Plasma metabolomics combined with personalized diagnosis guided by Chinese medicine reveals subtypes of chronic heart failure

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KEYWORDS
Metabolomics; Chinese medicine; Chronic heart failure

Abstract Background: Chronic heart failure (CHF) is characterized by insufficient blood supply from heart to meet the body’s metabolic demands. Integrating Western and traditional Chinese medicine to treat CHF has proved a validated therapeutic approach. In recent years, metabolomics has been regarded as a potential platform to provide biomarkers for disease-subtypes. Objective: To examine 38 patients, combined NMR plasma metabolomics and traditional Chinese medicine diagnosis in order to identify diagnostic biomarkers for two CHF syndrome subtypes. Methods: After processing the spectra, orthogonal partial least square discriminant analysis was performed, and the contributing NMR signals were analyzed using Y-scrambling statistical validation with good reliability. Results: Plasma metabolic patterns of yin deficiency and yang deficiency patients were clearly discriminated. The yin-deficiency group had increased level of lactate, glycoprotein, lipoprotein and lower levels of glucose, valine and proline. The yang-deficiency group had higher levels of lactate, glycoprotein and pyruvic acid, and lower levels of glucose and lipoprotein.

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Introduction

Chronic heart failure (CHF), a progressive clinical syndrome characterized by the inability of the heart to adequately pump blood to meet metabolic demands of the body. It represents the final common pathogenesis in various causes of heart damage. Despite a substantial improvement in the survival rate after the onset of CHF because of increasing use of pharmacological interventions, the mortality of patients suffering from CHF remains high. It is well recognized that the incidence and prevalence of CHF is expected to increase further with the aging population, better strategies for the prevention and treatment of CHF is required.

With progress in bioinformatics and medical science, the focus on health and disease in Western life sciences has shifted from standard protocol-based disease management to personalized medicine. Based on personalized health and systematical diagnostic principles, traditional Chinese medicine (TCM) has proved clinically effective at restoring the self-regulatory ability of the human system for thousands of years. Using integrated TCM and Western medicine approach to treat CHF has been reported to enhance heart function and reduce related clinical symptoms, including expiratory dyspnea and chronic fatigue, and subsequently improve systemic functions.

Materials and methods

Participants and study design

The study was designed as an explorative study without intervention. Patients with a history of coronary heart disease that met the CHF diagnostic criteria in accordance with the Guidelines for the Diagnosis and Management of Chronic Heart Failure established by the Chinese Society of Cardiology of the Chinese Medical Association in 2007 were enrolled. Eligibility criteria were age ≥45 years and left ventricular ejection fraction <50%. All patients were in New York Heart Association (NYHA) classes II–IV. All patients underwent our standardized recruitment and
management. They were diagnosed by two experienced doctors independently to reduce subjective factors. A pre-study screening involved a physical exam that included echocardiography and clinical laboratory tests and was performed immediately. Patients with end-stage renal or liver disease, ongoing infection and long-term immunosuppressive therapy were excluded.

Thirty-eight patients attending the Heart Diseases Clinic at Beijing University of Chinese Medicine Affiliated Hospital from January 2013 to September 2014 were finally enrolled in the study. Samples of venous blood were collected, and the detailed clinical data are shown in Table 1.

The study was approved by the Ethical Committee at the Affiliated Hospital of Beijing University of Chinese Medicine, and written informed consent was acquired from all participants recruited.

We used TCM to investigate general syndromes and classified them into two study groups: (1) yin-deficiency vs. non-yin-deficiency (Group 1); and (2) yang-deficiency VS non-yang-deficiency (Group 2). According to Clinical Terminology of Traditional Chinese Medical Diagnosis and Treatment—Syndromes, yin-deficiency is described as low fever, night sweats, afternoon zygomaticus red, dysphoria with feverish sensation of the chest palms and soles, dry mouth and throat, red tongue with little coating and thready rapid pulse. And yang-deficiency is a cluster of symptoms including an aversion to cold, dispirited feelings and lack of motivation, diarrhea before dawn, shortness of breath, frequent urination, edema, and liability to catch cold. Using these criteria, 15 patients were assessed as being in the yin-deficiency group and 7 patients as being in the yang-deficiency group, others of 38 patients were diagnosed as being in combined syndromes of CHF.

**Table 1** Demographic details of participants.

<table>
<thead>
<tr>
<th>Patients (n = 38)</th>
<th>Mean ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age/years</td>
<td>61.4 ± 5.13</td>
<td>45–79</td>
</tr>
<tr>
<td>BMI/kg m⁻²</td>
<td>28.8 ± 2.4</td>
<td>25.6–35.5</td>
</tr>
<tr>
<td>Sex M:F</td>
<td>21/17</td>
<td>—</td>
</tr>
<tr>
<td>Median NYHA class</td>
<td>3</td>
<td>—</td>
</tr>
<tr>
<td>Mean ejection fraction</td>
<td>54.3 ± 8.2</td>
<td>41–67</td>
</tr>
<tr>
<td>Etiology ischemic:</td>
<td>22/16</td>
<td>—</td>
</tr>
<tr>
<td>non-ischemic</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Hypertensive: non-hypertensive</td>
<td>20/18</td>
<td>—</td>
</tr>
<tr>
<td>Hyperlipidemia: non-hyperlipidemia</td>
<td>14/24</td>
<td>—</td>
</tr>
<tr>
<td>DM: non DM</td>
<td>15/23</td>
<td>—</td>
</tr>
<tr>
<td>Smoker: non-smoker</td>
<td>17/21</td>
<td>—</td>
</tr>
<tr>
<td>Na⁺</td>
<td>142.6 ± 4.3</td>
<td>127–147</td>
</tr>
<tr>
<td>K⁺</td>
<td>3.9 ± 0.6</td>
<td>0.9–4.8</td>
</tr>
<tr>
<td>Urea</td>
<td>7.1 ± 2.1</td>
<td>3–14</td>
</tr>
<tr>
<td>Creatinine</td>
<td>101 ± 32</td>
<td>43–229</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>128.7 ± 20.1</td>
<td>79–175</td>
</tr>
<tr>
<td>Beta-blokers Y:N</td>
<td>20/18</td>
<td>—</td>
</tr>
<tr>
<td>ACE inhibitors Y:N</td>
<td>14/24</td>
<td>—</td>
</tr>
<tr>
<td>Diuretics Y:N</td>
<td>19/19</td>
<td>—</td>
</tr>
</tbody>
</table>

**Sample collection and preparation**

Clinical parameters included gender, age, ejection fraction, creatinine, electrolyte, urea, B-type natriuretic peptide, platelet count, hemoglobin, fasting blood glucose, triglyceride levels and total cholesterol. Complications including diabetes, hypertension or dyslipidemia, and drug-taking information of patients were noted at inclusion.

Venous blood of 38 CHF patients at the Heart Failure Clinic were also collected in 5 mL Vacutainer tubes with chelating agent ethylene diamine tetraacetic acid (EDTA) and centrifuged at 3000 rpm for 10 min. The blood sample was then separated into equal aliquots and stored at −80°C until analysis.

For NMR analysis, plasma samples were thawed at room temperature. After being centrifuged at 13 000 rpm for 10 min, 200 μL samples of supernatant were removed. D₂O (400 μL) was added and the mixture was centrifuged again. Following centrifugation, 550 μL of supernatant was transferred to a 5-mm diameter specific NMR tube for NMR analysis. The reaction was performed using a Varian VNMRS 600 MHz NMR spectrometer (Varian Medical Systems, Inc., Palo Alto, CA, USA) at 25°C.

**1H-NMR spectroscopy**

The spectra were acquired by Carr–Purcell–Meiboom–Gill (CPMG) sequence D-[90°-(−180°-t)n-ACQ] and Longitudinal Eddy-Delay (LED) sequence. Both small molecular metabolites and lipid components in the plasma were observed respectively. The free induction decays were transferred into 64 K data points with a spectral width of 8000 Hz and 64 scans, then zero-filled to double size and multiplied before Fourier transformation, which was applied with an exponential window function to produce a 0.5 Hz broadening line. We identified plasma metabolites by comparison with chemical shifts, which is detailed in a previous report.

**Spectral and statistical analysis**

Spectra were manually phased, baseline corrected and normalized. Each spectrum was referenced using internal lactate CH₃ resonance at δ1.33 by Mest-ReNova7.1.0 software (Mestrelab Research, A Coruña, Spain). Signals from δ0.5 to δ9.0 for each sample were automatically binned with a 0.005 ppm width. Water and EDTA metal complex regions were excluded.

Prior to multivariate data analysis, statistical analyzes were performed on the data using SIMCA-P+12 software (Umetrics, Umea, Sweden) as variables and then mean-centered and pareto-scaled.

To analyze the NMR data holistically and discriminate CHF patients with different TCM syndrome types and controls, we applied both principal component analysis and orthogonal partial least-squares discriminant analysis (OPLS-DA).

Score and loading plots were calculated to demonstrate discriminatory metabolites for each group. Each point in a score plot pointed to the projection of a NMR spectrum (patient sample) on the predictive (horizontal axis) and
orthogonal components of the model (vertical axis). On the loading plot, positive signals represented those plasma metabolites revealed increased concentrations in CHF patients diagnosed with yin-deficiency or yang-deficiency syndrome. Accordingly, a negative signal corresponded to those down-regulated plasma metabolites.\textsuperscript{15}

The key metabolites resulting in discrimination were also analyzed by peak integration. And independent samples t-test were used to identify main differences in selected signals.

To obtain a more objective statistical estimation, we performed ‘Y-scrambling’ validation and calculated $R^2$ (correlation coefficients) and $Q^2$ (prediction properties) values to evaluate our OPLS-DA models.\textsuperscript{16}

Results

Clinical characteristics of participants

Detailed clinical data characteristics and plasma samples acquired from all CHF patients with different TCM syndrome types were collected. The groups showed no differences in any demographic characteristic, such as gender, age or body mass index.

Metabolomics analysis of plasma samples of CHF with TCM syndromes

As metabolomics has the advantage of being able to identify metabolomics biomarker profiles and reveal relationships among TCM syndrome subtypes, metabolic profiling coupled with multivariate analysis was applied in this study. Based on metabolic profiling, CHF patients with yin-deficiency or yang-deficiency and controls were able to be easily distinguished in principal component analysis score plots. The first two principal components were selected, which described 65.7% of the total variance of the plasma metabolome.

OPLS-DA is a newly developed data analysis method combining orthogonal signal correction and partial least squares, and has been widely used in clinical studies.\textsuperscript{10,17} Here, we also performed an OPLS-DA pattern recognition model with one predictive component and four orthogonal components to further identify plasma metabolites that differed in concentrations in CHF patients with different TCM syndrome types. OPLS-DA score plots revealed that yin-deficiency patients were statistically distinguishable from controls ($R^2 = 0.608$, $Q^2 = 0.327$). The former index shows the explainable ability of the syndrome classification, and the latter is the result of seven-fold cross-validation, and suggests that the OPLS-DA models were robust.\textsuperscript{18} The patterns of Group 1 and 2 are clearly distinct from that of the control group along the t[1]-axis direction of the first principle component, without any crossover or overlap (Figs. 1 and 2). This separating trend clearly indicates that metabolic profiling varied according to different TCM syndromes.

Further analysis of loading plots illustrated corresponding changes of metabolites of high variable importance, which accounted for metabolomics fingerprint changes and discrimination in score plots. Nine plasma metabolites of yin-deficiency patients and control groups could be determined in the 600 MHz one-dimensional CPMG and LED\textsuperscript{H} NMR spectra, ranked by the largest variable importance,\textsuperscript{19} to be significantly altered metabolites of CHF patients

Fig. 1 OPLS-DA score plots of Group 1. Black box: OPLS-DA score plots displaying discrimination between CHF yang-deficiency patients. Red circle: OPLS-DA score plots displaying discrimination between CHF yang-deficiency controls.
with yin-deficiency syndrome (i.e. potential biomarkers) (Figs. 3, 4 and Table 2A). CHF patients with yang-deficiency syndrome were examined as above. Nine metabolites were positively identified as potential biomarkers from variable importance values (Figs. 5, 6 and Table 2B).

**Statistical validation**

We performed 'Y-scrambling' statistical validation to correct chance correlation and evaluate the OPLS-DA model. The Y-variable of case and control group were randomly

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**Fig. 2** OPLS-DA score plots of Group 2. Black box: OPLS-DA score plots displaying discrimination between CHF yang-deficiency patients. Red circle: OPLS-DA score plots displaying discrimination between CHF yang-deficiency controls.

**Fig. 3** OPLS-DA loadings plots of key metabolites by CPMG sequence of Group 1. OPLS-DA loadings plots demonstrating discrimination of key metabolite levels between CHF yin-deficiency patients and controls.
permutated first and the statistical model was rebuilt. In addition, we recorded and analyzed trends of the predictive power and goodness of fit at each step. Two hundred rounds of reshuffling showed that the separation model was reliable, and that its high predictability was not affected by random or over-fitting of the data, as both permuted $R^2$ and $Q^2$ values were markedly lower than the corresponding original values (Fig. 7A, B). Even though this study may not include all possible confounding factors in the patients, our validation through randomization of the Y-variable suggests that these variations should not be key attributors for discrimination between case and control groups, or affect the predictability of our model.

### Discussion

CHF is clinically associated with high mortality and morbidity, decreased quality of life and substantial burden on health care systems. Despite advances in drug treatment strategies for CHF, the number of deaths resulting from this condition continues to rise.20 TCM pays particular attention to the integrity and holism of the human body and its interrelationship with nature. TCM also adheres to basic principle of treatment based on differentiation of symptoms and signs, treats the same disease by different methods and different diseases by the same method, and advocates individualized treatment, which vividly reflects the essence

#### Table 2A  Key metabolites differentiating CHF yin-deficiency patients and controls.

<table>
<thead>
<tr>
<th>No</th>
<th>Metabolite</th>
<th>(Chemical shift)</th>
<th>YIP</th>
<th>NYIP</th>
<th>$P$-value</th>
<th>VIP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Valine</td>
<td>1.04</td>
<td>0.2526 ± 0.0628</td>
<td>0.3031 ± 0.0507</td>
<td>0.007</td>
<td>1.76</td>
</tr>
<tr>
<td>2</td>
<td>VLDL/LDL</td>
<td>1.26, 1.3, 1.34</td>
<td>1.6539 ± 0.0885</td>
<td>1.5791 ± 0.0996</td>
<td>0.010</td>
<td>2.59</td>
</tr>
<tr>
<td>3</td>
<td>Lactate</td>
<td>1.33, 4.12</td>
<td>1.4396 ± 0.4708</td>
<td>1.1235 ± 0.2363</td>
<td>0.017</td>
<td>3.99</td>
</tr>
<tr>
<td>4</td>
<td>Alanine</td>
<td>1.48</td>
<td>0.2748 ± 0.0873</td>
<td>0.4077 ± 0.1251</td>
<td>0.000</td>
<td>2.11</td>
</tr>
<tr>
<td>5</td>
<td>Proline</td>
<td>3.33</td>
<td>0.0353 ± 0.0283</td>
<td>0.0511 ± 0.0226</td>
<td>0.029</td>
<td>3.93</td>
</tr>
<tr>
<td>6</td>
<td>Glucose</td>
<td>3.47; 3.72, 4.64, 5.23</td>
<td>4.1008 ± 0.4777</td>
<td>4.7331 ± 0.6542</td>
<td>0.007</td>
<td>3.68</td>
</tr>
<tr>
<td>7</td>
<td>Glycoprotein (N–Ac)</td>
<td>2.02</td>
<td>0.6093 ± 0.0223</td>
<td>0.4289 ± 0.0696</td>
<td>0.000</td>
<td>2.54</td>
</tr>
<tr>
<td>8</td>
<td>Carnitine</td>
<td>2.44</td>
<td>0.0725 ± 0.0138</td>
<td>0.0904 ± 0.0351</td>
<td>0.001</td>
<td>2.39</td>
</tr>
</tbody>
</table>

Abbreviations: YIP, yin-deficiency patients; NYIP, non-yin-deficiency patients; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein; HDL, high-density lipoprotein; Values expressed as the mean(SD) (range); $P$ values were calculated from the Independent-samples T Test; Variable importance in the projection (VIP) was acquired from the OPLS-DA model.
of TCM treatment. Treatment based on syndrome differentiation is at the core of TCM therapy for CHF.

In terms of the perspective of TCM, CHF may occur in all differentiation types, including yang deficiency, blood stasis and yin-deficiency, to name a few. Some Chinese herbs have been demonstrated to be safe and effective in the management of CHF in both animal models and in humans. Modern biological research has now begun integrating various research technologies and methods to tackle difficult biological problems at bio-molecular levels, which is exemplified by studies in the new scientific field of metabolomics. It is important that potential correlations
between TCM syndrome type and metabolites are investigated to develop novel therapeutic approaches for better treatment of CHF.

In this explorative study, we investigated plasma metabolites of CHF patients with yin-deficiency and yang-deficiency syndrome to examine new approaches to diagnosis and identify metabolic signatures of TCM syndromes in CHF. Our approach combined plasma metabolomics with TCM syndrome type diagnosis showed performances for each study group.

With the aim of evaluating the diagnostic performance of plasma metabolites in this study, we identified distinguishable metabolites able to differentiate CHF patients with each TCM syndrome from controls, including energy metabolites (glucose, lactate and glycoprotein), lipid/protein complexes (high-density lipoprotein (HDL), low-density lipoprotein; HDL, high-density lipoprotein; LDL, very low-density lipoprotein; VLDL, very low-density lipoprotein; HDL, high-density lipoprotein). Values are expressed as mean (SD) (range). *P*-values were calculated from independent samples t-tests. Variable importance in the projection (VIP) was acquired from the OPLS-DA model.

As with all new diagnostic approaches, this study had some limitations. First, there is the possibility of other confounding factors having an effect on metabolic profiles. Further, studies using larger cohorts are required to validate this method. Second, as plasma samples are representative of metabolic process in different organs, it is difficult to assign a metabolic fingerprint to specific metabolic processes. However, altered metabolite levels are at least partly reflected in CHF patients with certain TCM syndromes, which can be suggestive of markers of diseases. Further mechanistic studies are required on this point.

In the current study, yin-deficiency patients showed a distinct altered metabolism, including increased levels of lactate, glycoprotein and LDL/VLDL, and lower levels of glucose, valine, alanine, and carnitine compared with non-yin-deficiency patients.

Heart failure is characterized by alterations in energy metabolism, and a high level of lactate and a decreased level of glucose in the blood points to energy metabolism dysfunction in CHF patients with yin-deficiency. Glycoprotein is closely associated with the physiology and pathology of cell growth and can affect human metabolic energy supply and cellular immunity. We observed increased levels of LDL and VLDL in CHF patients with yin-deficiency, which were the most prominent factors differentiating yin-deficiency patients from the control groups. This metabolomics profile could be associated with lipolysis as a backup mechanism for energy utilization, because apolipoproteins are important in lipid metabolism.

It has been reported that increased levels of proline play a role in coronary atherosclerotic disease patients with yin-deficiency syndrome. The plasma levels of well-known essential and non-essential amino acids (such as alanine and valine) were observed to be low in CHF patients with yin-deficiency syndrome, which gradually breaks the internal equilibrium of the body. This observation aligns with research by Yan et al. (a metabolomics study using a rat model of myocardial ischemia with both qi-deficiency and yin-deficiency syndromes) that showed increased inositol and decreased valine, glycine and serine were closely associated with energy metabolism and oxidative stress response. Additionally, carnitine is an important substance involved in fat metabolism and energy supply, and was seen to reduce dramatically in these patients. Studies have confirmed that L-carnitine can increase the uptake of free fatty acids, which makes use of glucose as oxidative fuel in certain circumstances. If there is an insufficient supply of carnitine, the oxidation process in mitochondria will be affected, which leads to an imbalance in cellular metabolism and heart diseases. The tricarboxylic acid cycle, carbohydrates, proteins and fats are involved in the above metabolic processes, and the indicative of a complicated metabolic disorder in CHF patients with yin-deficiency syndrome.

Secondly, yin-deficiency patients showed higher levels of lactate, glycprotein, pyruvic acid, alanine and glutamate, and lower levels of glucose, LDL/VLDL and HDL compared with non-yin-deficiency patients. Based on TCM theory, yin-deficiency is associated with signs of chronic weakness, hypofunction, hypometabolism and degenerative symptoms and is commonly observed in late or severe stages of many diseases. The metabolism pattern in CHF patients with yin-deficiency showed decreases in glucose metabolism and increases in lactate, alanine and pyruvate, suggesting that carbohydrate and energy metabolism disorders in these patients was serious. There might be enhanced endogenous glucose production from gluconeogenesis and pyruvic acid change may indicate increased hepatic gluconeogenesis to provide extra pyruvate as a substrate for glucose.

Consistent with these findings, yin-deficiency syndrome typically occurs in patients with CHF at stage III and stage IV. Additionally, increased glycoprotein levels

### Table 2B Key metabolites differentiating CHF yin-deficiency patients and controls.

<table>
<thead>
<tr>
<th>No</th>
<th>Metabolite</th>
<th>(Chemical shift)</th>
<th>YADP</th>
<th>NYADP</th>
<th><em>P</em>-Value</th>
<th>VIP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>HDL</td>
<td>0.82</td>
<td>0.1447 ± 0.0439</td>
<td>0.1979 ± 0.0468</td>
<td>0.0180</td>
<td>2.51</td>
</tr>
<tr>
<td>2</td>
<td>Pyruvic acid</td>
<td>0.94</td>
<td>0.0967 ± 0.0181</td>
<td>0.0667 ± 0.0106</td>
<td>0.000</td>
<td>1.45</td>
</tr>
<tr>
<td>3</td>
<td>VLDL/LDL</td>
<td>1.26, 1.3, 1.34</td>
<td>1.5539 ± 0.0885</td>
<td>1.8791 ± 0.0996</td>
<td>0.010</td>
<td>2.50</td>
</tr>
<tr>
<td>4</td>
<td>Lactate</td>
<td>1.33, 4.12</td>
<td>1.4396 ± 0.4708</td>
<td>1.1235 ± 0.2363</td>
<td>0.017</td>
<td>3.70</td>
</tr>
<tr>
<td>5</td>
<td>Alanine</td>
<td>1.48</td>
<td>0.4748 ± 0.0873</td>
<td>0.2077 ± 0.1251</td>
<td>0.000</td>
<td>2.03</td>
</tr>
<tr>
<td>6</td>
<td>Glutamate</td>
<td>2.15, 2.52</td>
<td>0.0292 ± 0.0169</td>
<td>0.0586 ± 0.0189</td>
<td>0.000</td>
<td>1.95</td>
</tr>
<tr>
<td>7</td>
<td>Glucose</td>
<td>3.47, 3.72, 4.64, 5.23</td>
<td>4.1008 ± 0.4777</td>
<td>4.5371 ± 0.6542</td>
<td>0.007</td>
<td>3.01</td>
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<tr>
<td>8</td>
<td>Glycoprotein (N–Ac)</td>
<td>2.02</td>
<td>0.6093 ± 0.0223</td>
<td>0.4289 ± 0.0696</td>
<td>0.000</td>
<td>2.38</td>
</tr>
</tbody>
</table>

Abbreviations: YADP, yin-deficiency patients; NYADP, non-yin deficiency patients; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein; HDL, high-density lipoprotein. Values are expressed as mean (SD) (range). *P*-values were calculated from independent samples t-tests. Variable importance in the projection (VIP) was acquired from the OPLS-DA model.
Fig. 7  (A) Statistical validation of the OPLS-DA model of Group 1. A permutation test performed with 200 random permutations in a PLSDA model showing $R^2$ (green triangles) and $Q^2$ (blue boxes) values from permuted analysis (bottom left) as significantly lower than corresponding original values (top right).  (B) Statistical validation of the OPLS-DA model of Group 2. A permutation test performed with 200 random permutations in a PLSDA model showing $R^2$ (green triangles) and $Q^2$ (blue boxes) values from permuted analysis (bottom left) as significantly lower than corresponding original values (top right).
indicate immune defects in patients, while generally lower lipoprotein levels, including LDL/VLDL and HDL, suggest insufficient absorption and utilization of protein during these phases. Higher excretion levels of measured metabolites (glutamate and alanine) in Group 2 participants could indicate further more potential disturbances during these phases. Higher excretion levels of measured metabolites (glutamate and alanine) in Group 2 participants could indicate further more potential disturbances of renal function, resulting in these patients missing metabolites necessary for carbohydrate and energy metabolism.

A study in China investigated urinary metabolites of yang-deficiency syndrome in patients with chronic kidney disease and reported that key distinguishing metabolites differing between yang-deficiency syndrome patients and the control group included alanine, diethylamine and proline. As essential substances in cellular activities, such deficiencies will affect energy supply in all aspects of the human body. These alterations are likely important contributing factors to the altered metabolite profiling of CHF patients with yang-deficiency syndrome.

This study is an early phase investigation examining TCM syndrome types of CHF based on a small number of study participants. Importantly, this study has demonstrated that two TCM syndromes were able to be distinguished based on their plasma metabolic patterns. While the findings of this study are very promising, further research using larger cohort is required to confirm and validate the reliability of individualized treatment of CHF based on TCM subtypes.

Conclusion

The current study applied biology-based NMR plasma metabolomics with TCM personalized diagnosis to find metabolic subtypes in CHF. Two TCM syndrome subtypes of CHF were identified by plasma metabolomics and showed different metabolic patterns. Group 2 had lower levels of sugars, proteins, fats and amino acids compared with Group 1, indicating increased disturbances in and carbohydrate and energy metabolism and renal function in these patients. The identified plasma metabolites may be of special clinical relevance for the diagnosis of subtypes of CHF and for uncovering metabolomics pathways and prognosis in some cases, which could lead to a better understanding of, and improvement in, personalized interventions for CHF. Future studies are needed to validate the TCM subtypes identified in this study, and to assess intervention responses of these TCM subtypes to potential metabolic therapy or drugs.

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