UPLC-QTOF/MS-Based Lipidomic Profiling of Liver Qi-Stagnation and Spleen-Deficiency Syndrome in Patients with Hyperlipidemia

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Hyperlipidemia is a common disease caused by abnormal plasma lipid metabolism. Lipidomics is a powerful and efficient technology to study the integration of disease and syndrome of Chinese medicine. This study investigated specific changes in lipid metabolites from hyperlipidemia patients with syndrome of liver qi-stagnation and spleen-deficiency (SLQSD). Lipid profiles in plasma samples from 29 hyperlipidemia patients including 10 SLQSD and 19 non-SLQSD and 26 healthy volunteers (NC) were tested by UPLC-QTOF/MS. PLS-DA analysis and database searching were performed to discover differentiating metabolites. Differences in lipid metabolites between hyperlipidemia and healthy people mainly include phosphatidylcholines, phosphatidylethanolamines, phosphatidylinositols, triglycerides, diacylglycerols, lysophosphatidylethanolamines, sphingomyelins, lysophosphatidylcholines, and lactosylceramides. Hyperlipidemia patients with SLQSD and non-SLQSD could be differentiated by using identified lipid metabolites including phosphatidylcholines, phosphatidylethanolamines, phosphatidylinositol, triglycerides, diacylglycerols, lysophosphatidylethanolamines, sphingomyelins, lysophosphatidylcholines, and lactosylceramides. There were significant differences of lipid metabolism between different syndromes of the same disease such as hyperlipidemia which showed significant differences between SLQSD and non-SLQSD.

1. Introduction

Hyperlipidemia is a common disease caused by abnormal plasma lipid metabolism and is considered a high independent risk factor for atherosclerotic cardiovascular and cerebrovascular disease such as coronary heart disease and stroke. In traditional Chinese medicine (TCM), hyperlipidemia is called lipid turbidity and is treated based on syndrome differentiation. With the transformation of lifestyle hyperlipidemia showed a trend in young people and the syndrome of Chinese medicine changed from spleen-kidney deficiency to stagnation of liver qi and spleen deficiency (SLQSD) [1, 2]. The syndrome of liver depression and spleen deficiency is the main syndrome of hyperlipidemia [3, 4].

The investigation of syndrome essence is a key challenge in the field of Chinese medicine. Until now, due to limitations of the methods available, the progress towards understanding such complicated systems has been slow. As the most important section in the TCM system, syndrome differentiation based on the clinical manifestations from traditional four diagnostic methods naturally has biological foundation. Except for total cholesterol (TC), total triglyceride (TG), low density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C), does hyperlipidemia have any difference in lipid metabolites between syndrome of SLQSD and non-SLQSD?

Metabolomics has been widely applied to disease biomarker discovery, drug mechanism evaluation, and
2. Materials and Methods

2.1. Diagnostic Criteria and Syndrome Differentiation. The diagnostic criteria for hyperlipidemia were mainly obtained from “Guideline of Chinese adult dyslipidemia Prevention and Treatment (2007) [23].” Syndrome differentiation criteria were mainly obtained from the textbook “Diagnosis of Traditional Chinese Medicine [24]” and “TCM clinical diagnostic and treatment practices (2002) [25].” Criteria of syndrome of stagnation of liver qi and spleen deficiency included main symptoms, secondary symptoms, and syndrome determination. The characteristics of main symptoms were emotional depression or irritability, flank swelling and pain, poor appetite, loose stools, string, or thin pulse. The characteristics of secondary symptoms were paleness, tiredness and not wanting to talk, frequent sighing, abdominal painful distension, obesity, uncomfortable loose bowels or alleviation of abdominal pain after defecation, pale tongue, and white tongue coating. The characteristics of syndrome determination were three or more main syndromes or two main syndromes and three or more secondary symptoms and with the reference of tongue and pulse.

2.2. Patient Selection. All subjects were recruited from the First Affiliated Hospital of Guangdong Pharmaceutical University. There were 29 patients with primary hyperlipidemia including 10 cases with the symptom of SLQSD and 19 cases with non-SLQSD. Control group (normal group) consisted of 26 healthy volunteers with no cold and other acute diseases. Individuals who volunteered to be a subject signed an informed consent form; those who were aged 30 to 70 years and met the diagnostic criteria for hyperlipidemia were included. Secondary hyperlipidemic patients were excluded. Patients with colds, acute gastroenteritis, and other acute diseases in the survey period which interfered with the judgment of candidates were not included. Patients with cerebral infarction, myocardial infarction, other serious diseases, and mental illness or who could not cooperate with the investigation were excluded.

2.3. Reagents. HPLC grade acetonitrile was purchased from Merck Company. HPLC grade formic acid was purchased from Dima Company. HPLC grade ammonium acetate and chloroform was purchased from Tianjin Damao Company. HPLC grade methanol was purchased from B&J Company.

2.4. Sample Collection and Preparation. Plasma samples were taken after having fasted for more than 12 hours. The next morning 2 mL blood samples was collected from their median cubital vein and stored in 4 mL EDTA microcentrifuge tubes. The samples were centrifuged at 3,000 x g for 10 min at 4°C. Plasma was separated and kept frozen at −80°C until analysis.

A 0.1 mL aliquot of each plasma sample was transferred to 1.5 mL polypropylene tubes with a fixed amount of 0.3 mL 2:l (v/v) CHCl₃:MeOH. The mixture was settled at room temperature for 5s, and then 75µL ultra high purity water had been added to the tube and vortex-mixed. The mixture was centrifuged at 10,000 rpm for 5 min at 4°C. The under layer was transferred to another polypropylene tube and evaporated to dryness at room temperature under nitrogen gas. The final residue was redissolved in 120µL acetonitrile and then was centrifuged at 12000 rpm for 10 min. The supernatant was subjected to UPLC-QTOF/MS analysis.

2.5. UPLC Conditions. The samples were analyzed by a Waters Acquity Ultra Performance LC system (Waters, USA) equipped with Waters Xevo™ G2 QToF MS. Chromatographic separation was carried out at 30°C on an Acquity UPLC™ BEH C₁₈ (10 × 50 mm). Injection volume was 5µL. The total flow rate was 0.4 mL/min. The sample chamber temperature was kept at 4°C. The mobile phase consisted of acetonitrile water (0.1% formic acid, 1 mol/L ammonium acetate). The linear solvent gradient was shown in Table I.

2.6. Mass Spectrometry. The mass spectrometric detection was conducted by Q-TOF MS system. ESI ion source was used in both positive and negative ion modes and centroid mode was used to get signal acquisition and did the real-time simultaneous Lock-Mass mass correction with the correction fluid being chloramphenicol (500 pg/µL). Its precise charge to mass ratio was [M + H]⁺ = 345.0021 and [M-H]⁻ = 321.0045, respectively, in both positive and negative ion modes. Mass range was 300-1200m/z. In positive and negative ion modes MS conditions were as follows: capillary cone: 3200 V; sample cone: 39 V; extraction cone: 2.0 V; source temperature: 120°C; desolvation temperature: 250°C; cone gas: 60 L/hr; desolvation gas: 800 L/hr; ion energy: 1.0 V; collision energy: 10 V.

2.7. Statistical Analysis. The raw data were processed using the Micromass MarkerLynx Applications Manager version 4.0 (Waters Corp., Milford, USA). This application manager incorporates a peak deconvolution package that allows detection of the mass, retention time, and intensity of the peaks eluting in each chromatogram. The area of each peak, after being recognized and aligned, was normalized to the summed total ion intensity of each chromatogram. The resulting three-dimensional data, peak number (RT-m/z...
Table 1: Linear gradient composition.

| Time (min) | Water (0.1% formic acid, 1mol/L ammonium acetate) | Acetonitrile |
|-----------|--------------------------------------------------|-------------|
| 0         | 65                                               | 35          |
| 3         | 45                                               | 55          |
| 15        | 0                                                | 100         |
| 17        | 0                                                | 100         |
| 17.1      | 65                                               | 35          |
| 20        | 65                                               | 35          |

Table 2: Clinical characteristics.

| n     | Hyperlipidemia with syndrome of SLQSD | Hyperlipidemia with syndrome of non-SLQSD | Healthy volunteers |
|-------|--------------------------------------|------------------------------------------|-------------------|
| Age (X±SD) | 55.6±9.64* | 54.26±8.05** | 46.73±7.74 |
| Gender [Female (%)] | 60.0 | 63.2 | 53.8 |
| TC     | 4.90±0.69* | \(^{\ddagger\ddagger}\) | 5.88±0.72* | 4.37±0.60 |
| TG     | 2.24±0.84** | 2.00±1.27** | 1.02±0.26 |
| HDL-C  | 1.17±0.31 | 1.40±0.33 | 1.37±0.27 |
| LDL-C  | 2.80±0.56\(^{\ddagger}\) | 3.55±0.75** | 2.50±0.48 |
| VDL-C  | 0.96±0.25** | 0.93±0.50** | 0.49±0.14 |
| ApoA1  | 1.36±0.30 | 1.44±0.19 | 1.40±0.18 |
| ApoB   | 1.02±0.25 | 1.15±0.31* | 0.83±0.16 |

Gender was expressed as percentage and the other data were expressed as mean ± SD. *p<0.05 and **p<0.01 compared with healthy volunteer group. Hyperlipidemia with syndrome of SLQSD compared with hyperlipidemia with syndrome of non-SLQSD, \(^{\ddagger\ddagger}\)p<0.05, and \(^{\ddagger}\)p < 0.01.

3. Results and Discussion

3.1. Clinical Characteristics. The study population is 55 with 29 hyperlipidemia patients in which 10 had syndrome of stagnation of liver qi and spleen deficiency and 19 did not and 26 were healthy volunteers. Sex among the three groups showed no significant differences (P > 0.05). Age comparison showed differences between normal group and hyperlipidemia group (P = 0.01) whereas no significant differences between the two different syndrome groups of hyperlipidemia group (P = 0.68) indicated that the body was prone to abnormal lipid metabolism with age increasing. The clinical characteristics were shown in Table 2.

3.2. Chromatograms in Both Positive and Negative Ion Mode. As can be seen from Figure 1, there are significant differences in lipid metabolism in both positive and negative ion modes of healthy volunteers and patients with hyperlipidemia. The amounts of mass data obtained in the positive ion mode were more than that in the negative ion mode, indicating that the positive ion mode is more suitable for detecting plasma lipid metabolites. In order to get more comprehensive information on lipid metabolism, we selected both positive and negative ion modes to detect sample.

3.3. Plasma Samples Metabolic Profiles. The subtle changes could be found using a pattern recognition approach, such as PCA and PLS-DA. The supervised PLS-DA model was used to separate plasma sample into two blocks between patients with hyperlipidemia and healthy volunteers (Figure 2).

The supervised PLS-DA divided samples into two blocks and this method was applied to obtain a better discrimination between the two groups. Based on the differences in their metabolic profiles, the PLS-DA score plot analysis distinguished the plasma samples of hyperlipidemia patients with syndrome of SLQSD and hyperlipidemia patients with syndrome of non-SLQSD (Figure 3).

3.4. The Differential Lipids between Different Groups. 28 endogenous plasma lipid metabolites, contributing to the separation between the groups, were identified based on their molecular ion information as well as the fragments of corresponding product ion. The identification of the
Figure 1: Typical base peak intensity (BPI) chromatograms obtained from plasma of healthy volunteers group (NC), hyperlipidemia with syndrome of non-SLQSD group, and hyperlipidemia with syndrome of SLQSD group in positive ion mode (a) and negative ion mode (b).
Biomarker was submitted for database searching, either in-house or using the online Scripps Center for Metabolomics database (https://metlin.scripps.edu/), Lipid Maps (http://www.lipidmaps.org/), HMDB (http://www.hmdb.ca), and Chemspider (http://www.chemspider.com) data source. The variables (ions) were identified based on the metabolite identification strategy, and VIP values was also used for the selection of biomarkers (listed in Tables 3 and 4, Fact of Change >2 or <1). Compared with the healthy volunteers, the hyperlipidemia patients had higher concentrations of PC(16:0/18:2), PG(18:3/18:2), Cer(d18:0/16:0), PE(22:1/15:0), PE(15:0/24:1), PC(22:6/16:0) (Table 3 and Figure 4).

Our results indicate that there were a great many differences of lipid metabolism between different syndrome of the same disease, hyperlipidemia, and showed more obvious differences of the main syndrome of SLQSD. Comparing the hyperlipidemia with syndrome of non-SLQSD patients, the hyperlipidemia with syndrome of SLQSD patients exhibited elevated lipid metabolites including PE(22:2/15:0), PC(18:3/18:0), TG(14:0/18:3/15:0), LacCer(d18:1/12:0), PC(20:3/16:1), PC(18:3/18:0), SM(d18:1/20:0), PE(15:0/22:2), PC(22:6/22:6), DG(20:2/22:0/0:0), PE(22:5/20:1), PC(22:6/18:3), PE(24:0/20:3), PI(16:0/20:3), PC(22:4/20:5), and PI(16:0/20:4) (Table 4 and Figure 4).

According to the differences of metabolites, the different metabolic differences between sample content changes in each group were visualized. As shown in Figure 5, red indicated the higher level of the metabolites. The blue indicated the lower level of the metabolites.

4. Discussion

Under Chinese medicine principle guidance, TCM has been widely used in the clinic and has been considered an alternative therapy for the treatment of various diseases, such as hyperlipidemia, diabetes, hypertension, cardiovascular disease, kidney disease, and gastrointestinal disease [26–32]. TCM syndrome is the comprehensive analysis of clinical information gained by the four main diagnostic TCM procedures, observation, listening, questioning, and pulse analysis [33], and is built on the bases of long-term and substantial clinical practice [34]. The complete TCM process is known as BianZhengLunZhi [35]. TCM treatment is based on the traditional diagnose method to distinguish the TCM syndrome, not the disease. In the development process, TCM diagnosis and treatment system form two systems: disease differentiation and syndrome differentiation [36]. So there is a phenomenon in the relationship between TCM syndrome and disease, called different TCM syndrome for same disease [37]. Researchers used various means to research and explore the essence or modern scientific connotation of TCM syndrome [34,38].
SLQSD contains nerve, digestion, absorption, metabolism, immune, endocrine, nucleotide, matrix metalloproteinase, blood fluid rheology, and other aspects of change. Due to the complexity and integrity of the syndrome, it is difficult to use a single physiological and biochemical indicator to reveal its essence. So we used lipidomics technology to investigate the syndrome of the modern diseases (hyperlipidemia). The data demonstrated that PLS-DA showed a significantly separation between hyperlipidemia patients and healthy volunteers with the different lipids including PC, PE, PG, and Cer as well as between the hyperlipidemia patients with syndrome of SLQSD and the syndrome of non-SLQSD with the different lipids including PC, PE, PI, TG, DG, SM, LysoPC, LysoPE, and LacCer as shown in Tables 1 and 2. Interestingly, we found that PE(24:0/20:3) has a value of VIP more than 36, while and PC(22:4/20:5) elevated more than 23 folds between the SLQSD and non-SLQSD. The current study demonstrated many differences in lipid metabolism between different syndromes of the same disease such as hyperlipidemia and showed more obvious differences of the main syndrome of SLQSD.

Different types of lipids play different roles in the human body as phosphatidylethanolamine (PE) and phosphatidylcholine (PC) play crucial roles in the biological system to maintain the cellular environmental condition [39]. Oxidative stress and inflammation play a central part in the pathogenesis and progression of various diseases. Oxidative stress targets these phospholipids containing polyunsaturated fatty acids and accompanies the oxidized phospholipids [40–43]. Recent studies have suggested that oxidized phospholipids is associated with inflammation and might induce the atherosclerosis formation by the uptake of oxidized LDL through scavenger receptor as ligands [44]. Accumulated evidence has demonstrated that PC could improve insulin sensitivity and contribute to both proliferative growth and programmed cell death [45]. PC is also the biosynthetic precursor of lysoPC [13]. A number of studies have shown that lysoPC plays a critical role in glucose metabolism, lysoPC activates adipocyte glucose uptake and lowers blood glucose levels in murine models of diabetes [46], and the decreased plasma level of lysoPCs was found in Type 2 diabetes [47]. LysoPE, known as a relational protein, is involved in several motility-related processes such as angiogenesis and neurite outgrowth [48]. Glycosphingolipids are known to interfere with insulin signaling at elevated levels [49]. Lactosylceramide is highly expressed on the plasma membranes of human phagocytes and mediates several immunological and inflammatory reactions, including phagocytosis, chemotaxis,
| NO | Retention Time | Mass     | Group 1 Content | Group 2 Content | Factor of Change | VIP Value | Change Trend | Identified potential Biomarker | Lipid Class       | Ion   |
|----|----------------|----------|-----------------|-----------------|------------------|-----------|--------------|--------------------------------|-------------------|-------|
| 1  | 8.03           | 758.5651 | 4.7339±1.869*   | 0.6151±0.6942   | 7.7              | 4.76901   | ↑            | PE(22:2/15:0)                  | Glycerophospholipid| M+H   |
| 2  | 14.1           | 758.5656 | 24.4239±7.3675* | 16.0367±4.1211  | 1.5              | 19.6218   | ↑            | PC(16:0/18:2)                  | Glycerophospholipid| M+H   |
| 3  | 15.4           | 769.5915 | 2.8041±0.8225   | 2.3896±0.8705   | 1.2              | 7.40167   | ↑            | PC(18:3/18:2)                  | Glycerophospholipid| M+H   |
| 4  | 16.15          | 540.4826 | 0.2268±0.0674*  | 0.1447±0.0368   | 1.6              | 4.95507   | ↑            | PG(18:3/18:2)                  | Glycerophospholipid| M+H   |
| 5  | 18.05          | 760.5777 | 24.6892±1.4525* | 8.4016±1.6207   | 2.9              | 16.1507   | ↑            | Cer(d18:0/16:0)                 | Sphingomyelin      | M+H   |
| 6  | 14.32          | 832.6021 | 21.921±6.4255*  | 13.974±3.9031   | 1.6              | 4.2825    | ↑            | PE(22:1/15:0)                  | Glycerophospholipid| M+H   |
| 7  | 14.37          | 804.5628 | 44.7529±12.4215*| 25.7829±2.6471  | 1.7              | 2.48245   | ↑            | PC(22:6/16:0)                  | Glycerophospholipid| M+FA-H|

Group 1: hyperlipidemia. Group 2: healthy volunteers. ↑ indicated the concentrations compared to the other group are increasing. ↓ indicated the concentrations compared to the other group are reducing. * P<0.05 and ** P<0.01. PE: phosphatidylethanolamine; PC: phosphatidylcholine; PG: phosphatidylglycerol; Cer: ceramide.
Table 4: Identification results of varying ions and their change trend of hyperlipidemia with syndrome of SLQSD and hyperlipidemia with the syndrome of non-SLQSD.

| NO | Retention Time | Mass   | Group 3 Content | Group 4 Content | Factor of change | VIP value | Change Trend | Identified potential Biomarker | Lipid Class | Ion          |
|----|----------------|--------|-----------------|-----------------|------------------|-----------|--------------|--------------------------------|-------------|--------------|
| 1  | 5.53           | 544.3342 | 9.331±2.342       | 16.265±4.2760   | 0.6              | 7.2994    | ↓            | LysoPC(20:4)                  | Glycerophospholipid | M+H          |
| 2  | 12.28          | 784.5734 | 14.359±3.371       | 7.768±1.4901    | 1.8              | 10.021    | ↑            | PC(18:3/18:0)                 | Glycerophospholipid | M+H          |
| 3  | 12.34          | 787.5918 | 11.441±1.513       | 6.700±1.7245    | 3.1              | 6.0391    | ↑            | TG(14:0/18:3/18:0)            | Glycerolipids     | M+H          |
| 4  | 12.64          | 806.5652 | 5.772±1.104        | 2.293±0.7673    | 2.5              | 6.1168    | ↑            | LaCer(d18:1/12:0)             | Sphingomyelin     | M+H          |
| 5  | 15.81          | 782.5518 | 246.974±38.639      | 196.107±11.3457 | 1.3              | 63.2972   | ↑            | PC(20:3/16:1)                 | Glycerophospholipid | M+H          |
| 6  | 16.48          | 784.5824 | 38.277±5.452        | 8.407±0.3322    | 4.6              | 16.5178   | ↑            | PC(18:3/18:0)                 | Glycerophospholipid | M+H          |
| 7  | 16.69          | 759.5692 | 57.526±7.823        | 40.471±4.7738   | 1.4              | 12.2461   | ↑            | SM(d18:1/20:0)                | Sphingolipid      | M+H          |
| 8  | 16.86          | 810.5915 | 19.922±1.874        | 274.930±175.735 | 0.1              | 2.44919   | ↑            | PC(22:4/16:0)                 | Glycerophospholipid | M+H          |
| 9  | 18.30          | 758.5560 | 135.189±8.187       | 256.305±30.1737 | 0.5              | 36.2027   | ↑            | PE(22:2/15:0)                 | Glycerophospholipid | M+H          |
| 10 | 8.50           | 508.3418 | 43.099±4.123        | 79.117±18.238    | 0.5              | 3.0607    | ↑            | LysoPE(20:0/0:0)               | Sphingomyelin     | M+H          |
| 11 | 14.32          | 876.5754 | 7.735±2.439         | 4.788±0.1727    | 1.6              | 2.02951   | ↑            | PE(22:2/22:6)                 | Glycerophospholipid | M-H          |
| 12 | 14.38          | 351.2478 | 15.696±3.528        | 5.727±0.52700   | 2.8              | 2.29853   | ↑            | DG(20:2/22:0/0:2)             | Glycerolipids     | M-2H         |
| 13 | 15.05          | 800.5540 | 22.962±3.846        | 15.076±4.038    | 1.5              | 2.17423   | ↑            | PE(22:5/20:1)                 | Glycerophospholipid | M-H2O-H      |
| 14 | 15.3           | 826.6140 | 17.848±1.821        | 4.984±1.1049    | 3.6              | 1.14469   | ↑            | PC(22:6/18:3)                 | Glycerophospholipid | M-H          |
| 15 | 15.94          | 852.6908 | 17.848±1.821        | 4.984±1.1049    | 3.6              | 2.44998   | ↑            | PE(24:0/20:3)                 | Glycerophospholipid | M-H          |
| 16 | 16.00          | 841.5830 | 11.703±2.421        | 6.215±1.0354    | 1.9              | 1.77427   | ↑            | PI(16:0/20:3)                 | Phosphatidylinositol | M-H2O-H      |
| 17 | 16.25          | 854.6947 | 101.609±23.484      | 11.3739±0.8854  | 8.9              | 2.49232   | ↑            | PC(22:4/20:5)                 | Glycerophospholipid | M-H          |
| 18 | 16.45          | 857.5742 | 23.920±0.518        | 6.4069±1.4917   | 3.7              | 1.47236   | ↑            | PI(16:0/20:4)                 | Phosphatidylinositol | M-H          |

Group 3: hyperlipidemia with syndrome of SLQSD and Group 4: hyperlipidemia with the syndrome of non-SLQSD. ↑ indicated the concentrations compared to the other group are increasing, and ↓ indicated the concentrations compared to the other group are reducing. *P<0.05 and **P<0.01. PE: phosphatidylethanolamine; PC: phosphatidylcholine; TG: triglyceride; DG: diglyceride; PI: phosphatidylinositol; SM: sphingomyelin; LysoPC: lysophosphatidylcholine; LysoPE: lysophosphatidylethanolamine.
Figure 4: The statistical results of 25 biomarkers. (a, b) Comparison of 7 biomarkers peak relative signal intensities in hyperlipidemia and healthy volunteer. (c-e) Comparison of 18 biomarkers peak relative signal intensities in hyperlipidemia with syndrome of SLQSD and hyperlipidemia with the syndrome of non-SLQSD groups. Values are means ±SD, *P<0.05, and **P<0.01.
and superoxide generation [50]. Other studies proved that SLQSD mainly involves the decrease of thymus function and insufficient release of cytokines at early immune response stage and also involves the inhibition of cellular immunity and humoral immunity [51]. Cer(d18:0/16:0) has high sensitivity and specificity on the prognosis related to major adverse cardiovascular events after ST-segment elevation myocardial infarction [52].

The current study not only indicated that lipidomics was an effective method to distinguish different TCM syndromes of hyperlipidemia but also showed the changing trend of lipid metabolites between different syndromes. Future researches will focus on the discovery of specific lipid such as PE(24:0/20:3) and PC(22:4/20:5) of syndrome of SLQSD in other diseases and the validation of the explorative biomarkers. In addition, more efforts will be directed to the biological interpretation including investigating which pathway is involved in the lipids changes associated with the onset, development, and progression of hyperlipidemia and whether these changes are the same during onset and progression, or whether different changes of lipids occur of different syndrome. In the future, large sample studies are needed to reveal whether the biological basis of SLQSD is the oxidative stress and inflammatory reaction caused by PC(22:4/20:5) and PE(24:0/20:3). In addition to the clinical detection indicators of blood lipid, we need to know whether other lipids such as PC, PE, PG, and Cer can be new or early diagnostic indicators of dyslipidemia. Combined with systems biology and other techniques, it is possible to analyze the biological biomarkers of TCM syndrome more comprehensively.

**Data Availability**

The data used to support the findings of this study are available from the corresponding author upon request.

**Conflicts of Interest**

There are no any conflicts of interest regarding the publication of this manuscript.

**Authors’ Contributions**

Piao Shenghua and Tan Shuyu contributed equally to this work.

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References

[1] J. Guo and S. H. Piao, “The pathogenesis of hyperlipidemia in traditional Chinese medicine from the incidence trend of younger age of hyperlipidemia,” Journal of Information on Traditional Chinese Medicine, vol. 6, pp. 4–6, 2008.

[2] S. H. Piao, J. Guo, and W. Bai, “Dissertation on the position and role of the liver on the pathogenesis of hyperlipidemia,” Journal of New Chinese Medicine, vol. 2, pp. 1–3, 2011.

[3] S. H. Piao, J. Guo, and Z. P. Hu, “Clinical research of Chinese medicine syndromes of hyperlipidemia inpatients,” Chinese Journal of Integrative Medicine, vol. 32, no. 10, pp. 1322–1325, 2012.

[4] S. H. Piao, J. Guo, and Z. P. Hu, “Research on regularity of the TCM syndrome of hyperlipidemia based on literature,” Journal of Guangzhou University of Traditional Chinese Medicine, vol. 30, no. 5, pp. 609–614, 2013.

[5] D.-Q. Chen, H. Chen, L. Chen, D.-D. Tang, H. Miao, and Y.-Y. Zhao, “Metabolomic application in toxicity evaluation and toxicological biomarker identification of natural product,” Chemico-Biological Interactions, vol. 252, pp. 114–130, 2016.

[6] M. Wang, L. Chen, D. Liu, H. Chen, D.-D. Tang, and Y.-Y. Zhao, “Metabolomics highlights pharmacological bioactivity and biochemical mechanism of traditional Chinese medicine,” Chemico-Biological Interactions, vol. 273, pp. 133–141, 2017.

[7] Y. Y. Zhao, X. L. Cheng, N. D. Vaziri, S. Liu, and R. C. Lin, “UPLC-based metabolic applications for discovering biomarkers of diseases in clinical chemistry,” Clinical Biochemistry, vol. 47, no. 15, pp. 16–26, 2014.

[8] Z. Liu, Y. Zeng, and P. Hou, “Metabolomic evaluation of Euphorbia pekinensis induced ephrotoxicity in rats,” Pharmaceutical Biology, vol. 56, no. 1, pp. 145–153, 2018.

[9] H. Ji, Y. Liu, F. He, R. An, and Z. Du, “LC–MS based urinary metabolomics study of the intervention effect of aloe-emodin on hyperlipidemia rats,” Journal of Pharmaceutical and Biomedical Analysis, vol. 156, pp. 104–115, 2018.

[10] W. Wang, L. Zhao, Z. He et al., “Metabolomics-based evidence of the hypoglycemic effect of Ge-Gen-Jiao-Tai-Wan in type 2 diabetic rats via UHPLC-QTOF/MS analysis,” Journal of Ethnopharmacology, vol. 219, pp. 299–318, 2018.

[11] H. Miao, H. Chen, S. Pei, X. Bai, N. D. Vaziri, and Y.-Y. Zhao, “Plasma lipidomics reveal profound perturbation of glycerophospholipids, fatty acids, and sphingolipids in diet-induced hyperlipidemia,” Chemico-Biological Interactions, vol. 228, pp. 79–87, 2015.

[12] S. Kreimer, A. M. Belov, I. Ghiran, S. K. Murthy, D. A. Frank, and A. R. Ivanov, “Mass-spectrometry-based molecular characterization of extracellular vesicles: Lipidomics and proteomics,” Journal of Proteome Research, vol. 14, no. 6, pp. 2367–2384, 2015.

[13] Y.-Y. Zhao, X.-L. Cheng, and R.-C. Lin, “Lipidomics applications for discovering biomarkers of diseases in clinical chemistry,” International Review of Cell and Molecular Biology, vol. 313, pp. 1–26, 2014.

[14] H. Lee, J. M. Choi, J. Cho, T. Kim, H. J. Lee, and B. H. Jung, “Regulation of endogenous metabolites by rosuvastatin in hyperlipidemia patients: An integration of metabolomics and lipidomics,” Chemistry and Physics of Lipids, vol. 214, pp. 69–83, 2018.

[15] H. Miao, Y.-H. Zhao, N. D. Vaziri et al., “Lipidomics biomarkers of diet-induced hyperlipidemia and its treatment with poria cocos,” Journal of Agricultural and Food Chemistry, vol. 64, no. 4, pp. 969–979, 2016.

[16] Y. Y. Zhao, S. P. Wu, S. Liu, Y. Zhang, and R. C. Lin, “Ultra-performance liquid chromatography-mass spectrometry as a sensitive and powerful technology in lipidomic applications,” Chemico-Biological Interactions, vol. 220, pp. 181–192, 2014.

[17] H. Miao, H. Chen, X. Zhang et al., “Urinary metabolomics on the biochemical profiles in diet-induced hyperlipidemia rat using ultra-performance liquid chromatography coupled with quadrupole time-of-flight SYNAPT high-definition mass spectrometry,” Journal of Analytical Methods in Chemistry, vol. 2014, Article ID 184162, 9 pages, 2014.

[18] H. Chen, L. Chen, D. Liu et al., “Combined clinical phenotype and lipidomic analysis reveals the impact of chronic kidney disease on lipid metabolism,” Journal of Proteome Research, vol. 16, no. 4, pp. 1566–1578, 2017.

[19] Y.-T. Liu, J.-B. Peng, H.-M. Jia et al., “UPLC-Q/TOF MS standardized Chinese formula Xin-Ke-Shu for the treatment of atherosclerosis in a rabbit model,” Phytomedicine, vol. 21, no. 11, pp. 1364–1372, 2014.

[20] Y.-Y. Zhao, H. Miao, X.-L. Cheng, and F. Wei, “Lipidomics: Novel insight into the biochemical mechanism of lipid metabolism and dysregulation-associated disease,” Chemico-Biological Interactions, vol. 5, no. 240, pp. 220–238, 2015.

[21] H. Miao, “The antihyperlipidemic effect of Fu-Ling-Pi is associated with abnormal fatty acid metabolism as assessed by UPLC-HDMS-based lipidomics,” RSC Advances, vol. 5, no. 79, pp. 64208–64219, 2015.

[22] Z.-H. Zhang, N. D. Vaziri, F. Wei, X.-L. Cheng, X. Bai, and Y.-Y. Zhao, “An integrated lipidomics and metabolomics reveal nephroprotective effect and biochemical mechanism of Rheum officinale in chronic renal failure,” Scientific Reports, vol. 6, Article ID 22151, 2016.

[23] Joint committee on guidelines for the prevention and treatment of adult dyslipidemia in China, “Joint committee on guidelines for the prevention and treatment of adult dyslipidemia in China,” Chinese Journal of Cardiovascular Disease, vol. 35, no. 5, pp. 390–413, 2007.

[24] J. X. Chen, Diagnostics of Traditional Chinese Medicine, Chinese Press of Traditional Chinese Medicine, Beijing, Chinese, 9th edition, 2015.

[25] X. Y. Zheng, Guidelines for Clinical Research on Chinese New Herbal Medicines (Trial implementation), Medical Science and Technology Publishing House of China, Beijing, China, 2002.

[26] M. Wang, D.-Q. Chen, L. Chen et al., “Novel RAS Inhibitors Poricoic Acid ZG and Poricoic Acid ZH Attenuate Renal Fibrosis via a Wnt/β-Catenin Pathway and Targeted Phosphorylation of smad3 Signaling,” Journal of Agricultural and Food Chemistry, vol. 66, no. 8, pp. 1828–1842, 2018.

[27] M. Wang, D.-Q. Chen, M.-C. Wang et al., “Poricoic acid ZA, a novel RAS inhibitor, attenuates tubulo-interstitial fibrosis and podocyte injury by inhibiting TGF-β/Smad signaling pathway,” Phytomedicine, vol. 36, pp. 243–253, 2017.

[28] K. H. Wong, G. Q. Li, M. K. Li, V. Razmovski-Naumovski, and K. Chan, “Kudzu root: traditional uses and potential medicinal benefits in diabetes and cardiovascular diseases,” Journal of Ethnopharmacology, vol. 134, no. 3, pp. 584–607, 2011.

[29] R. Khoogar, B. Kim, J. Morris, and M. J. Wargovich, “Chemoprevention in gastrointestinal physiology and disease. Targeting the progression of cancer with natural products: a focus on gastrointestinal cancer,” American Journal of Physiology-Gastrointestinal and Liver Physiology, vol. 310, no. 9, pp. G629–G644, 2016.
[30] W.-Q. Wang, Y.-P. Yin, L. Jun, and L.-J. Xuan, "Halimane-type diterpenoids from Vitex rotundifolia and their anti-hyperlipidemia activities," *Phytochemistry*, vol. 146, pp. 56–62, 2018.

[31] H. Chen, T. Yang, M.-C. Wang, D.-Q. Chen, Y. Yang, and Y.-Y. Zhao, "Novel RAS inhibitor 25-O-methylalisol F attenuates epithelial-to-mesenchymal transition and tubulo-interstitial fibrosis by selectively inhibiting TGF-β-mediated Smad3 phosphorylation," *Phytomedicine*, vol. 42, pp. 207–218, 2018.

[32] Y.-Y. Zhao, X.-L. Cheng, J.-H. Cui et al., "Effect of ergosta-4,6,8(14),22-tetraen-3-one (ergone) on adenine-induced chronic renal failure rat: A serum metabolomic study based on ultra performance liquid chromatography/high-sensitivity mass spectrometry coupled with MassLynx i-FIT algorithm," *Clinica Chimica Acta*, vol. 413, no. 19-20, pp. 1438–1445, 2012.

[33] H. Sun, A. Zhang, and X. Wang, "Potential role of metabolomic approaches for Chinese medicine syndromes and herbal medicine," *Phytotherapy Research*, vol. 26, no. 10, pp. 1466–1471, 2012.

[34] C.-L. Lu, X.-Y. Qv, and J.-G. Jiang, "Proteomics and syndrome of Chinese medicine," *Journal of Cellular and Molecular Medicine*, vol. 14, no. 12, pp. 2721–2728, 2010.

[35] M. Jiang, C. Lu, C. Zhang et al., "Syndrome differentiation in modern research of traditional Chinese medicine," *Journal of Ethnopharmacology*, vol. 140, no. 3, pp. 634–642, 2012.

[36] F. Li, S. L. Ji, and J. Liu, "Syndrome differentiation and individualized health care," *Journal of Medicine and Philosophy*, vol. 22, no. 9, pp. 953–959, 2001.

[37] Z. Guo, S. Yu, Y. Guan et al., "Molecular mechanisms of same TCM syndrome for different diseases and different TCM syndrome for same disease in chronic hepatitis B and liver cirrhosis," *Evidence-Based Complementary and Alternative Medicine*, vol. 2012, Article ID 120350, 9 pages, 2012.

[38] X. Lu, Z. Xiong, J. Li, S. Zheng, T. Huo, and F. Li, "Metabonomics study on 'Kidney-Yang Deficiency syndrome' and intervention effects of Rhizoma Drynariae extracts in rats using ultra performance liquid chromatography coupled with mass spectrometry," *Talanta*, vol. 83, no. 3, pp. 700–708, 2011.

[39] Y.-Y. Zhao, X.-L. Cheng, R.-C. Lin, and F. Wei, "Lipidomics applications for disease biomarker discovery in mammal models," *Biomarkers in Medicine*, vol. 9, no. 2, pp. 153–168, 2015.

[40] D.-Q. Chen, G. Cao, H. Chen et al., "Gene and protein expressions and metabolomics exhibit activated redox signaling and wnt/β-catenin pathway are associated with metabolic dysfunction in patients with chronic kidney disease," *Redox Biology*, vol. 12, pp. 505–521, 2017.

[41] H. Chen, G. Cao, D. Q. Chen et al., "Metabolomics insights into activated redox signaling and lipid metabolism dysfunction in chronic kidney disease progression," *Redox Biology*, vol. 10, pp. 168–178, 2016.

[42] Y.-Y. Zhao, H.-L. Wang, X.-L. Cheng et al., "Metabolomics analysis reveals the association between lipid abnormalities and oxidative stress, inflammation, fibrosis, and Nrf2 dysfunction in aristolochic acid-induced nephropathy," *Scientific Reports*, vol. 5, Article ID 12936, 2015.

[43] D.-Q. Chen, H. Chen, L. Chen et al., "The link between phenotype and fatty metabolism in advanced chronic kidney disease," *Nephrology Dialysis Transplantation*, vol. 32, no. 7, pp. 1154–1166, 2017.

[44] S. Hisaka and T. Osawa, "Lipid hydroperoxide-derived adduction to amino-phospholipid in biomembrane," *Subcellular Biochemistry*, vol. 77, pp. 41–48, 2014.

[45] N. D. Ridgway, "The role of phosphatidylcholine and choline metabolites to cell proliferation and survival," *Critical Reviews in Biochemistry and Molecular Biology*, vol. 48, no. 1, pp. 20–38, 2013.

[46] K. Yea, J. Kim, J. H. Yoon et al., "Lysophosphatidylcholine activates adipocyte glucose uptake and lowers blood glucose levels in murine models of diabetes," *The Journal of Biological Chemistry*, vol. 284, no. 49, pp. 33833–33840, 2009.

[47] M. N. Barber, S. Risis, C. Yang et al., "Plasma lysophosphatidylcholine levels are reduced in obesity and type 2 diabetes," *PloS ONE*, vol. 7, no. 7, p. e41456, 2012.

[48] W. H. Moolenaar and A. Perrakis, "Insights into autotaxin: How to produce and present a lipid mediator," *Nature Reviews Molecular Cell Biology*, vol. 12, no. 10, pp. 674–679, 2011.

[49] D. N. Obanda, Y. Yu, Z. Q. Wang, and W. T. Cefalu, "Modulation of sphingolipid metabolism with calorie restriction enhances insulin action in skeletal muscle," *The Journal of Nutritional Biochemistry*, vol. 26, no. 7, pp. 687–695, 2015.

[50] K. Iwabuchi, H. Nakayama, A. Oizumi, Y. Suga, H. Ogawa, and K. Takamori, "Role of ceramide from glycosphingolipids and its metabolites in immunological and inflammatory responses in humans," *Mediators of Inflammation*, vol. 2015, 2015.

[51] R. H. Zhao, M. Xie, and C. Li, "Changes of immune functions in rat models of liver depression syndrome, spleen deficiency syndrome and syndrome of liver depression andspleen deficiencsy," *Mediators of Inflammation*, vol. 36, no. 12, pp. 821–824, 2013.

[52] L. Huang, T. Li, Y.-W. Liu et al., "Plasma metabolic profile determination in young ST-segment elevation myocardial infarction patients with ischemia and reperfusion: Ultra-performance liquid chromatography and mass spectrometry for pathway analysis," *Chinese Medical Journal*, vol. 129, no. 9, pp. 1078–1086, 2016.