidine, pseudouridine,5-Methylcytosine, thymine</td>
<td>0.0005</td>
</tr>
<tr>
<td>Choline metabolism in cancer</td>
<td>PC(16:0/16:0), SOPC</td>
<td>0.0019</td>
</tr>
<tr>
<td>Protein digestion and absorption</td>
<td>L-Phenylalanine, L-Lysine, L-Histidine</td>
<td>0.0027</td>
</tr>
<tr>
<td>Aminoacyl-tRNA biosynthesis</td>
<td>L-Phenylalanine, L-lysine, L-histidine</td>
<td>0.0037</td>
</tr>
<tr>
<td>Retrograde endocannabinoid signaling</td>
<td>PC(16:0/16:0), SOPC</td>
<td>0.0058</td>
</tr>
<tr>
<td>Arginine and proline metabolism</td>
<td>5-Aminopentanoic acid, 4-Guanidinobutyric acid, D-proline</td>
<td>0.0115</td>
</tr>
<tr>
<td>Linoleic acid metabolism</td>
<td>PC(16:0/16:0), SOPC</td>
<td>0.0125</td>
</tr>
<tr>
<td>Central carbon metabolism in cancer</td>
<td>L-Phenylalanine, L-histidine</td>
<td>0.0214</td>
</tr>
<tr>
<td>alpha-Linolenic acid metabolism</td>
<td>PC(16:0/16:0), SOPC</td>
<td>0.0296</td>
</tr>
<tr>
<td>Glycerophospholipid metabolism</td>
<td>PC(16:0/16:0), SOPC</td>
<td>0.0403</td>
</tr>
</tbody>
</table>

SOPC: 1-stearoyl-2-oleoyl-sn-glycerol 3-phosphocholine

**Figures**

**Figure 1**

The effects of aortic constriction on the hypertrophy and fibrosis of cardiomyocytes. Three weeks after TAC surgery, HE and Masson staining were performed to observe the pathologic changes. Cardiomyocyte cross-sectional area and level of myocardial fibrosis increased significantly in the TAC group than the sham group. *P<0.05. Bar: 50 µm.
Metabolic pattern analysis by OPLS-DA score plot. A and B: OPLS-DA score plot under positive- (A) and negative-ion modes (B). C and D: Validated model plots obtained by permutation test: positive- (C) and negative-ion mode (D). OPLS-DA analysis of the two-dimensional distribution patterns of the metabolites showed that in both positive- and negative-ion modes, there was significant separation of metabolite patterns when the TAC group was compared with the sham group. The R2 and Q2 were 0.977 and 0.324, respectively, in positive mode and 0.993 and 0.435 in negative mode.

**Figure 2**
Figure 3

Unsupervised hierarchical clustering heat map of metabolites obtained from the plasma of rats after TAC surgery
Figure 4

Enriched KEGG pathways between sham and TAC group presented by bubble diagram based on significant different metabolites.
Figure 5

ROC analysis for the metabolites associated with lysine degradation pathway

Supplementary Files

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