Identifications of Metabolic Differences between Hedysari Radix Praeparata Cum Melle and Astragali Radix Praeparata cum Melle for spleen-qi deficiency rats: A comparative study

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Highlights

1. Hedysari Radix Praeparata Cum Melle (HRPCM) and Astragali Radix Praeparata cum Melle (ARPCM) improve spleen-qi deficiency (SQD) syndrome including the gastrointestinal dysfunction and decreased immunity.
2. HRPCM and ARPCM treat SQD rats by regulating the lipid metabolism, amino acid metabolism, nucleotide metabolism, sugar metabolism, and energy metabolism.
3. The differences in metabolic profiling between HRPCM and ARPCM for SQD rats

are mainly in the synthesis of L-glutamine in amino acid metabolism.

Abbreviations

AR, Astragali Radix. HR, Hedysari Radix. HRPCM, Hedysari Radix Praeparata Cum Melle. ARPCM, Astragali Radix Praeparata Cum Melle. SQD, spleen-qi deficiency. BZYQP, Bu-Zhong-Yi-Qi Pills. HPLC, high performance liquid chromatography. SPF, specific pathogen free. UPLC-MS/MS, ultra high performance liquid chromatography-triple quadrupole tandem mass spectrometry. ELISA, enzyme-linked immunosorbent assay. MS, mass spectrometer. ESI, electrospray ionization. QC, quality control. GAS, gastrin. IL, interleukin. TNF, tumor necrosis factor. PCA, Principal component analysis. OPLS-DA, orthogonal partial least squares discriminant analysis. RPT, response permutation testing. VIP, variable important inprojection. FC, fold change.

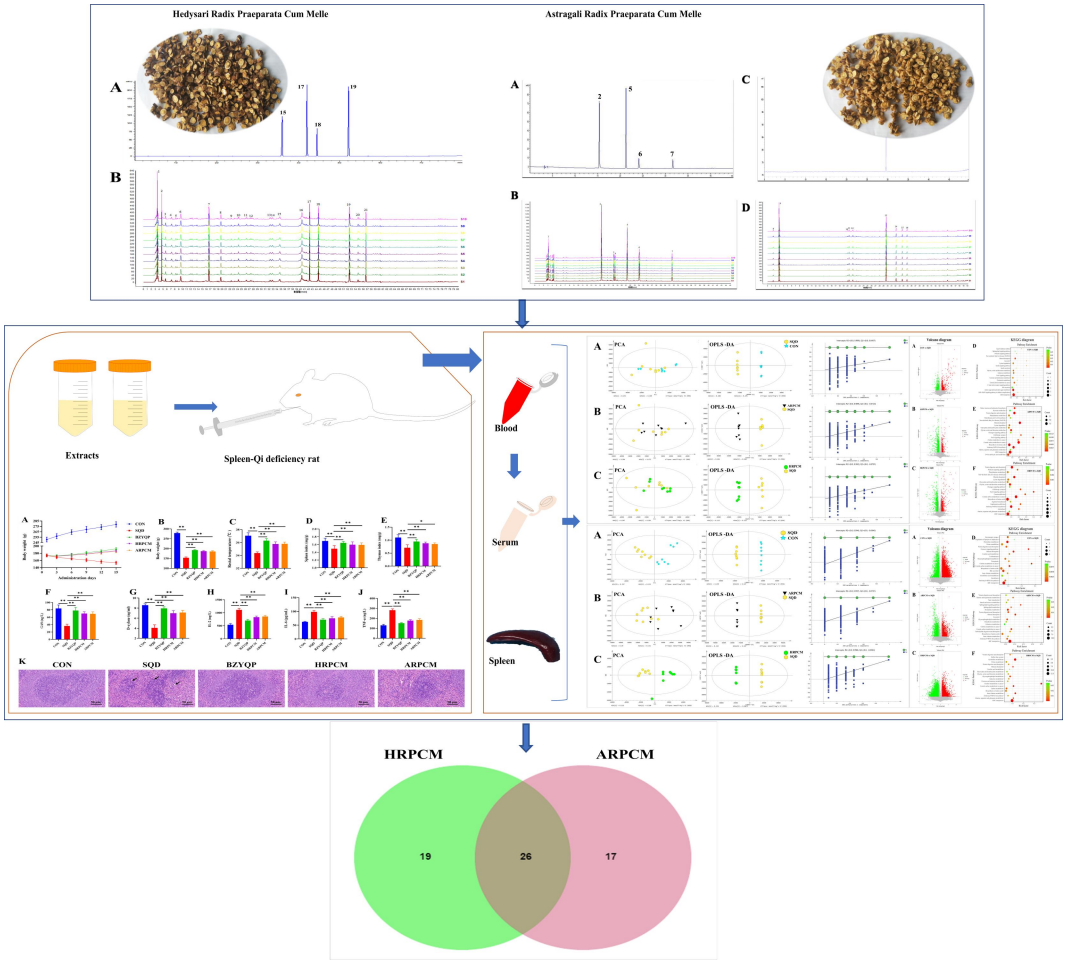
ABSTRACT

Hedysari Radix Praeparata Cum Melle (HRPCM) and Astragali Radix Praeparata cum Melle (ARPCM) are capable of improving spleen-qi deficiency (SQD) syndrome especially in the gastrointestinal dysfunction and decreased immunity in traditional Chinese medicine clinically. This study aims to compare and reveal the metabolic differences between HRPCM and ARPCM for SQD rats. Firstly, HRPCM (12.6 g/kg) and ARPCM (12.6 g/kg) were used to intervene SQD rats to further evaluate the effect. The results showed that HRPCM and ARPCM were able to improve the spleen pathology, increase the body weight, the rectal temperature, the spleen index, the thymus index, the levels of GAS and D-xylose in serum, and decrease the levels of IL-2, IL-6 and TNF- α in serum for SQD rats. Then, the studies of metabolic differences in serum and spleen were carried out using UPLC-Q-TOF-MS. The findings emphasized that HRPCM and ARPCM not only regulated metabolic profiling of serum and spleen in SQD rats, but also existed differences. HRPCM and ARPCM regulated metabolic pathways mainly including lipid metabolism, energy metabolism, amino acid metabolism, nucleotide metabolism, sugar metabolism and other types of

metabolism for SQD rats. However, the metabolite profiles in SQD rats changed significantly, mainly involving abnormal glycine synthesis occurred in SQD rats. The expression trends of metabolites in HRPCM and ARPCM intervention for SQD rats were partly the same. Interestingly, there are similarities and differences in metabolic profiling between HRPCM and ARPCM for SQD rats. The differences were mainly in the synthesis of L-glutamine in amino acid metabolism.

Keywords: Hedysari Radix Praeparata Cum Melle; Astragali Radix Praeparata cum Melle; spleen-qi deficiency; metabolomics; metabolic differences; comparative study

Graphical Abstract



1 Introduction

Astragali Radix (AR) is the dried root of the leguminous *Astragalus membranaceus* (Fisch.) Bge. var. *mongholicus* (Bge.) Hsiao or *Astragalus membranaceus* (Fisch.) Bge. Hedysari Radix (HR) is the dried root of the leguminous *Hedysarum polybotrys* Hand.-Mazz. AR and HR are both genuine medicinal materials in Gansu, China. They belong to the same family but different genera. HR and AR have many common components such as polysaccharides, flavonoid, saponins, amino acid and microelement. Meanwhile, HR and AR have common pharmacological effects such as anti-tumor, anti-aging, immunoregulation, the protection of cardiovascular and cerebrovascular, and so on [1, 2]. HR, thus, is still used instead of AR in Gansu, Qinghai, and Hong Kong in China[3].

HR and AR processed with honey play a synergistic effect to improve the effects of invigorating Qi. Honey-processed Hedysari Radix and honey-processed Astragali Radix are called as Hedysari Radix Praeparata Cum Melle (HRPCM) and Astragali Radix Praeparata Cum Melle (ARPCM) in Chinese. HRPCM and ARPCM only have the effects of invigorating spleen-stomach and replenishing qi. Thus, they are commonly used to treat spleen-qi deficiency (SQD) syndrome including fatigue, poor appetite, diarrhoea and weakened immunity[4]. The research on components for HRPCM and ARPCM also focus on polysaccharides, flavonoid, saponins, amino acid and microelement [5, 6]. However, Some studies have suggested that polysaccharides [7], saponins including astragaloside IV and astragaloside I [8] as well as flavonoids including calycosin-7-glucoside [8] are the key components of HRPCM and ARPCM to enhance the effect of invigorating spleen-stomach and replenishing qi. At present, studies on the effects of HRPCM and ARPCM in invigorating spleen-stomach and replenishing qi are mostly focused on anti-oxidation and immune enhancement [9]. Our previous study found that HRPCM and ARPCM are capable of improving the gastrointestinal dysfunction and decreased immunity in SQD rats and HRPCM is more effective than ARPCM [10]. As mentioned above, the main chemical composition and the effects of invigorating spleen-stomach and replenishing qi for

SQD syndrome between HRPCM and ARPCM are similar. Therefore, it is vital that to explore the reason why HRPCM is more effective than ARPCM in improving the gastrointestinal dysfunction and decreased immunity for SQD rats.

Traditional Chinese medicine (TCM) has the characteristics of multiple components. The therapeutic effect of TCM can usually be attributed to the combination or formula of multi-components [11]. It is difficult to evaluate the therapeutic effect of TCM with clear composition changes [12]. For this reason, it is necessary to introduce metabolomics, a classical method, to clarify the reason for the therapeutic effect of TCM. ncomplements this approach by providing a comprehensive understanding of the metabolic changes induced by TCM treatments and the interactions between TCM and the human body. After TCM intervention, metabolomics focuses on the comprehensive analysis of metabolites which present in biological systems. These metabolites include various small molecules such as sugars, amino acids, lipids, and organic acids that are involved in the chemical reactions and processes within a living organism [13]. These reactions and processes finally reveal the main reasons for the therapeutic effect of TCM.

Although our previou research revealed that HRPCM is more effective than ARPCM in improving the gastrointestinal dysfunction and decreased immunity for SQD rats, the metabolic differences that give rise to this phenomenon have not yet been revealed. This study aims to compare and reveal the metabolic differences between HRPCM and ARPCM for SQD rats by metabolomics analysis, which hope to provide a basis for better promotion of the rational clinical replacement application of HRPCM and ARPCM.

2 Materials and methods

2.1 Experimental herbs

HR and AR samples were collected from Micang Mountain, Wudu District, Longnan City, Gansu Province, China. The two herbs were authenticated by Professor Wang Mingwei from the Department of Chinese Medicine Identification, School of Pharmacy, Gansu University of Chinese Medicine. Bu-Zhong-Yi-Qi Pills (Batch No.

19C38) were purchased from Lanzhou Foci Pharmaceutical Co., Ltd. Rhubarb (*Rheum palmatum* L.) medicinal decoction pieces were purchased from Affiliated Hospital of Gansu University of Chinese Medicine.

2.2 Preparation, Chemical compositions and quality analysis of HRPCM and ARPCM

The preparation methods of HRPCM and ARPCM referred to our previous study [10]. The chemical compositions were analyzed by the ultra high performance liquid chromatography-triple quadrupole tandem mass spectrometry (UPLC-MS/MS) method. The quality analysis of HRPCM and ARPCM were also performed by high performance liquid chromatography (HPLC) according to our previous method [10]. The specific methods of preparation, chemical compositions and quality analysis are showed in online **Supplementary Methods**.

2.3 Animals

60 six-week-old specific pathogen free (SPF) male SD rats, weighing 180±20 g, were provided by the Animal Experiment Center of Gansu University of Chinese Medicine. In a barrier environment, the rats were raised in separate cages, 5 in each cage. The ambient temperature of the breeding room was controlled to 23 ± 2 °C, and its relative humidity was controlled to 45%–50%. This animal experiment satisfied the requirements of the Experimental Animal Ethics Committee and was approved by the Ethics Committee (Committee No. 2019-203).

2.4 Experimental protocols and Drug intervention

The experimental protocol is showed in **Fig. 1.** After 7 days of adaptive feeding, 60 SD male rats were randomly divided into a control group (CON, n=10) and building model group (BM, n=50). The CON rats were routinely raised without model conditions. The BM rats were used to limit food intake, make diarrhoea and fatigue to establish the model of SQD [14]. The modeling methods of SQD are listed in our previous literature [10]. After 15 days of continuous model building (Note: ten rats were sacrificed during model building), the BM rats were randomly divided into the SQD model group (SQD, n=10), Bu-Zhong-Yi-Qi Pill group (BZYQP is used to treat the SQD syndrome and becomes a kind of positive active Chinese patent medicine in

this study. n=10), HRPCM intervention group (HRPCM, n=10) and ARPCM intervention group (ARPCM, n=10).

The equivalent dose was converted from the body surface area of humans and rats and the optimal administration dose for rats was determined based on the previous research [10, 15] combined with pilot experiment. Drug intervention was started on the 16th day of the experiment. During the drug intervention period, The BZYQP rats were given 1.89 $g \cdot kg^{-1} \cdot d^{-1}$ BZYQP water-soluble substance by intragastric administration at 8 o'clock every morning. Both HRPCM and ARPCM groups were given 12.6 $g \cdot kg^{-1} \cdot d^{-1}$ by intragastric administration of HRPCM and ARPCM water extract, the gavage volume is 10 $ml \cdot kg^{-1}$. The CON and SQD rats were orally administered an equal dose of distilled water. Once a day for 15 days, excluding the CON rats, the other groups continued to apply the modelling conditions.

2.5 Sample collection

Exactly 15 days after extract administration, rats in each group were fasted but given free access to drinking water. At 6:00 PM on the same day, the rats in each group were weighed, and the rectal temperature was measured. On the 16th day after drugs administration, rats in each group were given 1 $mL \cdot kg^{-1}$ 5% D-xylose solution. After 1 h of gavage, 5 mL of blood samples was collected from the abdominal aorta into non-anticoagulated vacuum blood collection tubes. After refrigeration at 4 °C for 1 h, the rats were centrifuged at 3000 rpm and 4 ℃ for 15 min, the supernatant was placed in a 2 mL cryotube and frozen for later use. After blood collection, the rats were sacrificed. The spleens and thymus were immediately removed and accurately weighed. After weighing, the spleen tissue was quickly divided into two parts: one part was fixed in the pre-prepared 4% paraformaldehyde fixative solution for pathological observation, and the other part was placed in the cryotube for the metabolomic study of spleen. Liquid nitrogen was used to freeze the samples, which were then placed in the refrigerator at −80 ℃ for later use.

2.6 H&E staining Pathological Analysis

The spleen tissues were precisely weighed and fixed in 4% paraformaldehyde fixative solution for 1 week, embedded in paraffin, sliced, baked, stained with HE and

observed under an inverted microscope at 200x.

2.7 Enzyme-linked immunosorbent assay

The serum samples were obtained from the abdominal aorta and assayed for IL-2, IL-6, TNF-α, GAS and D-xylose (Nos. MM-0192R1, MM-0190R1, MM-0180R1. MM-20284R1, and ml015649. The kits of L-2, IL-6, TNF-α and GAS were purchased from Jiangsu Meimian industrial Co., Ltd, Jiangsu, China. The kit of D-xylose was purchased from Enzyme-linked Biotechnology Co., Ltd, Shanghai, China.) by using the enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions.

2.8 Metabolomics analysis

The serum and spleen samples were separated using Agilent 1290 Infinity LC UHPLC system and HILIC column and were analyzed by the Triple-TOF5600 mass spectrometer (MS). Each sample was tested in positive- and negative-ion mode using electrospray ionisation (ESI). The specific processing of LC-MS tests is listed in Online **Supplementary Methods**. After completing the LC-MS tests and data conversion, metabolite structure identification was achieved by accurate mass matching (<25 ppm) and secondary spectral matching to retrieve HMDB, Massbank (http://www.massbank.jp.) and laboratory self-built databases. For the extracted data, delete ion peaks with missing values >50% in the group, normalize the total peak area of the positive and negative ion data, integrate the positive and negative ion peaks and apply the software SIMCA-P 14.1 (Umetrics, Umea, Sweden) to perform Pattern recognition, after the data is preprocessed by Pareto scaling, multivariate statistical analysis is performed.

2.9 Statistical methods

The data showed a normal distribution and their variance was uniform, the results are expressed as mean±standard deviation ($\bar{x}$ ±s), and comparisons between groups were conducted by using one-way ANOVA. If the variance was not uniform and the data did not have a normal distribution, the W-H rank sum test was used. All of the data were statistically analyzed using Paragraph Prism version 8.0.2 (GraphPad Software, La Jolla, CA) software. $p<0.05$ was considered to indicate statistically

significant differences.

3 Results

3.1 Qualitative analysis of HRPCM and ARPCM

HPLC fingerprints of HRPCM and ARPCM were established (**Figure 2 and Figure 3**). The indicative components in HRPCM and ARPCM were also identified and quantified (**Table 1**). These results met the requirements of Chinese Pharmacopoeia 2020 edition, thereby the following efficacy evaluation and metabolomics can be continued.

3.2 The Effect of HRPCM and ARPCM on SQD rats

The body weight increased gradually in all treatment groups, while decreased gradually in SQD goup during drug intervention (**Figure 4A**). After drug intervention, compared with the CON group, the body weight, the rectal temperature, the spleen index, the thymus index, and the levels of GAS and D-xylose in serum significantly decreased (**Figure 4B-G,** $p<0.01$), but the levels of IL-2, IL-6 and TNF- α in serum significantly increased (**Figure 4H-J,** $p<0.01$). The effect of HRPCM and ARPCM for SQD rats were evaluated and found that HRPCM treatment and ARPCM treatment reversed effectively the changes of the above indexes (**Figure 4B-J,** $p<0.01$ or $p<0.05$). Meanwhile, the pathological analysis indicated that HRPCM treatment and ARPCM treatment relieved pathological injury of spleen in SQD rats (**Figure 4K**). These results demonstrated that HRPCM and ARPCM have the effect to treat the SQD rats.

3.3 Metabolomics analysis

3.3.1 HRPCM and ARPCM treatments Regulated metabolic profiling of serum and spleen in SQD rats.

The ion mode diagrams of the quality control (QC) samples and all samples were showed in **Supplementary Figure S1,2,3**. The retention time and peak intensity were consistent, indicating that mass spectrometry had better signal stability when the same sample was detected at different times. Therefore, the methods were suitable for subsequent sample analyses. The annotated data of all metabolites were listed in **Supplementary Table S1**. Principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA) were used to evaluation the

separation effects in serum metabolism (**Figure 5**) and spleen metabolism (**Figure 6**) between CON and SQD, between ARPCM and SQD, and between HRPCM and SQD. OPLS-DA had the excellent separation and 200 response permutation testing were controlled to validate the OPLS-DA models. According to the permutation testing, the abscismal of permutation testing represents the correlation between Y of the randomized group and Y of the original group; the ordinate represents the scores of R2 and Q2, where the intercept of Q2 is greater than 0.05 [16], indicating that these OPLS-DA models did no over-fitting and had good predictive abilities. The above analysis results showed significant differences in serum metabolite and spleen metabolite between CON and SQD, between ARPCM and SQD, and between HRPCM and SQD.

3.3.2 Comparison of the levels of the common differential metabolites in serum and spleen amongst groups

To compare the level of change for the common differential metabolites in serum and spleen after model replication and drugs intervention, comparison of the main common differential metabolites was carried out (**Table 2 and Table 3**). There were 18 common differential metabolites in serum (**Table 4**) and 16 common differential metabolites in spleen (**Table 5**) were screed respectively amongst CON, SQD, ARPCM and HRPCM. The expression of 9 metabolites (i.e. 10-hydroxydecanoic acid, LPC(18:1), deoxycarnitine, serine, nicotinamide, oxidised glutathione, D-Mannose-6-phosphate, allantoin and glutamine) in the serum and spleen of SQD rats was significantly down-regulated. The expression of 11 metabolites of 10-hydroxydecanoic acid, LPC(18:1), deoxycarnitine, serine, nicotinamide, betaine, glutathione, D-Mannose-6-phosphate, allantoin, hypotaurine and pantothenic acid in the serum and spleen of rats with SQD was significantly down-regulated, whereas the expression of four metabolites of glucose, uric acid, lactic acid and glycine was significantly up-regulated. After ARPCM intervention, the expression of the first 11 metabolites was significantly up-regulated, and the expression of the latter 4 metabolites was significantly down-regulated. Therefore, the above-mentioned 15 metabolites may be important effect metabolites of ARPCM in the intervention of

SQD rats. The expression of 7 metabolites (such as N-acetylglycine, glucose, L-phenylalanine, uric acid, lactic acid, L-alanine and D-gluconic acid) was significantly up-regulated. After HRPCM intervention, the expression of the first 9 metabolites was significantly up-regulated, whereas the expression of the latter 7 metabolites was significantly down-regulated. Therefore, we speculated that the 16 above-mentioned metabolites may be important effect metabolites of HRPCM in the intervention of SQD rats.

3.3.3 Screening of Differential Metabolites and Pathways in Serum and Spleen between HRPCM and ARPCM for SQD rats

In this study, VIP>1, FC>2 or FC<0.5 and p <0.05 were used as the screening criteria for differential metabolites. Volcano diagrams were used to finish the screening and the top 20 of KEGG enrichment diagrams were used to forecast the metabolic pathway. Heatmaps were used to exhibit the relative expressions of differential metabolites between groups in serum and spleen (**Supplementary Figure S4,5**). Metabolic differentials between groups in serum (**Figure 7A-C**) and spleen (**Figure 8A-C**) were obtained by volcano diagrams analysis. The screened metabolic differentials in serum and spleen between CON and SQD were listed in **Supplementary Table S2** and **Supplementary Table S5,** respectively. The screened metabolic differentials in serum and spleen between ARPCM and SQD were listed in **Supplementary Table S3** and **Supplementary Table S6,** respectively. The screened metabolic differentials in serum and spleen between ARPCM and SQD were listed in **Supplementary Table S4** and **Supplementary Table S7,** respectively. The metabolic pathways among these groups were enriched according to these differential metabolites.

The metabolic pathways these groups in serum and spleen were showed in **Figure 7D-F** and **Figure 8D-F,** respectively. The significant difference metabolites in serum between SQD and CON mainly involved metabolic pathways including ATP-binding cassette transporter, amino sugar and nucleotide sugar metabolism, mineral absorption, glycine, serine and threonine metabolism and galactose metabolism (**Figure 7D**). The significant difference pathways in spleen between SQD and CON

mainly involved metabolic pathways including ATP-binding cassette transporter, bile secretion, amino acid biosynthesis, nucleotide metabolism and lipid metabolism (**Figure 8D**). The significant difference metabolites in serum between ARPCM and SQD mainly involved metabolic pathways including ATP-binding cassette transporter, amino acid biosynthesis, protein digestion and absorption, mineral absorption, aminoacyl-tRNA biosynthesis, glycine, serine and threonine metabolic pathways such as amino acid metabolism and phenylalanine metabolism. The significant difference metabolites in spleen between ARPCM and SQD mainly involved metabolic pathways ATP-binding cassette transporter, pyrimidine metabolism, amino acid biosynthesis, mineral absorption, lipid metabolism and other metabolic pathways. The significant difference metabolites in serum between HRPCM and SQD mainly involved metabolic pathways including ATP-binding cassette transporter, amino sugar and nucleotide sugar metabolism, protein digestion and absorption, mineral absorption, glycine, serine and threonine metabolism, 2-oxocarboxylic acid metabolism and other metabolic pathways (**Figure 7F**). The significantly different metabolites in spleen between HRPCM and SQD mainly involved metabolic pathways including ATP-binding cassette transporter, nucleotide metabolism, amino acid biosynthesis, lipid metabolism, β-alanine metabolism and other pathways (**Figure 8F**). These results showed that there were differences in metabolic pathways between HRPCM and ARPCM after intervention in SQD rats, respectively. The common metabolic pathways in serum between ARPCM and HRPCM for SQD rats were ATP-binding cassette transporter, protein digestion and absorption, mineral absorption, and glycine. The common metabolic pathways in spleen between ARPCM and HRPCM for SQD rats were ATP-binding cassette transporter and mineral absorption.

3.4 Identification of Potential Biomarkers

3.4.1 Identification of Potential Biomarkers of ARPCM for SQD rats

After ARPCM intervention, a total of 27 differential metabolites in serum and a total of 22 differential metabolites in spleen were screened respectively from ARPCM and SQD (**Supplementary Table S3,6**). The comparison found six repeated different

metabolites pathway including lipid metabolism, nucleotide metabolism, amino acid metabolism, sugar metabolism, tricarboxylic acid cycle and other types of metabolism. Forty-three differential metabolites corresponding to these pathways (**Table 4**) may be potential biomarkers for ARPCM to interfere with SQD rats.

3.4.2 Identification of Potential Biomarkers of HRPCM for SQD rats

After HRPCM intervention, a total of 27 differential metabolites in serum and 24 differential metabolites in spleen were screened respectively from the HRPCM and SQD (**Supplementary Table S4,7**). The comparison found that 6 repeated different metabolites pathway mainly involved in lipid metabolism, nucleotide metabolism, amino acid metabolism, sugar metabolism, tricarboxylic acid cycle and other types of metabolism. Forty-five differential metabolites corresponding to these pathways (**Table 5)** may be potential biomarkers for HRPCM to interfere with SQD rats.

3.5 Differences of biomarkers between HRPCM and ARPCM for SQD rats

Forty-three potential biomarkers between ARPCM and SQD as well as Forty-five potential biomarkers between HRPCM and SQD were interacted with venn analysis (http://jvenn.toulouse.inra.fr/app/example.html). The results of venn analysis showed that nineteen biomarkers were the unique to HRPCM for SQD rats, seventeen biomarkers were the unique to ARPCM for SQD rats, and twenty-six biomarkers were the common between HRPCM and ARPCM for SQD rats (**Figure 9, Table 6**). So far, the differences of biomarkers between HRPCM and ARPCM for SQD rats have been identified.

4 Discussion

In this study, LC-MS/MS was used to identified the chemical components in HRPCM and ARPCM. What's more, the difference of the chemical components was compared between HRPCM and ARPCM (**Table 2**), which, moreover, provided 2 differential classes of chemical components including phenols and plyphenols between HRPCM and ARPCM. These differences may be one of the reasons for the difference between HRPCM and ARPCM. Whereafter, HPLC fingerprints of HRPCM and ARPCM were established and the indicative components were determined for

quality control. The results of quality control of HRPCM and ARPCM provided a basis for the follow-up study. On the basis of SQD model, the spleen index and thymus index, the contents of D-xylose, GAS, IL-2, IL-6 and TNF-α in serum were used as evaluation indexes. The effects of HRPCM and ARPCM on the SQD rats were evaluated from gastrointestinal function, immune function and inflammation [17, 18]. HRPCM and ARPCM not only help to repair the spleen injury of SQD rats, but also improve the gastrointestinal function, immune function and intestinal inflammation of SQD rats. However, HRPCM was better than ARPCM in promoting gastrointestinal function, immune function and anti-intestinal inflammation, which is consistent with the previous study [10, 15].

In this study, the differential metabolites from serum and spleen of SQD rats were screened including amino acids, choline, sterols and fatty acids (**Supplementary Table S2,5**). They were mainly involved in lipid metabolism, amino acid metabolism, nucleotide metabolism, sugar metabolism and other pathways (**Figure 6D, 7D)**. Amongst them, betaine and choline are the raw materials for glycine synthesis [19], whilst N-acetylglycine is the product of glycine synthesis. In SQD group, the expression levels of betaine, choline, phosphocholine and LPC (18:1) were all down-regulated, whilst the expression levels of glycine and N-acetylglycine were up-regulated, indicating abnormal glycine metabolism in SQD rats (**Supplementary Table S2,5**). Normal glycine metabolism produces glutathione, creatine and serine through many ways. However, inhibition of glycine metabolism in SQD rats affects the production of glutathione, creatine and serine [20], which indicating that glycine metabolism plays an important role in the pathological formation of SQD syndrome. Glutathione, a tripeptide compound containing sulfhydryl groups, participates in glycometabolism and tricarboxylic acid cycle and promotes the energy production [21]. Creatinine is a metabolite of creatine. The process of creatine metabolism to produce creatinine is accompanied with the production of ATP [22]. D-Gluconic acid is the product of glucose oxidation. Glucose can release more energy through the glycolysis pathway, tricarboxylic acid cycle and biological oxidation. It is the main energy supply substance of the human body. Various intermediate products produced

during its metabolism provides precursors for the metabolism of other nutrients [23]. Lecithin also plays a very important role in the metabolic process. When the body is in a state of strenuous exercise, lecithin can be used as a substance with information transmission and material transfer functions to provide muscle cells with required nutrients and energy. lecithin is also an important nutrient for the brain nerves, which is conducive to eliminating fatigue [24]. Deoxycarnitine is the precursor of carnitine. As a key substance in lipid metabolism, carnitine transport activates long-chain fatty acids through the inner membrane of mitochondria and is oxidised in the mitochondria for energy supply. Therefore, carnitine is the key substance to improve the maximal exercise level [25]. 2'-Deoxyinosine is the deoxygenated product of inosine. Inosine is a component of ATP, coenzyme A, ribonucleic acid and other important substances in human body. It is the precursor of adenylate and guanylic acid during de novo synthesis of purines, as well as a key substance in the material metabolism and energy metabolism [26]. Pantothenate is the precursor substance for the synthesis of coenzyme A. Coenzyme A is the coenzyme for acetylation of the body and the precursor for the synthesis of acetyl-coenzyme A. Acetyl-coenzyme A is the key substance for the tricarboxylic acid cycle and oxidative phosphorylation. The tricarboxylic acid cycle and oxidative phosphorylation are the key pathways for sugar and lipid metabolism, and slao important pathway of ATP production. The expression of glucose and D-gluconic acid in SQD group was up-regulated (**Supplementary Table S5**), indicating that SQD rats had abnormally elevated blood sugar and abnormal glucose metabolism. By contrast, the expression levels of glutathione, creatinine, lecithin, deoxycarnitine, 2'-deoxyinosine, inosine, pantothenic acid and coenzyme A were all down-regulated (**Supplementary Table S5**), indicating that creatine metabolism was reduced, energy metabolism was abnormal. After that, the energy supply was insufficient and brain neurotrophic substances were reduced. This may be one of the main causes of fatigue, lethargy, slow response and laziness in SQD rats.

10-Hydroxydecanoic acid acts on the tumour suppressor gene p53, directly inactivates the NLRP3 inflammatory pathway, indirectly promotes autophagy and

significantly reduces the level of pro-inflammatory mediators to achieve anti-inflammatory effects [27]. Linoleic acid promotes simultaneously the expression of inflammatory factors such as TNF-α and promote the inflammatory response [28]. Nicotinamide is a coenzyme component of the energy metabolism. Nicotinamide plays a crucial part in carbohydrate and protein metabolism and lipid synthesis. Moreover, Nicotinamide can block the activation of inflammatory cells by regulating the energy metabolism and inhibit the release of inflammatory factors such as IL-6 [29]. D-Mannose-6-phosphate is the phosphorylation product of mannose. Mannose is a monosaccharide that is loaded into cells by a glucose transporter and participates in a variety of sugar metabolism pathways [30]. Mannose has anti-tumour function and the effect of bi-directional regulation of the inflammatory reaction [31]. In SQD group, the expression levels of 10-hydroxydecanoic acid, nicotinamide, mannose and D-Mannose-6-phosphate were all down-regulated, but the expression of linoleic acid was up-regulated (**Supplementary Table S2**), which suggested that the immune function of SQD rats impaired, the expression of inflammatory factors such as TNF-α and IL-6 increased, and the inflammatory reaction occurred. This result was consistent with the the earlier research [15]. Glutamate-conjugated chenodeoxycholic acid is a compound of glutamate and chenodeoxycholic acid. Chenodeoxycholic acid is one of the primary bile acids. Cholic acid is an organic acid with a steroid structure, which can promote fat dissolution. A proper amount of cholic acid helps the function of digestion, whereas excessive cholic acid has a strong damaging effect on tissue mucosa [32, 33]. Glutamate-conjugated chenodeoxycholic acid and cholic acid in SQD group were abnormally increased (**Supplementary Table S2**), indicating abnormal bile acid metabolism in a main reason for the abnormal digestive function.

After HRPCM intervention, the metabolite profiling changed significantly. Compared with the SQD rats, forty-five different metabolites were noted in HRPCM group (**Table 5**). Amongst them, both succinic acid and citraconic acid are important intermediates in the tricarboxylic acid cycle. The activity of succinate dehydrogenase in the small intestine tissue of SQD rats is reduced, and inhibits the tricarboxylic acid cycle, thereby leading to the accumulation of succinic acid [34]. Studies have shown

that citraconic acid is down-regulated in urine metabolism for the SQD rats [35]. In the present study, the expression of succinic acid in HRPCM group was down-regulated and the expression of citraconic acid was up-regulated (**Supplementary Table S4**), indicating that HRPCM contributed to restored the activity of succinate dehydrogenase, promoting the tricarboxylic acid cycle and the energy supplement.

Allantoin is produced by purine bases in animals. Allantoin not only has the effect of preventing the occurrence and development of gastric ulcers, but improves oxidative stress and inflammatory damage in kidney tissues [36]. The up-regulated expression of allantoin in HRPCM group (**Supplementary Table S4,7**), indicating that HRPCM promoted the synthesis of allantoin for SQD rats, thereby inhibiting inflammation, preventing gastric ulcer and protecting the spleen and gastrointestinal tract. In HRPCM group, the expression of N-acetylglycine was down-regulated, whilst choline and LPC (18:1) were up-regulated (**Supplementary Table S4,7**), which indicating that HRPCM could improve abnormal glycine metabolism in SQD rats. Studies have shown that threonine helps to alleviate fatigue and promote growth and development; its absence or low levels can lead to growth retardation and low immunity [37]. The expression of threonine in HRPCM group was up-regulated (**Supplementary Table S4,7**), which indicating that HRPCM could regulate the threonine metabolism, enhance the immunity o, thereby relieving fatigue and the symptoms of SQD rats.

Phenylalanine is one of the essential amino acids. Phenylalanine metabolism is essential to maintain the normal physiological functions. And, most of phenylalanine are metabolised into tyrosine by phenylalanine hydroxylase [38]. After HRPCM intervention, L-phenylalanine concentration significantly decreased (**Supplementary Table S2,6**), indicating that HRPCM could regulate L-phenylalanine metabolism and restore normal physiological functions. Serine is mainly synthesised from 3-phosphoglycerate acid and glutamic acid. A metabolite of serine is ethanolamine. Studies have shown that serine, promotes the fat and fatty acid metabolism and muscle growth, plays an important role in protein synthesis and nucleotide metabolism [39]. The expression of serine was down-regulated in SQD group, whilst

491 the expression levels of L-glutamate, serine and phosphatidylethan olamine 17 were
492 up-regulated in HRPCM (**Supplementary Table S2,6**). Hence, serine metabolism
493 was abnormal in SQD rats, whereas HRPCM could regulate serine metabolism
494 effectively.

495 Glutamine can be synthesised by glutamate, valine and isoleucine. It is important
496 to maintain the homeostasis [40]. Glutamine can reduce the intestinal inflammatory
497 response [41] by reducing the permeability of the intestinal mucosa [42] and
498 enhancing the intestinal immune function [43]. It has protective and reparative effects
499 on gastrointestinal mucosal damage [44]. The expression of glutamine in HRPCM
500 group was up-regulated compared with the SQD group (**Supplementary Table S2,6**),
501 which indicated that ARPCM could promote the synthesis of glutamine in SQD rats,
502 enhance the functions of immune, reduce the inflammatory reaction of the
503 gastrointestinal tract, and repair gastrointestinal mucosa injury.

504 Lactate is produced in the cytoplasm and transported into mitochondria. Lactate
505 is reconverted to pyruvate in the mitochondria by mitochondrial lactate
506 dehydrogenase, which then oxidize to acetyl-coa in response to pyruvate
507 dehydrogenase and enters the tricarboxylic acid cycle [45]. A Study have shown that
508 fatigue may occur when the body's lactic acid is abnormally elevated [46]. The
509 contents of pyruvate acid and lactic acid significantly increased, and the content of
510 coenzyme A significantly decreased in SQD group, which indicated that the activity
511 of pyruvate dehydrogenase in SQD rats may be inhibited and the metabolism of
512 pyruvate was abnormal. The content of lactic acid in ARPCM was significantly
513 reduced (**Supplementary Table S2,3,5,6**), suggesting that ARPCM may help activate
514 pyruvate dehydrogenase activity, promote pyruvate metabolism and regulate the
515 pyruvate and lactic acid metabolism balance. This may be the reason why ARPCM
516 relieves fatigue caused by excessive lactic acid for SQD rats.

517 Uric acid is a product of purine metabolism. In mammals, uric acid is oxidised to
518 allantoin by uric acid enzyme and excreted together with urea. Abnormally elevated
519 uric acid in body can lead to hyperuricemia and cause gouty arthritis, gouty
520 nephropathy and other diseases [47]. The intestinal flora can affect the expression of

inflammatory factors in the intestinal mucosal immune response, participate in the immune response and promote the excretion of uric acid [48] The overexpression of uric acid and the underexpression of xanthine and allantoin in SQD rats indicated that the purine metabolism and urase activity may be inhibited in SQD rats. After ARPCM intervention, the expression of uric acid was down-regulated while allantoin was up-regulated, which suggested that ARPCM could regulate the metabolic disorder of purine, help to uricase activity and promote the decomposition of uric acid to produce allantoin. Allantoin can prevent the occurrence and development of gastric ulcer, improve oxidative stress, and inhibit inflammatory injury in renal tissue, which may be one of the mechanisms by which ARPCM improved the immune function and reduced the inflammatory reaction for SQD rats.

Glycine expression was down-regulated in ARPCM, whereas serine, L-glutamic acid, threonine, betaine, phosphocholine, sn-Glycero-3-phosphocholine, LPC (16:0) and LPC (18:1) were all up-regulated (**Supplementary Table S3,6**). These results indicated that ARPCM regulates effectively the disorder of glycine synthesis, promotes the metabolism of glycine, and restores the physiological functions. Tryptophan is a functionally essential amino acid, which is essential for animals to maintain normal food intake, growth, immunity and other physiological functions. It has the effects of promoting the growth and development and improving immunity [49]. Studies have shown that a lack of tryptophan leads to a series of problems in animals such as decreased food intake, reduced body mass and neurological dysfunction [50].

Amino acids in animals are derived from the synthesis and ingestion of the intestinal microflora, which regulates the tryptophan metabolic pathway. Amino acids in animals are derived from the synthesis of the intestinal flora and the food. The intestinal flora is an important microbial group that regulates the tryptophan metabolism pathway [51]. The down-regulation of tryptophan expression in SQD rats indicated that the gut microbiota homeostasis was destroyed and the synthesis of tryptophan was reduced. This may be one of the important reasons for the loss of body weight in SQD rats. The expression of tryptophan in ARPCM group was up-

regulated (**Supplementary Table S3**), which indicated that ARPCM restores tryptophan synthesis by regulating the gut microbiota homeostasis and restoring the food intake of SQD rats, thereby increasing the body weight.

In summary, the metabolite profiles of serum and spleen in SQD rats changed significantly, mainly involving lipid metabolism, energy metabolism, amino acid metabolism, nucleotide metabolism, sugar metabolism and other types of metabolism. Abnormal glycine synthesis occurred in SQD rats. Moreover, the production of glutathione, creatine and serine was blocked and the tricarboxylic acid cycle and glucose metabolism were abnormal, resulting in energy metabolism disorder and insufficient energy supply. Abnormal nucleotide metabolism and lipid metabolism led to decreased synthesis of anti-inflammatory substances, increased expression of pro-inflammatory substances, weakened immune function and aggravation of inflammatory reactions. The metabolism of bile acids is abnormal, the homeostasis of the intestinal flora may be destroyed and the synthesis of amino acids such as tryptophan may be hindered, resulting in weakened gastrointestinal digestion and absorption function. HRPCM and ARPCM have the ability to regulate the above-mentioned metabolic dysfunction in SQD rats, thereby treating the SQD syndrome. After HRPCM and ARPCM intervened for SQD rats, the expression trends of serum and spleen metabolites in HRPCM and ARPCM rats were partly the same. The intervention mechanisms of HRPCM and ARPCM were similar but some differences were noted. The differences were mainly in the synthesis of L-glutamine in amino acid metabolism.

5 Conclusion

There are similarities and differences in chemical composition and metabolic profiling between HRPCM and ARPCM for SQD rats. The metabolic profiling with lipid metabolism, amino acid metabolism, nucleotide metabolism, carbohydrate metabolism and energy metabolism changes significantly in SQD rats. HRPCM and ARPCM regulates the expression of metabolites in SQD rats and the above metabolic pathways for SQD rats. However, there were differences in the partial mechanisms like the synthesis of L-glutamine in amino acid metabolism between HRPCM and

ARPCM in regulating metabolism.

Conflicts of interest

No conflicts of interest exit in this manuscript.

Author Contributions

Yuefeng Li conceived and designed the study. Yuefeng Li and Yugui Zhang finished the original manuscript. Jiangtao Niu and Yugui Zhang rearranged the manuscript and finished the revision. Yugui Zhang and Rui Cao took part in the data processing. Yugui Zhang, Tiantian Bian, Zhe Wang, Dingcai Ma, and Maomao Wang performed the experiments. Yuefeng Li and Xingke Yan funded this study. All authors read and approved the manuscript for publication.

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742 **Tables**

743 **Table 1 The results of indicative components in HRPCM and ARPCM（n=10, $\bar{x}\pm s$）**

Components (μg·g^{-1})	HRPCM	ARPCM
Calycosin-7-O-beta-D-glucoside	52.65±1.14	246.97±2.04
Ononin	91.15±2.21	55.55±0.50
Calycosin	79.95±1.13	136.99±3.55
Formononetin	145.42±2.35	31.67±0.77
Astragaloside IV	-	1297.60±9.03

744 **Table 2 Changes in levels of the common differential metabolites in serum amongst groups**

Differential metabolites	SQD vs CON	HRPCM vs SQD	ARPCM vs SQD
Glucose	Up	Down	Down
10-Hydroxydecanoic acid	Down	Up	Up
Deoxycarnitine	Down	Up	Up
LPC 18:1	Down	Up	Up
Serine	Down	Up	Up
Nicotinamide	Down	Up	--
N-Acetylglycine	Up	Down	--
Betaine	Down	--	Up
L-Phenylalanine	Up	Down	--
Deoxyuridine	--	Up	Up
FA 12:1	--	Up	Up
FA 18:3	--	Up	Up
N-Acetylhistidine	--	Up	Up
sn-Glycero-3-phosphocholine	--	Up	Up
Threonine	--	Up	Up
Allantoin	--	Up	Up
Succinic acid	--	Down	Down
Tryptophan	--	Up	Up

745

746 **Table 3 Changes in levels of the common differential metabolites in spleen amongst groups**

Differential metabolites	SQD vs CON	HRPCM vs SQD	ARPCM vs SQD
Oxidized glutathione	Down	Up	Up
Allantoin	Down	Up	Up
D-Mannose-6-phosphate	Down	Up	Up
Serine	Down	Up	Up
Uric acid	Up	Down	Down
Lactic acid	Up	Down	Down
Glutamine	Down	Up	--
L-alanine	Up	Down	--

D-Gluconic acid	Up	Down	−−
Hypotaurine	Down	−−	Up
Pantothenate	Down	−−	Up
Deoxycarnitine	Down	−−	Up
Glycine	Up	−−	Down
Betaine	−−	Up	Up
2'-Deoxycytidine	−−	Up	Up
Phosphocholine	−−	Up	Up

747

748 **Table 4 Potential biomarkers of ARPCM intervention for SQD rats**

Related metabolic pathways	Differential metabolites	ARPCM vs SQD
Fat metabolism	10-Hydroxydecanoic acid	Up
	Deoxycarnitine	Up
	FA 12:1	Up
	FA 18:3	Up
	LPC 16:0	Up
	LPC 18:1	Up
	Palmitoylcarnitine	Up
	Propionylcarnitine	Up
	sn-Glycero-3-phosphocholine	Up
	Hypotaurine	Up
	Phosphocholine	Up
	sn-Glycerol 3-phosphate	Up
	O-Phosphoethanolamine	Up
	Lactic acid	Down
Nucleotide Metabolism	5-Hydroxymethylcytidine	Up
	Deoxyuridine	Up
	2'-Deoxycytidine	Up
	5-Methylcytosine	Up
	Uric acid	Down
Amino acid metabolism	Glycine	Down
	4-aminovaleric acid betaine	Up
	Betaine	Up
	L-Glutamic acid	Up
	Serine	Up
	Threonine	Up
	N-Acetylhistidine	Down
	N-acetylneuraminate	Down
	L-5-Oxoproline	Down
	Tryptophan	Up
	Tyr	Down
	L-asparagine	Down
Glucose metabolism	D-fructose	Up
	Glucose	Down

	D-Mannose-6-phosphate	Up
	Mannose	Up
	N-Acetylglucosamine	Down
Tricarboxylic acid cycle	Succinic acid	Down
Other metabolism	Nicotinamide	Up
	Allantoin	Up
	Hippurate	Down
	Pantothenate	Down
	Riboflavin	Up
	Oxidized glutathione	Up

749 **Table 5 Potential biomarkers of HRPCM intervention for SQD rats**

Related metabolic pathways	Differential metabolites	HRPCM vs SQD
Fat metabolism	10-Hydroxydecanoic acid	Up
	16-Hydroxyhexadecanoic acid	Up
	Choline	Up
	Acetylcarnitine	Up
	Deoxycarnitine	Up
	FA 12:1	Up
	FA 18:3	Up
	LPC 18:1	Up
	sn-Glycero-3-phosphocholine	Up
	Trans-Vaccenic acid	Up
	Phosphatidylethanolamine 17	Up
	Phosphocholine	Up
	sn-Glycerol 3-phosphate	Up
	Lactic acid	Down
Nucleotide Metabolism	Uric acid	Down
	5-Hydroxymethylcytidine	Up
	Deoxyuridine	Up
	2'-O-Methylinosine	Up
	Cytosine	Up
	Xanthosine	Down
	2'-Deoxyinosine	Down
Amino acid metabolism	L-alanine	Down
	Glutamine	Up
	Glycine	Down
	L-Phenylalanine	Down
	N-acetylglycine	Down
	D-ornithine	Down
	L-Norvaline	Down
	L-Glutamic acid	Up
	N-Acetylhistidine	Up
	Serine	Up

	Threonine	Up
	Pyroglutamic acid	Down
	N-acetylneuraminate	Down
	Tryptophan	Up
	Betaine	Up
Tricarboxylic acid cycle	Succinic acid	Down
	Citraconic acid	Up
Glucose metabolism	Glucose	Down
	D-Gluconic acid	Down
	D-Mannose-6-phosphate	Up
Other metabolism	Nicotinamide	Up
	Allantoin	Up
	glutathione	Up
	Hippurate	Down

750 **Table 6 Comparison of potential biomarkers in HRPCM and ARPCM for SQD rats.**

No.	Metabolites	Detection results		No.	Metabolites	Detection results	
		HRPCM	ARPCM			HRPCM	ARPCM
1	16-Hydroxyhexadecanoic acid	**Yes**	No	32	Mannose	No	**Yes**
2	Choline	**Yes**	No	33	N-Acetylglucosamine	No	**Yes**
3	Acetylcarnitine	**Yes**	No	34	Pantothenate	No	**Yes**
4	Trans-Vaccenic acid	**Yes**	No	35	Riboflavin	No	**Yes**
5	Phosphatidylethanolamine 17	**Yes**	No	36	Oxidized glutathione	No	**Yes**
6	2'-O-Methylinosine	**Yes**	No	37	10-Hydroxydecanoic acid	**Yes**	**Yes**
7	Cytosine	**Yes**	No	38	Deoxycarnitine	**Yes**	**Yes**
8	Xanthosine	**Yes**	No	39	FA 12:1	**Yes**	**Yes**
9	2'-Deoxyinosine	**Yes**	No	40	FA 18:3	**Yes**	**Yes**
10	L-alanine	**Yes**	No	41	LPC 18:1	**Yes**	**Yes**
11	Glutamine	**Yes**	No	42	sn-Glycero-3-phosphocholine	**Yes**	**Yes**
12	L-Phenylalanine	**Yes**	No	43	Phosphocholine	**Yes**	**Yes**
13	N-acetylglycine	**Yes**	No	44	sn-Glycerol 3-phosphate	**Yes**	**Yes**
14	D-ornithine	**Yes**	No	45	Lactic acid	**Yes**	**Yes**
15	L-Norvaline	**Yes**	No	46	Uric acid	**Yes**	**Yes**
16	Pyroglutamic acid	**Yes**	No	47	5-Hydroxymethylcytidine	**Yes**	**Yes**
17	Citraconic acid	**Yes**	No	48	Deoxyuridine	**Yes**	**Yes**
18	D-Gluconic acid	**Yes**	No	49	Glycine	**Yes**	**Yes**
19	glutathione	**Yes**	No	50	L-Glutamic acid	**Yes**	**Yes**
20	LPC 16:0	No	**Yes**	**51**	N-Acetylhistidine	**Yes**	**Yes**
21	Palmitoylcarnitine	No	**Yes**	52	Serine	**Yes**	**Yes**
22	Propionylcarnitine	No	**Yes**	53	Threonine	**Yes**	**Yes**
23	Hypotaurine	No	**Yes**	54	N-acetylneuraminate	**Yes**	**Yes**
24	O-Phosphoethanolamine	No	**Yes**	55	Tryptophan	**Yes**	**Yes**
25	2'-Deoxycytidine	No	**Yes**	56	Betaine	**Yes**	**Yes**

26	5-Methylcytosine	No	**Yes**	57	Succinic acid	**Yes**	**Yes**
27	4-aminovaleric acid betaine	No	**Yes**	58	Glucose	**Yes**	**Yes**
28	L-5-Oxoproline	No	**Yes**	59	D-Mannose-6-phosphate	**Yes**	**Yes**
29	Tyr	No	**Yes**	60	Nicotinamide	**Yes**	**Yes**
30	L-asparagine	No	**Yes**	61	Allantoin	**Yes**	**Yes**
31	D-fructose	No	**Yes**	62	Hippurate	**Yes**	**Yes**

Tables

Figure 1 Overview of the experimental protocol.

Figure 2 HPLC fingerprint of HRPCM. (A) Mixed reference. **(B)** HPLC fingerprint. Peak 15, 17, 18 and 19 are Calycosin-7-O-beta-D-glucoside, ononin, calycosin and formononetin, respectively.

Figure 3 HPLC fingerprint of ARPCM. (A) Mixed reference in HPLC-UV. **(B)** HPLC fingerprint HPLC-UV. **(C)** Mixed reference in HPLC-ELSDA. **(D)** HPLC fingerprint HPLC-ELSAD. Peak 2, 5, 6, 7 and 13 are Calycosin-7-O-beta-D-glucoside, ononin, calycosin, formononetin and Astragaloside IV, respectively.

Figure 4 The Effects of HRPCM and ARPCM on SQD rats. (**A**) Weight change during drugs treatment. (**B**) Body weight, **(C)** rectal temperature, **(D)** spleen index, **(E)** thymus index, and the levels of **(F)** GAS, **(G)** D-xylose, **(H)** IL-2, **(I)** IL-6 and **(J)** TNF-α in serum after drugs administration. **(K)** Pathological observations of spleen. The values are presented as mean ± standard deviation (SD). Compared with the sqd group, $*p < 0.05$, $**p < 0.01$.

Figure 5 Multivariate statistical analysis of metabolomics in serum. (**A**) CON vs SQD. (**B**) ARPCM vs SQD. (**C**) HRPCM vs SQD. PCA is shown on the left, OPLS-DA in the middle, and validation of the OPLS-DA model is shown on the right.

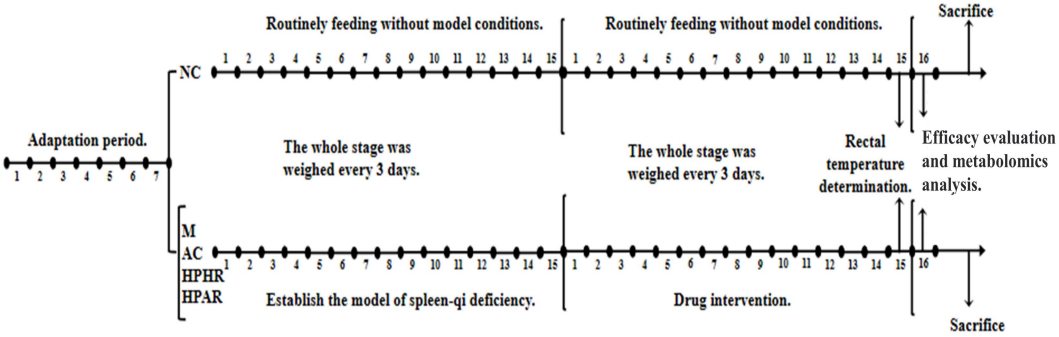
Figure 6 Multivariate statistical analysis of metabolomics in spleen. (**A**) CON vs SQD. (**B**) ARPCM vs SQD. (**C**) HRPCM vs SQD. PCA is shown on the left, OPLS-DA in the middle, and validation of the OPLS-DA model is shown on the right.

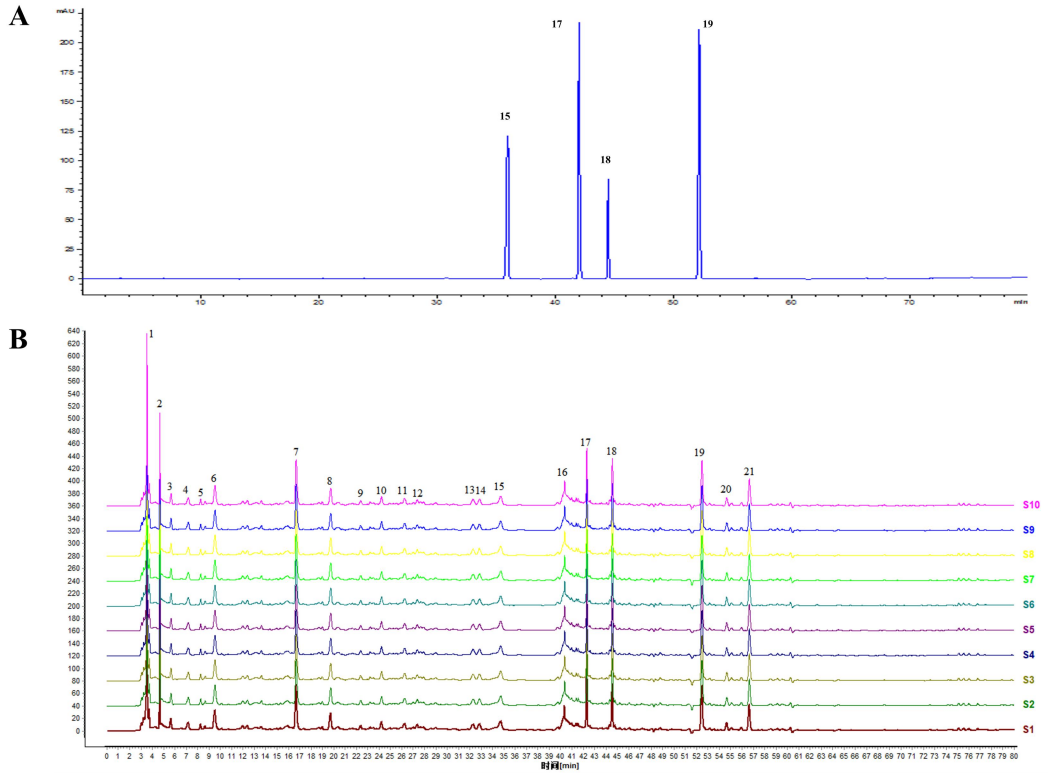
Figure 7 Differential metabolite analysis and KEGG pathway enrichment of metabolomics in serum. (A, D) CON vs SQD. (B, E) ARPCM vs SQD. (C, F)

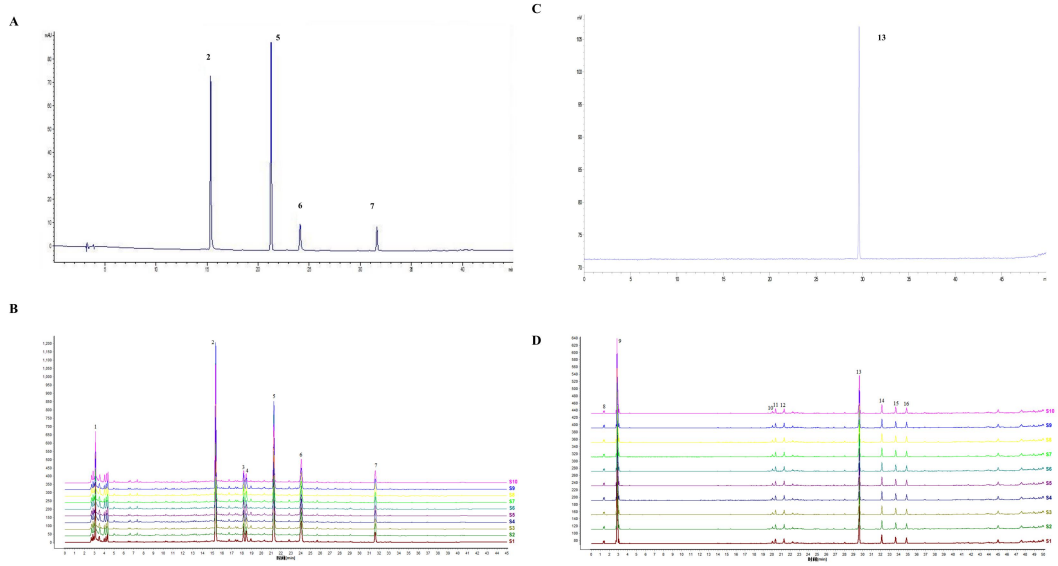
HRPCM vs SQD. The volcano diagram is shown on the left and the top 20 of KEGG enrichment diagram is shown on the right.

Figure 8 Differential metabolite analysis and KEGG pathway enrichment of metabolomics in spleen. (A, D) CON vs SQD. (B, E) ARPCM vs SQD. (C, F) HRPCM vs SQD. The volcano diagram is shown on the left and the KEGG enrichment diagram is shown on the right.

Fig. 9 Venn analysis of biomarkers differences between HRPCM and ARPCM for SQD rats.







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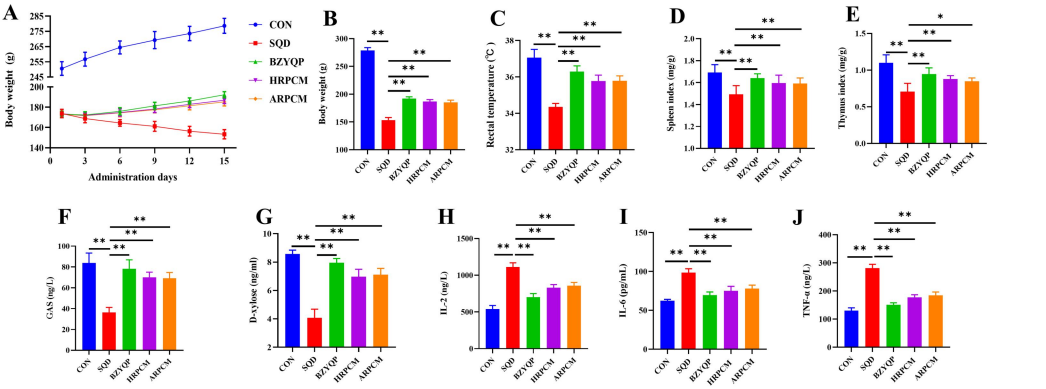

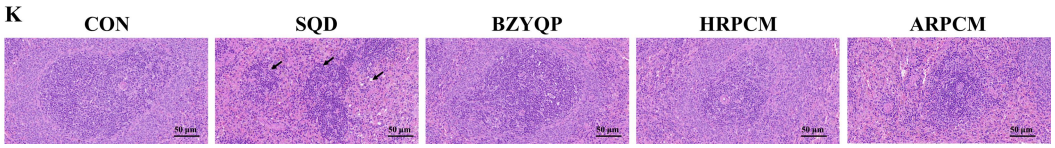

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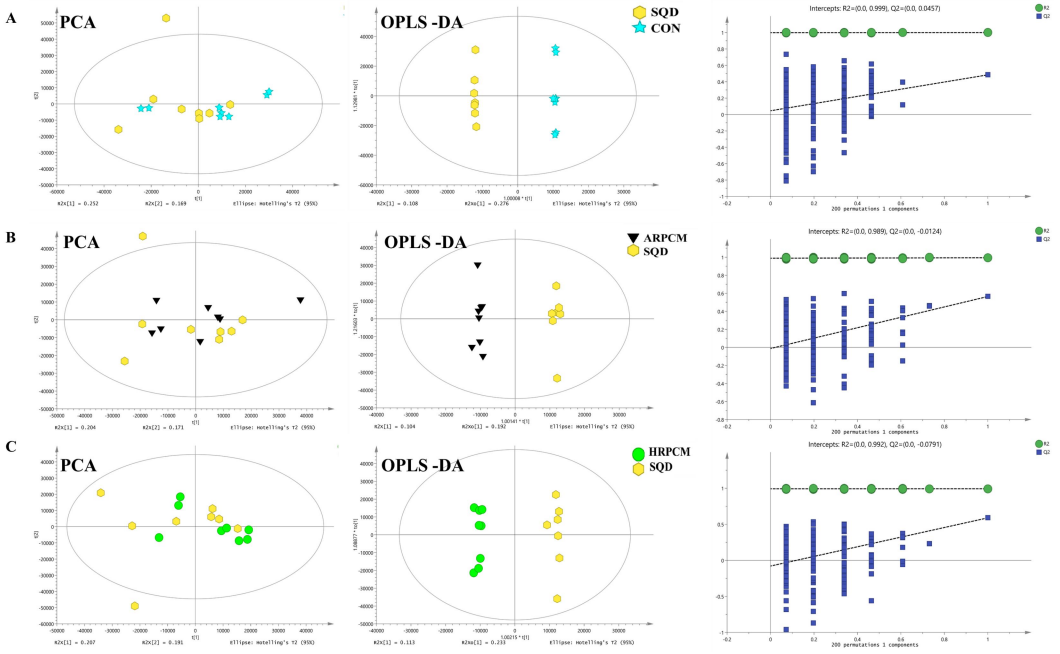

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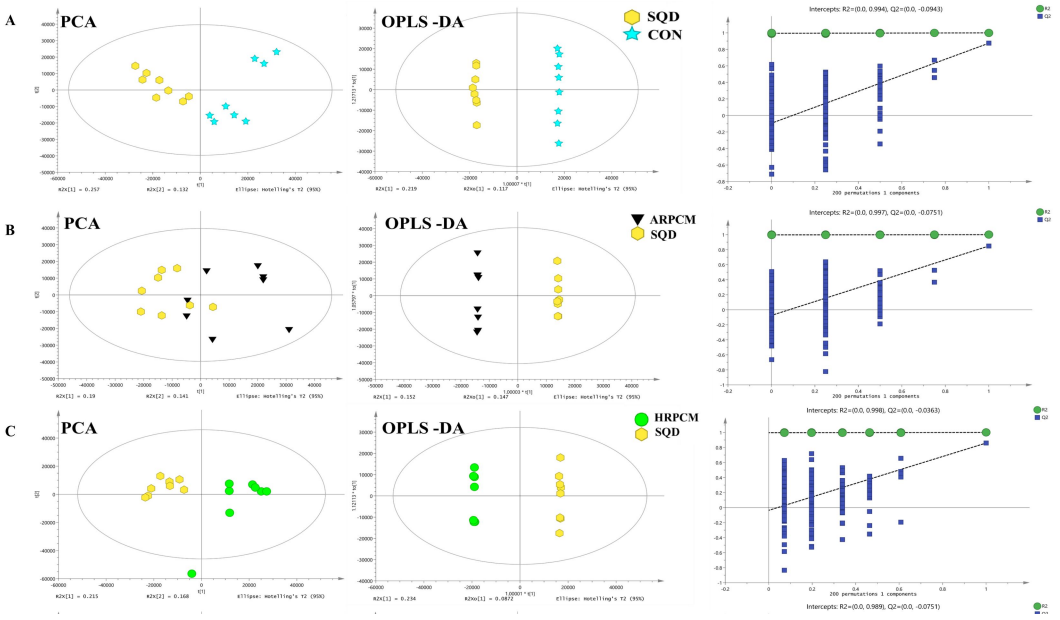

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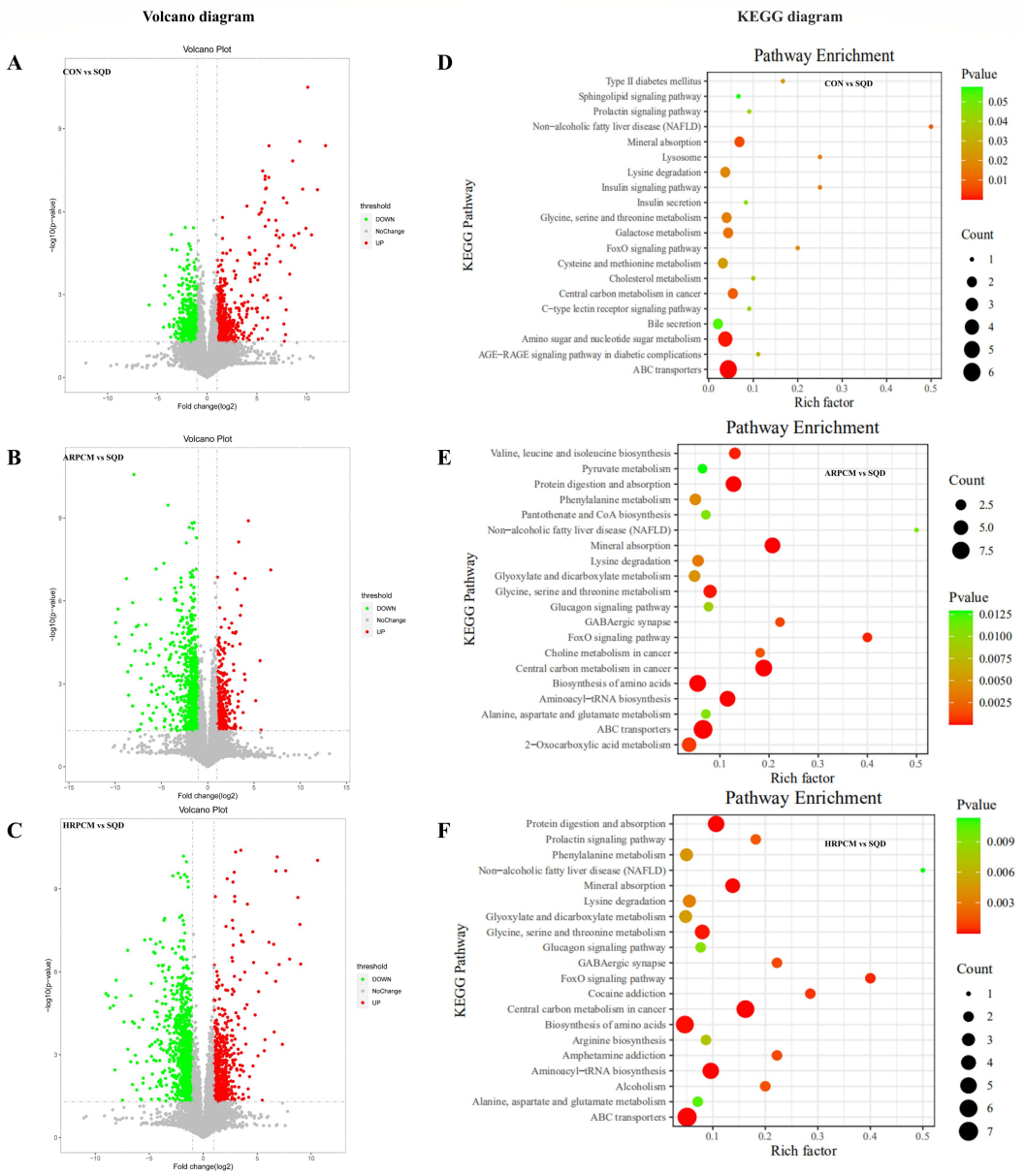
Volcano diagram
KEGG diagram
A
Volcano Plot
CON vs SQD
−log10(p−value)
Fold change(log2)
threshold
DOWN
NoChange
UP
B
Volcano Plot
ARPCM vs SQD
−log10(p−value)
Fold change(log2)
threshold
DOWN
NoChange
UP
C
Volcano Plot
HRPCM vs SQD
−log10(p−value)
Fold change(log2)
threshold
DOWN
NoChange
UP
D
Pathway Enrichment
CON vs SQD
KEGG Pathway
Type II diabetes mellitus
Sphingolipid signaling pathway
Prolactin signaling pathway
Non−alcoholic fatty liver disease (NAFLD)
Mineral absorption
Lysosome
Lysine degradation
Insulin signaling pathway
Insulin secretion
Glycine, serine and threonine metabolism
Galactose metabolism
FoxO signaling pathway
Cysteine and methionine metabolism
Cholesterol metabolism
Central carbon metabolism in cancer
C−type lectin receptor signaling pathway
Bile secretion
Amino sugar and nucleotide sugar metabolism
AGE−RAGE signaling pathway in diabetic complications
ABC transporters
Rich factor
Pvalue
0.05
0.04
0.03
0.02
0.01
Count
1
2
3
4
5
6
E
Pathway Enrichment
ARPCM vs SQD
KEGG Pathway
Valine, leucine and isoleucine biosynthesis
Pyruvate metabolism
Protein digestion and absorption
Phenylalanine metabolism
Pantothenate and CoA biosynthesis
Non−alcoholic fatty liver disease (NAFLD)
Mineral absorption
Lysine degradation
Glyoxylate and dicarboxylate metabolism
Glycine, serine and threonine metabolism
Glucagon signaling pathway
GABAergic synapse
FoxO signaling pathway
Choline metabolism in cancer
Central carbon metabolism in cancer
Biosynthesis of amino acids
Aminoacyl−tRNA biosynthesis
Alanine, aspartate and glutamate metabolism
ABC transporters
2−Oxocarboxylic acid metabolism
Rich factor
Count
2.5
5.0
7.5
Pvalue
0.0125
0.0100
0.0075
0.0050
0.0025
F
Pathway Enrichment
HRPCM vs SQD
KEGG Pathway
Protein digestion and absorption
Prolactin signaling pathway
Phenylalanine metabolism
Non−alcoholic fatty liver disease (NAFLD)
Mineral absorption
Lysine degradation
Glyoxylate and dicarboxylate metabolism
Glycine, serine and threonine metabolism
Glucagon signaling pathway
GABAergic synapse
FoxO signaling pathway
Cocaine addiction
Central carbon metabolism in cancer
Biosynthesis of amino acids
Arginine biosynthesis
Amphetamine addiction
Aminoacyl−tRNA biosynthesis
Alcoholism
Alanine, aspartate and glutamate metabolism
ABC transporters
Rich factor
Pvalue
0.009
0.006
0.003
Count
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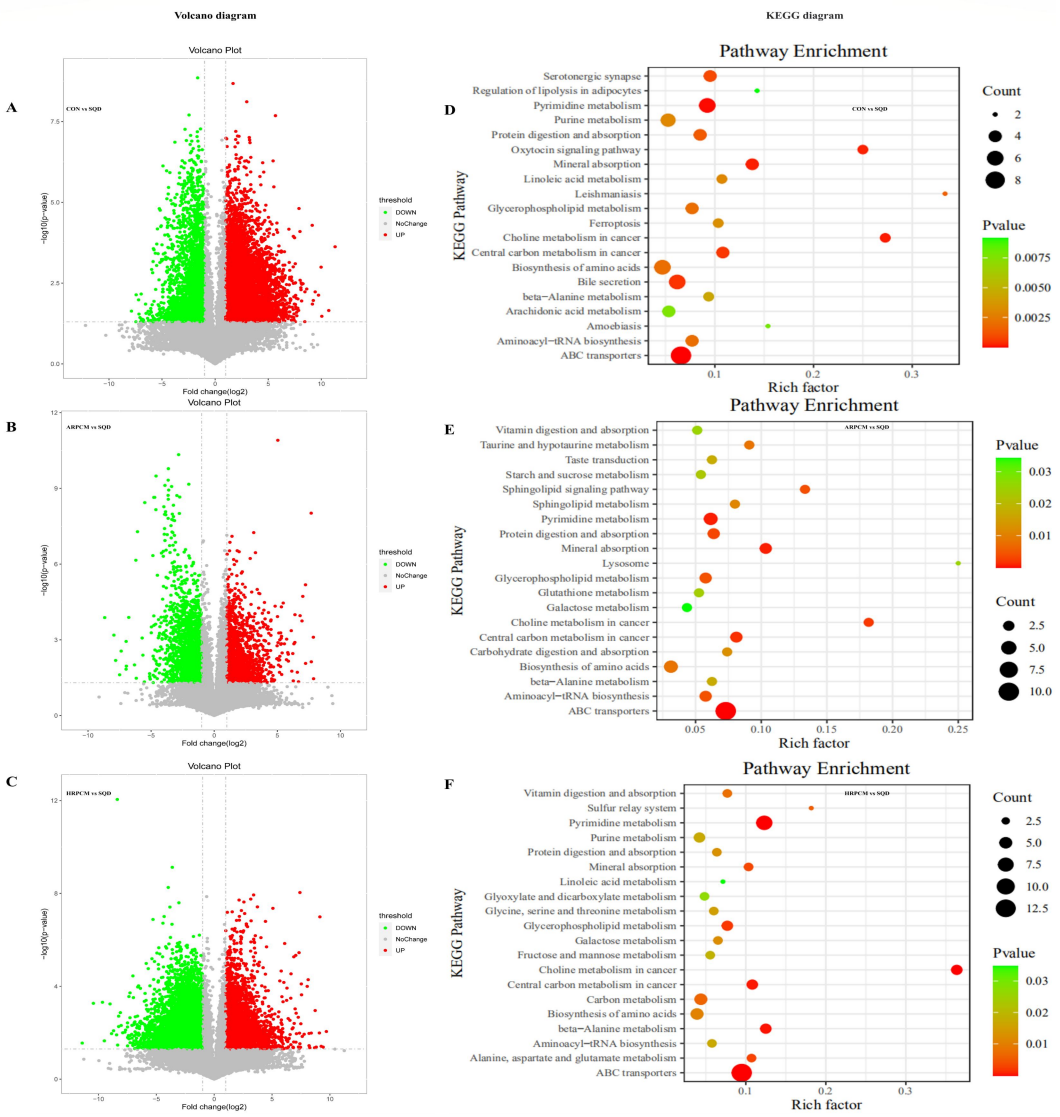
2
3
4
5
6
7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Rich factor
Count
Pvalue
Serotonergic synapse
Regulation of lipolysis in adipocytes
Pyrimidine metabolism
Purine metabolism
Protein digestion and absorption
Oxytocin signaling pathway
Mineral absorption
Linoleic acid metabolism
Leishmaniasis
Glycerophospholipid metabolism
Ferroptosis
Choline metabolism in cancer
Central carbon metabolism in cancer
Biosynthesis of amino acids
Bile secretion
beta−Alanine metabolism
Arachidonic acid metabolism
Amoebiasis
Aminoacyl−tRNA biosynthesis
ABC transporters
Vitamin digestion and absorption
Taurine and hypotaurine metabolism
Taste transduction
Starch and sucrose metabolism
Sphingolipid signaling pathway
Sphingolipid metabolism
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Protein digestion and absorption
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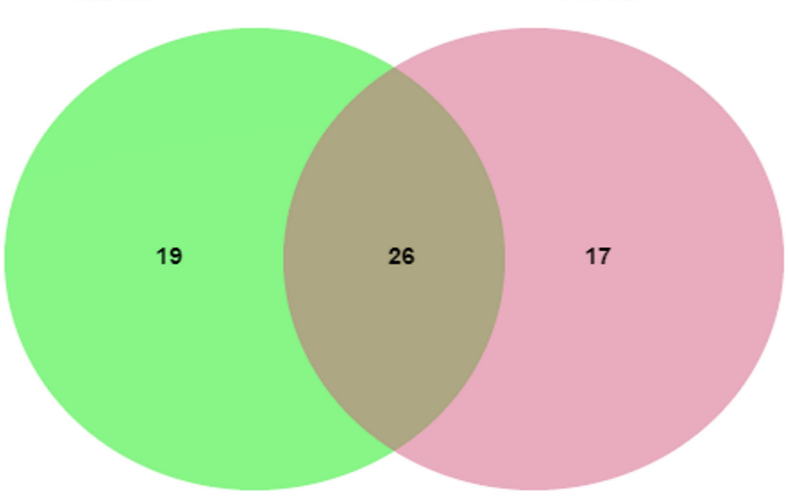
HRPCM
ARPCM
19
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17