Metabonomic Study on the Plasma of High-Fat Diet-Induced Dyslipidemia Rats Treated With Ge Gen Qin Lian Decoction By Ultra High Performance Liquid Chromatography–Mass Spectrometry

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Research

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Abstract

Background: Gegen Qinlian decoction (GGQLD) has a definite effect on T2DM in clinic, and it has the effect of lowering blood sugar, improving insulin resistance, and improve the blood lipid level of rats with dyslipidemia, but the intervention mechanism of GGQLD on dyslipidemia has not been clarified.

Methods: Based on ultra high performance liquid chromatography coupled with quadrupole-time-of-flight tandem mass spectrometry (UHPLC-Q-TOF-MS), the metabolic profiles of rat serum samples were collected. The rat model of dyslipidemia was induced by 60% fat-fed high-fat diet. After feeding the rats with high-fat diet for 4 weeks, the dyslipidemia appeared. After 5 weeks of GGQLD (14.85g•kg⁻¹) administration, the metabonomics of rats plasma samples in normal group, model group and administration group were analyzed. Mass profiler professional (MPP), SIMCA-P 14.1 and Graphpad prism 6.0 software were used combined with METLIN biological database and human metabolite database HMDB to screen and identify endogenous biomarkers. Metaboanalyst 4.0 software was used by combining with HMDB and KEGG databases, the enrichment and metabolic pathway of biomarkers were analyzed to explore the metabolic mechanism of dyslipidemia rats induced by high-fat diet and the intervention mechanism of Gegen Qinlian decoction.

Results: After 5 weeks of administration of GGQLD, the levels of serum TC and TG were significantly decreased (P < 0.05, P < 0.01), while HDL-C and LDL-C were not significantly affected. After administration, the food intake of rats in administration group decreased gradually, and the change trend of body weight gradually slowed down. The metabonomics of rat plasma samples results showed that 23 potential biomarkers including α-linolenic acid, arachidonic acid and lysophosphatidylcholine were significantly changed in positive ion mode.

Conclusion: Studies have shown that GGQLD has significant lipid-lowering effect on dyslipidemia rats induced by high-fat diet, and its preventive mechanism is related to tryptophan metabolism, fatty acid biosynthesis, α-linolenic acid metabolism, arachidonic acid, glycerophosphatidyl metabolism pathway.

Background

Type 2 diabetes mellitus (T2DM) accounts for 90% of the total population of diabetes mellitus, but most patients do not have obvious symptoms, especially in the early stage of diabetes mellitus, which is accompanied by varying degrees of overweight or obesity and dyslipidemia. Dyslipidemia refers to the disorder of lipid metabolism in the body and the disorder of plasma lactone exceeding the normal range, accompanied by the increase of total cholesterol (TC), triglyceride (TG), low density lipoprotein-cholesterol (LDL-C) and the decrease of high density lipoprotein-cholesterol (HDL-C). With the popularization of Western fast food, high fat and high calorie diet and other bad habits, dyslipidemia and obesity have become a common phenomenon. Epidemiological studies show that the incidence rate of dyslipidemia in T2DM patients is 50%. [1]
Liu et al. [2] showed that with the increase of TC and TG levels, the prevalence of T2DM increased significantly. Dyslipidemia is also a risk factor for diabetic complications [3, 4]. Dyslipidemia is an important inducement of insulin resistance and plays an important role in the occurrence and development of T2DM [5]. Adiels et al. [6] studies have shown that dyslipidemia is an early event of T2DM. The results of UK Prospective Diabetes Study (UKPDS) show that dyslipidemia is an independent risk factor of T2DM, and there is a significant dyslipidemia in the early stage of the disease.

Blood lipid parameters can predict the occurrence of type 2 diabetes to a certain extent. The close attention to the level of blood lipid and timely intervention when abnormal blood lipid occurs are of great significance to delay and control the occurrence and development of type 2 diabetes mellitus and its complications.

Gegen Qinlian Decoction originated from Zhang Zhongjing’s Treatise on Typhoid Fever. It is composed of four herbs: *Pueraria lobata* (Willd.) Ohwi (Ge-Gen), *Scutellaria baicalensis* Georgi (Huang-Qin), *Coptis Chinensis* Franch (Huang-Lian), and *Glycyrrhiza uralensis* Fisch (Gan-Cao). As an important herbal medicine in compound prescription, *Pueraria lobata* has been widely used in the treatment of cardiovascular diseases and endocrine and metabolic diseases [7–11], among which dyslipidemia is one of the representatives [12]. Modern pharmacological studies have shown that berberine, after administration of berberine (an effective component of *Coptis chinensis*) for a period, the contents of TG, TC and LDL-C decreased significantly, while the contents of HDL-C increased significantly in hyperlipidemic rats, berberine can inhibit cholesterol synthesis, promote cholesterol transport and removal, and reduce cholesterol [13–16].

The total flavonoids contained in *Scutellaria baicalensis* have the effect of lowering blood cholesterol. Preliminary clinical studies have shown that the total flavonoids of *Scutellaria baicalensis* have a certain preventive and therapeutic effect on dyslipidemia [17–19].

In summary, the pharmacological studies on the single drug or monomer components in the compound and compound prescriptions suggest that Gegen Qinlian decoction has a certain effect on improving blood lipid.

The preliminary experimental studies of the research group show that Gegen Qinlian decoction has a definite effect on T2DM in clinic, and it has the effect of lowering blood sugar and improving insulin resistance [20–22]. However, the metabolic mechanism and GGQLD intervention mechanism of dyslipidemia rats in the early stage of diabetes have not been clarified.

In recent years, metabonomics has been widely used in the research of disease phenotype, biomarker discovery of drug toxicology, metabolism phenotype and physiological function of experimental animals, comprehensively depicting the overall characteristics of metabolism [23, 24]. In order to study the metabolic mechanism of dyslipidemia and try to find the key biomarkers in the intervention of Gegen Qinlian decoction on dyslipidemia. In this experiment, the model of dyslipidemia was established by feeding rats with high-fat diet. And the metabolic mechanism of dyslipidemia induced by high fat diet
was explored, Gegen Qinlian decoction as the intervention drug was used to find the endogenous markers of dyslipidemia rats, and the possible mechanism of Gegen Qinlian Decoction intervention in dyslipidemia rats, which provides scientific basis to prevent and treat T2DM.

**Methods**

**Animals and ethical statement**

Animal studies were performed according to the Principles of Laboratory Animal Care (World Health Organization, Geneva, 1985). 48 male Sprague-Dawley rats (180 ± 20 g) were purchased from the Jiangxi University of Traditional Chinese Medicine (Certificate: SCXK(Gan)2017-0004, Nanchang, China). The rats were housed in a specific pathogen-free breeding room (temperature: 20 ± 2 °C; humidity: 60 ± 5%; 12 h light–dark cycle). All of the rats were provided with free access to tap water. Ordinary rat feed was purchased from Laboratory Animal Science and Technology Center of Jiangxi University of Traditional Chinese Medicine. High-fat feed (D12492): 60% fat, 20% protein and 20% sugar. The formula is from Research Diets Company (USA).

**Instruments**

Modular p800 Biochemical analyzer (Roche, Germany), High-speed refrigeration centrifuge (Thermo Scientific, Germany), BT225-Electronic analytical balance (Sartorius, Germany), SpeedVac® SPD131 Centrifuge enrichment system (Thermo Scientific, Germany), Milli-Q Advantage A10 Ultra-pure Water Purifier (Millipore, USA), Agilent 6538A Ultra High Performance Liquid Chromatography-time-of-flight mass Spectrometry (UHPLC-Q-TOF-MS) (Agilent, USA).

**Reagents**

Cholesterol and triglyceride were purchased from Roch (700487-01, Germany), High and low density lipoprotein (HL8108m) and glucose reagent (GL8322) were obtained from Purebio Biotechnology (Ningbo, China). HPLC-grade formic acid was purchased from Dikma (Richmond Hill, NY, USA). HPLC-grade methanol and acetonitrile were obtained from Tedia (Fairfield, OH, USA). Deionized water was used. GGQLD was prepared our lab and performed quality control by Agilent 1260 HPLC. The fingerprint of Gegen Qinlian Decoction for this study can be found in the **Supplementary Materials**.

**Experimental methods**

**Establishment of rat model with dyslipidemia**

24 male SD rats were adaptively fed for 5 d, they were randomly divided into model group (16 rats) and normal group (8 rats) according to body weight. Normal rats were fed with ordinary feed, while model group rats were fed with 60% high-fat feed.
The changes of dietary intake, phenotypic characteristics and body weight were observed every day, body length was observed and Lee's index was calculated every week. After four weeks, four blood lipids, serum insulin were measured and IR index was calculated. The blood lipid levels and FPG, Fins, IR index levels of rats in each group after grouping as showed in Table 1 and Table 2.

### Table 1

| Group          | n   | TC (mmol/L) | TG (mmol/L) | HDL-C (mmol/L) | LDL-C (mmol/L) |
|----------------|-----|-------------|-------------|----------------|---------------|
| Control group  | 8   | 1.53 ± 0.22 | 1.26 ± 0.23 | 0.67 ± 0.11    | 0.29 ± 0.10   |
| Model group    | 8   | 1.91 ± 0.44*| 1.77 ± 1.14 | 0.50 ± 0.05**  | 0.60 ± 0.31** |
| Administrated group | 8   | 1.96 ± 0.14**| 1.21 ± 0.53 | 0.56 ± 0.06**  | 0.86 ± 0.24** |

Note: vs. normal group, *P < 0.05** P < 0.01

### Table 2

| Group     | n   | FPG (mmol/L) | Fins (mU/L) | IR index |
|-----------|-----|--------------|-------------|----------|
| Control   | 8   | 5.04 ± 0.91  | 21.79 ± 4.78| 4.83 ± 1.18 |
| Model     | 8   | 5.36 ± 1.73  | 23.85 ± 4.69| 5.58 ± 1.92 |
| Administrated | 8   | 5.34 ± 0.95  | 23.42 ± 2.65| 5.60 ± 1.43 |

### Treatment of animal

After 5 weeks of administration, rats in each group were fasting for 12 hours, and orbital blood was collected for 1.2 mL to prepare serum and plasma. Blood samples were placed in normal eppendorf (EP) tubes and EP tubes containing 0.1% heparin sodium, respectively. The blood samples were kept at 4 °C for 3 h, and centrifuged at 4 °C for 15 min (3500 rpm). The supernatant in normal EP tube was absorbed and stored at 80 °C for the detection of biochemical indicators. Meanwhile the EP tubes containing 0.1% heparin sodium for plasma samples.

### Sample preparation

Frozen plasma samples were thawed at room temperature. Then, 100 µL of sample was placed in EP tubes, and 300 µL of methanol was added. The tubes were vortexed for 1 min, incubated for 3 h at 4°C, and then centrifuged (15,000 rpm, 10 min, 4 °C). The supernatants were collected and dried by
SpeedVac®, and the residues were reconstituted in 200 µL of methanol: water (15: 85). Then, the samples were vortexed for 1 min and centrifuged (18,000 rpm, 15 min, 4 °C). The supernatants were collected and subsequently analyzed following a previously described UHPLC-Q-TOF-MS -based untargeted metabolic profiling strategy[25].

Data processing and statistical analyses

The collected data were extracted by molecular feature extraction with the retention time, m/z value, and the volume for each compound. The data were converted into the .cef format using Profinder B.06.0 (Agilent Technologies), and they were processed by Mass Profiler Professional (MPP) v12.1 (Agilent Technologies). To compare the metabolite profiles of the three groups, the Student's t-test and analysis of variance were conducted. Compounds that satisfied \( P < 0.05 \) and had a fold change \( \geq 2.0 \) were selected as preliminary potential biomarkers, and unsupervised principal component analysis (PCA) was performed. The CSV format file with peak area value was derived, and partial least squares discriminant analysis (PLS-DA) was carried out with SIMCA-P software. Compounds with VIP > 1 were screened again as potential biomarkers. The potential biomarkers were then returned to original data for matching the different variable compounds in each group. The endogenous biomarkers were identified by comparing the HMDB database with MS/MS information following a previously described method[25]. MetaboAnalyst 3.0 was used to analyze related metabolic pathway.

Results

Establishment of rat model with dyslipidemia and changes of blood lipid level in rats after 5 weeks of Gegen Qinlian Decoction administration

After feeding 60% fat-fed high-fat diet for 4 weeks, the weight of rats increased significantly, TC and LDL-C increased significantly, HDL-C decreased significantly, and TG increased without statistical difference. A rat model of abnormal lipid metabolism was established. At this time, there was no disturbance of glucose metabolism in rats. After 12 weeks of feeding, TC, TG and LDL-C of rats were significantly higher than those of the normal group, and HDL-C was significantly lower than that of the normal group. High-fat diet had a greater impact on HDL-C of rats.

Five weeks after GGQLD administration, the levels of TC and TG in model group were significantly higher than those in normal group \( (P < 0.01) \), while HDL-C was significantly lower \( (P < 0.01) \). Compared with the model group, the levels of TC and TG in the administrated group were significantly decreased \( (P < 0.05, P < 0.01) \), as shown in Table 3. Compared with the normal group, the blood sugar in the model group increased significantly \( (P < 0.01) \), but remained stable in the range of \( (6.1–7.9 \text{ mmol/}) \). Compared with the model group, the administrated group significantly prevented the increase of blood sugar \( (P < 0.05, P < 0.01) \).

LC-MS analysis of metabolic profiles of plasma samples
UHPLC-Q-TOF/MS was used to analyze all the plasma samples. LC-MS total ion chromatograms (TIC) of a plasma sample in positive-ion mode is showed in Fig. 1. In metabonomics experiments, it is very important to investigate the repeatability of analytical methods because many samples need to be processed.

The quality control (QC) sample was used to monitor the reliability of analytical methods, which was prepared by blending the same volume of liquid from all plasma samples and injecting every five samples to supervise the stability of the analysis in the total run. Relative standard deviations of the retention time and peak area of six selected peaks in quality control samples were < 0.17% and < 3.58%, respectively (Supplementary Table 1). Resulting data showed that the precision and repeatability of the proposed method were satisfactory for this analysis.

Observation of Principal Component Analysis

The endogenous metabolites in plasma samples were detected by UHPLC-Q-TOF/MS in positive full scan mode, to study the effect of Gegen Qinlian Decoction on endogenous substance metabolism in rats with dyslipidemia after administration. The PCA was performed on MPP; the clustering results of each group of samples are shown in the Fig. 2. In the PCA plot, every point represents one plasma sample, and the spatial distribution of the sample represents the metabolic status of different groups of samples.

Partial Least Squares Discriminant Analysis

Compared with unsupervised PCA, supervised partial least squares discriminant analysis (PLS-DA) pays more attention to the compounds contributing to clustering. This experiment further used PLS-DA to screen potential biomarkers.

In this study, PLS-DA was used to analyze the differences among the three groups of samples. The evaluation index R2X of the model indicated the percentage of variation of implicit variable reflecting independent variable X; R2Y indicated the percentage of variation of implicit variable reflecting variable Y; Q2 was the cumulative percentage of difference between X and Y obtained by the model after cross-validation, and predicted the effect of new data. In many cases, the larger the values of R2X, R2Y and Q2, and the closer is the ratio of R2Y and Q2 to 1, the more stable and reliable the model is. On the contrary, it means that the model is over-fitting. In order to better evaluate the predictability of the model, it is necessary to test whether the reaction of permutation is over-fitting. From the score chart (Fig. 3), we can see that the normal group (C), model group (M) and Gegen Qinlian decoction administrated group (A) were obviously separated (model parameters were R2X = 0.662, R2Y = 0.994, Q2 = 0.903). The normal group (C) and model group (M) were separated along t [1] axis, indicating that the endogenous metabolites of the two groups changed significantly. After treatment with Gegen Qinlian decoction, the serum profile of the administrated group moved towards the normal group, indicating that Gegen Qinlian decoction had a reversal effect on the rats with dyslipidemia.
In order to eliminate the over-fitting effect of the model, 200 tests were arranged. Generally, the Q2 value on the left side was lower than the original point on the right side, and the intersection point of the vertical axis on the left side and the regression line on the Q2 point was at or below zero. The R2 value on the left side was lower than the original point on the right side, which indicated the validity of the original model. Figure 4 shows that the model has not been fitted, and the model validation is established. The difference between the normal group and the model group is further analyzed by OPLS-DA. The score chart is shown in Fig. 5. The model parameters are R2Y = 0.993, Q2 = 0.873, and the arrangement test is shown in Fig. 6. The quality of the model is good. VIP values (the importance of variables in mapping) were extracted from OPLS-DA model, and the variables with VIP > 1 (Fig. 7) were used as the final potential biomarkers for further identification.

Identification of potential biomarkers

The list of variables (P < 0.05, FC (fold change) > 2, VIP > 1) was exported to excel, matching the database HMDB with MS/MS information. Mammals-Rattus norvegicus (rat) (KEGG) metabolic pathway analysis showed that potential biomarkers were mainly involved in the biosynthesis of unsaturated fatty acids, α-linolenic acid metabolism, glycerophospholipid metabolism, arachidonic acid metabolism, tryptophan metabolism and fatty acid biosynthesis. In this experiment, the changes of biomarkers in plasma of rats in different groups and involved metabolic pathways were analyzed. The results showed that the contents of some endogenous compounds in rat plasma changed in the model group compared with these of normal rats. After administration of GGQLD, the contents of 16 endogenous compounds (listed in Table 4) in plasma of rats in the administration group returned to normal rats to some extent compared with those in the model group (Fig. 8). Through the identification and analysis of 16 biomarkers, the results showed that GGQLD may play a pharmacodynamic role through fatty acid biosynthesis, α-linolenic acid metabolism, arachidonic acid metabolism, glycerophospholipid metabolism, tryptophan metabolism pathway.
| Biomarker                          | Formula         | m/z       | RT(min) | VIP | Change trend | Model group | Administered group |
|----------------------------------|-----------------|-----------|---------|-----|--------------|-------------|-------------------|
| Indole acetaldehyde              | C10 H9 N O      | 160.0743  | 1.71    | 1.34 | ↓##          | ↑           |                   |
| Acylglycine                      | C9 H9 N O3      | 180.0646  | 4.17    | 1.65 | ↓##          | ↑**         |                   |
| Iso-L-carnitine                  | C11 H21 N O4    | 232.1534  | 2.39    | 1.28 | ↓##          | ↓           |                   |
| α-Linolenic acid                 | C18 H30 O2      | 279.2301  | 14.55   | 1.62 | ↓##          | ↑*          |                   |
| (Z)-13- octadecenoic acid        | C18 H34 O2      | 283.2628  | 17.16   | 1.64 | ↑##          | ↓*          |                   |
| Androstenane 3α, 17β - diol      | C15 H28 N6      | 293.2458  | 13.51   | 1.47 | ↓##          | ↑           |                   |
| Arachidonic acid                 | C20 H32 O2      | 305.2472  | 15.51   | 1.24 | ↑##          | ↓           |                   |
| Octadecanoic acid                | C18 H36 O2      | 307.2604  | 16.39   | 1.06 | ↑##          | ↓**         |                   |
| L- Octyl carnitine               | C16 H25 N5      | 310.1997  | 5.74    | 1.74 | ↑##          | ↓           |                   |
| Docosapentaenoic acid            | C22 H34 O2      | 331.2622  | 12.41   | 1.41 | ↑#           | ↑           |                   |
| Arachidonic acid ethanolamine    | C22 H37 N O2    | 348.2889  | 16.32   | 1.30 | ↑#           | ↓           |                   |
| Ethyl acetate arachidonic acid   | C22 H36 O2      | 355.2614  | 6.85    | 1.08 | ↓##          | ↑**         |                   |
| Tetracosahexaenoic acid          | C24 H36 O2      | 357.2768  | 7.98    | 1.28 | ↓##          | ↓           |                   |
| N- Acetylethanolamine            | C24 H36 O3      | 373.2722  | 6.81    | 1.45 | ↓##          | ↑*          |                   |
| 7- Ketodeoxycholic acid          | C24 H38 O5      | 407.2781  | 6.47    | 1.32 | ↓#           | ↑*          |                   |
| Stearyl carnitine                | C21 H45 N7 O2   | 428.3719  | 9.32    | 1.60 | ↑##          | ↓           |                   |

Note: “↓” means decreased, “↑” means increased; compared with normal group, #P < 0.05, ##P < 0.01; compared with model group, *P < 0.05, **P < 0.01

VIP: Variable importance in the projection.
| Biomarker                  | Formula                  | m/z         | RT(min) | VIP | Change trend | Model group | Administered group |
|---------------------------|--------------------------|-------------|---------|-----|--------------|-------------|-------------------|
| LysoPC(14: 0/0: 0)        | C24 H41 N3 O6            | 468.307     | 7.67    | 1.32| ↓#           | ↓**         |                   |
| LysoPE(20: 4 (5Z, 8Z, 11Z, 14Z) / 0: 0) | C25 H44 N07P            | 502.2919    | 8.49    | 1.60| ↑#           | ↓**         |                   |
| LysoPE(20: 1(11Z) / 0: 0) | C27 H45 N3 O6            | 508.3371    | 8.72    | 1.59| ↓##          | ↓           |                   |
| LysoPC(20: 5 (5Z, 8Z, 11Z, 14Z, 17Z )) | C30 H43 N3 O6            | 542.3227    | 7.82    | 1.65| ↓##          | ↑           |                   |
| LysoPC (22:4 (7Z, 10Z, 13Z, 16Z )) | C32 H49 N3 O6            | 572.3698    | 9.89    | 1.59| ↑##          | ↑           |                   |
| LysoPC (24: 1(15Z))       | C35 H55 N7 O2            | 628.4311    | 17.04   | 1.51| ↓##          | ↓           |                   |
| SM (D18: 1/14: 0)         | C39 H70 N4 O5            | 697.5252    | 19.17   | 1.67| ↑##          | ↓           |                   |

Note: “↓” means decreased, “↑” means increased; compared with normal group, #P<0.05, ##P<0.01; compared with model group, *P<0.05, **P<0.01

VIP: Variable importance in the projection.

Metabolic pathway analysis

16 potential biomarkers were screened and analyzed by MetaboAnalysis 4.0 combined with Pathway-associated metabolite sets (SMPDB) and KEGG for Enrichment Analysis and Pathway Analysis.

Enrichment Analysis

The results of SMPDB enrichment analysis showed that the potential biomarkers were mainly enriched in six metabolic pathways: α-linolenic acid and linoleic acid metabolism, long-chain saturated fatty acid mitochondrial beta-oxidation, acetal phospholipid synthesis, short-chain saturated fatty acid mitochondrial beta-oxidation, arachidonic acid metabolism and tryptophan metabolism(Fig. 9, Table 5).
### Table 5
Potential biomarker enrichment analysis table

| Metabolite Set                                      | Total | Hits | Expected | P   | Holm P | FDR  |
|----------------------------------------------------|-------|------|----------|-----|--------|------|
| Alpha Linolenic Acid                               | 19    | 5    | 0.38     | 0.002 | 0.002  | 0.002|
| Linoleic Acid Metabolism                           |       |      |          |      |        |      |
| Mitochondrial Beta-Oxidation of Long Chain Saturated Fatty Acids | 28    | 2    | 0.55     | 0.10 | 1.0    | 1.0  |
| Plasmalogen Synthesis                              | 26    | 1    | 0.51     | 0.41 | 1.0    | 1.0  |
| Mitochondrial Beta-Oxidation of Short Chain Saturated Fatty Acids | 27    | 1    | 0.53     | 0.42 | 1.0    | 1.0  |
| Tryptophan Metabolism                              | 60    | 1    | 1.17     | 0.71 | 1.0    | 1.0  |
| Arachidonic Acid Metabolism                        | 69    | 1    | 1.32     | 0.76 | 1.0    | 1.0  |

Pathway Analysis

The results of metabolic pathway analysis by using mammals Rattus norvegicus (rat) (KEGG) showed that the potential biomarkers are mainly related to the biosynthesis of unsaturated fatty acids, α linolenic acid metabolism, glycerophospholipid metabolism, arachidonic acid metabolism and tryptophan metabolism. 6 metabolic pathways, namely metabolism and fatty acid biosynthesis, are shown in Fig. 10 and Table 6.

### Table 6
Analysis Table of Potential Biomarker Metabolic Pathways

| Pathway Name                               | MatchStatus | P   | Holm P | Impact |
|--------------------------------------------|-------------|-----|--------|--------|
| Biosynthesis of unsaturated fatty acids    | 5/42        | 0.00| 0.00   | 0.0    |
| alpha-Linolenic acid metabolism            | 1/9         | 0.07| 1.0    | 0.2    |
| Glycerophospholipid metabolism             | 1/30        | 0.21| 1.0    | 0.05   |
| Arachidonic acid metabolism                | 1/36        | 0.25| 1.0    | 0.4    |
| Tryptophan metabolism                      | 1/41        | 0.28| 1.0    | 0.03   |
| Fatty acid biosynthesis                     | 1/43        | 0.30| 1.0    | 0.0    |

**Discussion**

Biosynthesis of fatty acid
In this experiment, compared with the normal group, the contents of α-linolenic acid, docosahexaenoic acid, arachidonic acid, octadecanoic acid, docosahexaenoic acid and stearic acid in the plasma of rats in the model group were significantly changed. After GGQLD was administrated, compared with the model group, the contents of the above components in the plasma of the rats in the administration group all returned to the level of the normal group.

Reported studies have shown that polyunsaturated fatty acids (PUFAs) are important components of cell membrane phospholipids. The synthesis of PUFAs is based on the catalysis, dehydrogenation and prolongation of stearic acid through a series of enzymes[26]. In mammalian livers, eicosapentaenoic acid is catalyzed by Δ5 and Δ7 lengthening enzymes for two times to form 24-carbon pentaenoic acid, which is catalyzed by Δ6 desaturase to form 24-carbon hexaenoic acid, which is then transferred to peroxidase body through endoplasmic reticulum for a beta oxidation to form docosahexaenoic acid. Omega 3 fatty acids such as docosahexaenoic acid can reduce the levels of TC, TG and LDL-C, which are beneficial to cardiovascular health[27].

The mechanism of lipid-lowering effect of GGQLD is related to the biosynthesis of fatty acids in vivo, ω-3 polyunsaturated fatty acids mainly include α-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which have the functions of protecting cardiovascular system, regulating blood lipid, anti-inflammation, anti-allergy, anti-tumor and improving immune regulation. The effects of ω-3 polyunsaturated fatty acids on blood lipids were different in different studies, but the effects on TG were similar. ω-3 polyunsaturated fatty acids have a direct inhibitory effect on the synthesis of TG, which may be related to the increase of beta oxidation and the clearance of TG-rich lipoproteins. ALA, as a maternal body, can be used as a beneficial supplement in diet. It can significantly reduce the TC, TG, LDL-C and body weight of rats induced by high-fat diet, increase the level of HDL-C, and prevent and treat hyperlipidemia[28]. Studies[29] have shown that ALA may play a role in reducing body weight and blood lipids by accelerating lipid oxidation, affecting the activities of key metabolic enzymes and reducing visceral fat accumulation. After high-fat diet, the circulatory alpha-linolenic acid in rats decreased significantly, which increased the risk of cardiovascular disease. After GGQLD intervention, the level of alpha-linolenic acid was significantly reversed, and the concentration of TC and TG was decreased by regulating the metabolism of alpha-linolenic acid in rats.

Arachidonic acid metabolism

Arachidonic acid (AA) metabolism is the core of inflammatory metabolic network. The two main metabolic pathways are cyclooxygenase (COX) pathway and perlipoxygenase (LOX) pathway. Protaglandins (PGs), thromboxans (TXs), leukotrienes (LTs) and lipid peroxides produced by COX pathway are potential targets for anti-inflammatory research[30].

AA metabolic abnormalities are associated with dyslipidemia and coronary heart disease. Thromboxane A2 and prostaglandin I2, metabolites of AA, promote platelet aggregation and agglutination respectively. Their balance maintains smooth blood circulation and protects endothelial cells from damage. When blood lipid is abnormal, AA synthesizes thromboxane A2 more quickly, which decreases the ability of
blood vessel wall to synthesize prostaglandin I2. It was found that the ratio of plasma lipid peroxide and thromboxane A2/prostaglandin I2 increased in hyperlipidemic rats[31]. Studies have shown that thromboxane A2 and prostaglandin I2 are associated with TC and TG. Puerarin can promote vascular endothelial growth factor-like effect and inhibit the increase of thromboxane A2/prostaglandin I2 ratio induced by high-fat diet[32].

In this study, after GGQLD administration, the AA content in plasma of rats in the administration group decreased to the normal group as compared with that in the model group, and its lipid-lowering effect may be one of the mechanisms affecting arachidonic acid metabolism.

Glycerophospholipid metabolism

Lysophosphatidylcholine is a kind of phosphatidylcholine which contains a fatty acid chain[33]. It mainly refers to lysophosphatidylcholine (LPC), also known as hemolytic lecithin, followed by lysophosphatidylcholine ethanolamine (LPE), which participates in glycerol phospholipid metabolism. LPC is formed by the hydrolysis of phosphatidylcholine by phospholipase A2 or by the hydrolysis of lecithin-cholesterol acyltransferase (LCAT), producing fatty acids such as arachidonic acid, which is closely related to inflammation. LPC plays a role in lipid signaling by acting on LPC receptors, which are members of the G protein-coupled receptor family and participate in many kinds of cell-to-cell signaling. LPC is closely related to metabolic diseases such as dyslipidemia, diabetes mellitus and cardiovascular diseases[34].

Studies have shown that LPC is the core component of oxidized low density lipoprotein, which can change endothelial cell permeability and damage endothelial cells[35]. Many studies have reported a significant increase in plasma LPC in obesity or T2DM[36–38]. Barber[37] and other studies showed that the plasma LPC level significantly decreased after high-fat induction in rats. Regression analysis confirmed that part of LPC was related to IR and that diet and obesity were the main factors affecting blood LPC. LPC is not only involved in cell proliferation, tumor cell invasion and inflammation, but also in glucose metabolism. Yea et al. [39] showed that LPC (16:0) and LPC (14:0) could stimulate the glucose uptake of 3T3-L1 adipocytes and significantly reduce the blood sugar level of T2DM mice. In this study, after GGQLD administration, the plasma lysophosphatidylcholine level of rats in the administration group was reversed from that of the model group to that of the normal group, possibly through regulating the metabolism of glycerophospholipids to play its hypoglycemic and lipid-lowering role. Its specific mechanism of action and the diverse biological functions of lysophosphatidylcholine deserve further study.

Tryptophan metabolism

Indoleacetaldehyde (IAALD) belongs to indole derivatives and participates in tryptophan metabolism. Tryptophan (TRP) in humans and animals is mainly brought in by diet, mainly metabolized by kynurenine (KYN), and small amounts of which were metabolized through 5-hydroxytryptamine and indole-retaining pathway.
In this study, IAALD production was significantly reduced in the plasma of model group rats, suggesting that KYN pathway metabolized by indole 2, 3-dioxygenase (IDO) or tryptophan 2, 3-dioxygenase (TDO) increased, which is related to inflammation and cardiovascular disease. IDO was positively correlated with age, body mass index and negatively correlated with HDL-C. Studies have shown that blood TRP, KYN and KYN/TRP ratios are associated with obesity.

The abnormal blood lipid and obesity of organisms make it in a chronic low-grade inflammatory state. Proinflammatory cytokines induce the increase of IDO expression, enhance the decomposition of TRP, and increase the production of the toxic metabolites in the KYN pathway. Recently, a prospective study analyzed the correlation between tryptophan metabolism and T2DM, indicating that TRP and its metabolites increased significantly in the early stage of T2DM and decreased in the complete state of T2DM, predicting IR and assessing the risk of T2DM.

In addition, as an essential amino acid, the metabolism of TRP is closely related to intestinal microflora. In the intestine, intestinal microorganisms can convert dietary tryptophan into indole and indole derivatives, including indole propionic acid, indole acetic acid, indole acetaldehyde, etc. The changes of indole derivatives in vivo after high-fat diet suggest the imbalance of intestinal microflora. The main components of GGQLD have the anti-inflammatory effect, they can increase the contents of TRP metabolites to a certain extent. GGQLD may play a role in regulating TRP metabolism and intestinal homeostasis by inhibiting the activation of IDO.

Metabolism of arachidonic acid ethanolamine

Endogenous cannabinoid system (ECS) includes two cannabinoid receptors: CB1 and CB2. Two endogenous ligands, arachidonic ethanolamine (AEA) and arachidonic glycerol (2-AG), are also known as endocannabinoids. AEA mainly binds to CB1 receptor and acts as a partial agonist. The content level of AEA in blood can reflect the change of ECS in vivo to a certain degree. ECS participates in the regulation of food intake, lipid metabolism and energy metabolism, and plays a direct role in adipogenesis. In the pathological model induced by high-fat diet, ECS is over-activated, the expression of endogenous cannabinoid is increased, the biosynthesis of fatty acids and triglycerides in liver is induced, and the differentiation and maturation of preadipocytes are promoted. Adipocytes inhibit fat breakdown and promote obesity. The results showed that the expression of CB1 in peripheral tissues increased in obese groups and animal models induced by high fat diet, which eventually led to the disorder of glucose and lipid metabolism. The level of TC and TG could be effectively reduced by giving CB1 antagonists to diet-fattening mice. In this experiment, after 5 weeks of intervention with Gegen Qinlian Decoction, the appetite of rats decreased, the weight gain began to slow down, TC, TG and FPG decreased significantly, GGQLD could reduce the level of AEA in plasma of rats with dyslipidemia to a certain extent. It may inhibit appetite by inhibiting the expression of cannabinoid receptors in the central and peripheral nervous system, and accelerating the metabolism of peripheral fat, so as to improve the disorder of glycolipid metabolism.

Acyl carnitines
L-carnitine lipid is the key substance of lipid metabolism. Fatty acids combine with L-carnitine to form aliphatic carnitine, which passes through the mitochondrial membrane and enters the mitochondrial matrix under the mediation of aliphatic carnitine transferase, and then oxidizes and decomposes to release energy. L-carnitine can accelerate fat metabolism, improve heart function, reduce TC, TG, increase HDL-C and reduce body weight. If the fatty acid is not effectively oxidized by beta, it will cause accumulation of fatty acid, easily produce lipid toxicity and promote the development of inflammation. The increase of free fatty acids in rats with dyslipidemia and obesity requires more L-carnitine for effective beta oxidation. The decrease of L-carnitine may not be enough to compensate for the increase of free fatty acids by beta oxidation, leading to lipid metabolism disorder. Incomplete oxidation-derived acyl carnitine is significantly associated with metabolic diseases, such as long-chain acyl carnitine C18 in diet-induced obese rats. In this study, the plasma levels of L-octyl carnitine and long-chain acyl carnitine stearyl carnitine (C18:0) in the model group were significantly higher than those in the normal group, which was consistent with the changes of acyl carnitine metabolic profiles in patients with pre-T2DM[43]. Studies have shown that the increase of long-chain acyl carnitine in circulation can interfere with insulin signal transduction in cell membranes, which is related to the occurrence and development of insulin resistance, but the exact mechanism of action has not yet been elucidated[44]. Gegen Qinlian Decoction can regulate the level of acyl carnitine to a certain extent, which may promote the beta oxidation of fatty acids through many ways. Meanwhile, the specific contribution of acyl carnitine of various chain lengths to its biological activity deserves further study.

Sphingolipid and bile acid metabolism

In this experiment, the contents of sphingolipids and bile acids in plasma of rats in each group were significantly changed. Sphingolipids are an important component of cell membranes and participate in important physiological processes such as cell growth, differentiation and apoptosis[45]. In obese individuals, sphingolipids accumulate abnormally in tissues and cells, and circulatory levels increase abnormally. Increased sphingolipids can induce insulin resistance by interfering with signal transduction of insulin signaling pathway and promoting cell apoptosis. Bile acids are steroid acids mainly found in mammalian bile. Bile acid can regulate the digestion and absorption of intestinal and liver lipids, and its transformation is closely related to intestinal flora[46]. Bile acid involves key enzymes that balance cholesterol in vivo. Bile acid participates in glycolipid and energy metabolism[47] through different pathways. In this experiment, compared with the normal group, the increase of sphingolipid content in plasma and the decrease of bile acid content in the model group suggest that the obstacle of energy consumption, the accumulation of energy accelerate fat synthesis, and promote abnormal blood lipid and weight gain. Studying the changes of sphingolipid metabolism and bile acid levels in organisms has been widely applied to the study of metabolic diseases such as dyslipidemia, obesity and T2DM, and provides new ideas for preventing obesity and metabolic diseases related to obesity.

Conclusion
Metabolomic studies have shown that 60% fat-fed high-fat diet involves changes of substances in hemolytic phospholipids, sphingomyelin, bile acid, acyl carnitine, unsaturated long-chain fatty acids, saturated long-chain fatty acids. Gegen Qinlian Decoction has a significant lipid-lowering effect on dyslipidemia rats induced by high-fat diet. Its preventive mechanism is related to tryptophan metabolism, fatty acid biosynthesis, alpha-linolenic acid metabolism, arachidonic acid and glycerophospholipid metabolism pathway.

**Abbreviations**

GGQLD
Gegen Qinlian decoction; T2DM:Type 2 diabetes mellitus; UHPLC-Q-TOF-MS:ultra high performance liquid chromatography quadrupole time-of-flight mass spectrometry; TC:total cholesterol; TG:triglyceride, LDL-C:low density lipoprotein- cholesterol; HDL-C:high density lipoprotein- cholesterol; QC:quality control; ANOVA:one-way analysis of variance; PCA:principal component analysis; PLS-DA:partial least-squares-discriminate analysis; OPLS-DA:orthogonal to partial least-squares discriminate analysis; TIC:total ion chromatogram; VIP:variable importance in the projection; ALA:α-linolenic acid, EPA:eicosapentaenoic acid, DHA:docosahexaenoic acid; MetPA:metabolic pathway analysis.

**Declarations**

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Not applicable.

**Authors’ contributions**

ZX, YS and QZ designed the experiments. ZX and YS performed the experiments. BL and ZZ analyzed the data, while the other authors assisted in experiments, ZX, QZ and YS wrote the manuscript. QZ, YS, LJ and GX discussed the results and revised the final manuscript. All authors approved the final manuscript.

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**Data availability statement**

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.
Ethics statement

Animal studies were performed according to the Principles of Laboratory Animal Care (World Health Organization, Geneva, 1985)

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Table 3
Table 3 is not available in this version of the manuscript.

Figures
Figure 1

TIC of plasma sample in positive scan mode

Figure 2

PCA chart of normal group (C), model group (M), and Adminstrated group (M)
Figure 3

PLS-DA scores of normal group (C), model group (M), and Administrated group (M)

Intercepts: $R^2 = (0.0, 0.827)$, $Q^2 = (0.0, -0.244)$

Figure 4

Model verification diagram of three groups
Figure 5

OPLS-DA score map of normal group (C) and model group (M)

Intercepts: $R^2 = (0.0, 0.899), Q^2 = (0.0, -0.262)$

Figure 6

Model verification diagram of normal group (C) and model group (M)
Figure 7

Variable screening chart
Figure 8

The contents of 16 endogenous compounds in plasma of rats in the control group, model group and administration group. Note: vs. normal group, #P<0.05; ##P<0.01; vs. model group, *P<0.05; **P<0.01
Figure 9

Biomarkers enrichment analysis

Figure 10

Biomarkers metabolic pathway map

Supplementary Files
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