Diabetes, Metabolic Syndrome and Obesity

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ORIGINAL RESEARCH

Metabolomics Analysis of the Mechanism of Yuquan Capsules in the Treatment of T2DM Rats Using UPLC/MS

Mengli Zhang[1,*], Zhien Tan[1], Min Ou[1], Taiping Li[1,2], Xiaonan Yang[1], Lu Chen[1], Rui Xiao[3], Na Lu[1], Xijun Wang[2], Fangfang Wu[1,3,*]

[1]National Engineering Research Center for Southwest Endangered Medicinal Materials Resources Development, Guangxi Botanical Garden of Medicinal Plants, Nanning, People's Republic of China; [2]National Chinmedomics Research Center, National TCM Key Laboratory of Serum Pharmacochemistry, Chinmedomics Research Center of State Administration of TCM, Laboratory of Metabolomics, Department of Pharmaceutical Analysis, Heilongjiang University of Chinese Medicine, Harbin, People's Republic of China; [3]Engineering Research Center of Innovative Traditional Chinese, Zhuang and Yao Materia Medica, Ministry of Education, Guangxi University of Chinese Medicine, Nanning, People's Republic of China

*These authors contributed equally to this work

Correspondence: Fangfang Wu; Xiaonan Yang, Email wfftn@163.com; nannangood33@163.com

Purpose: Yuquan capsule (YQC) is a well-known proprietary Chinese medicine used for the treatment of type 2 diabetes mellitus. The aim of this study was to investigate the potential mechanism and efficacy of YQC in the treatment of T2DM by means of metabolomics.

Methods: Thirty-two male SD rats were randomly divided into four groups of control, type 2 diabetic mellitus (T2DM), metformin (Met), and YQC. Establishment of the T2DM model by high-fat diet (HFD) and streptozotocin (STZ). Fasting blood glucose (FBG) and weight were measured weekly, urine output was collected and recorded. The blood, kidney, pancreas, and liver tissue samples were collected at the end of the experiment. Blood samples were analyzed with methods of ELISA, pancreas, and liver tissues were analyzed by pathological sections, and serum was analyzed by metabolomics using ultra-performance liquid chromatography quadrupole time-of-flight coupled with mass spectrometry (UPLC-Q/TOF-MS).

Results: It was observed that YQC could reduce blood glucose levels by modulating blood lipid and transaminase indices, and by diminishing the concentration of inflammatory factors within hepatic and pancreatic tissues. Furthermore, YQC restores homeostasis by regulating lipid and amino acid metabolism, engaging 21 biomarkers and 10 metabolic pathways.

Conclusion: YQC has the capacity to enhance blood lipid and transaminase levels, suppress the expression of inflammatory factors, and foster the homeostatic progression of metabolic circulation in rats with T2DM.

Keywords: type 2 diabetes mellitus, mechanism, lipid metabolism, blood lipid balance

Introduction

Type 2 diabetes mellitus (T2DM) is a metabolic disorder characterized by insulin resistance (IR) and pancreatic islet cell dysfunction.[1] According to the IDF and WHO, the global prevalence of diabetes in people aged 20–79 years was 10.5% in 2021. China is the country with the largest number of diabetic patients in the past 10 years (2011~2021), and the number of diabetic patients has increased from 90 million to 140 million in the same period, a 56% rise.[2,3] In 2022, the number of adults aged 18 and over with diabetes worldwide reached 828 million, an increase of more than four times from 200 million in 1990. Among them, China and India have the world's top two diabetes patients, with 148 million and 212 million respectively.[4] The increase in the number of people living with diabetes, especially among young people, has placed a huge burden on the family economy and the national health care system.[4] Metabolomics is an emerging systems biology technique for monitoring the dynamics of endogenous small molecule biomarkers in living organisms. It allows the dissection of the overall changes of metabolites in the blood, body fluids, feces, and tissues, and has been widely used in biomarker discovery, as well as the evaluation of drug efficacy and toxicity.[5,6]

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Traditional Chinese medicine (TCM) treatment has the characteristics of multi-ingredient, multi-targeted multi-pathway, and has unique advantages in the treatment of various diseases.[7] The multi-component of TCM can synergistically enhance its therapeutic effects while potentially reducing side effects. The multi-target treatment approach of TCM is often more effective than the single-target treatment of diseases, prompting researchers to make it a fundamental principle of research.[8] In addition, the multi-pathway aspect of TCM emphasizes the ability of these treatments to modulate multiple biological pathways simultaneously, which is particularly advantageous in addressing complex diseases characterized by multiple dysfunctional biological processes.[8–11] Yuquan capsules (YQC) have a long history of use in the treatment of diabetes in TCM. This preparation was inherited from the famous ancient prescriptions, which was a collection of Yuquan Pills (YQP) in "Wanbinghuichun" and "Shou Shi Bao Yuan" written by Gong Yanxian in the Ming Dynasty.[12] As a representative Chinese patent medicine, YQC has been approved by the China Food and Drug Administration (approval number: Z20090631) for the treatment of diabetes and its complications. YQC is comprised of ten herbs: *Trichosanthis Radix* (Tianhuafen), *Puerariae Lobatae Radix* (Gegen), *Astragali Radix* (Huangqi), *Ginseng Radix et Rhizoma* (Renshen), Mume Fructus (Wumei), *Glycyrrhizae Radix et Rhizoma* (Gancao), *Ophiopogonis Radix* (Maidong), *Poria* (Fuling), *Schisandrae Chinensis Fructus* (Wuweizi) and *Rehmanniae Radix* (Dihuang). A reliable and stable diabetic rat model induced by a high-fat -diet (HFD) combined with a low dose of STZ injection was used to mimic various physiological functions of T2DM in humans.[1] In this study, we applied an advanced UPLC-G2Si-HDMS instrument system and combined metabolomics strategies to explore the altered metabolic markers and pathways to determine the pathogenesis of T2DM. In this study, the data identified 21 endogenous differential metabolites and elaborated the intervention of YQC with the tricarboxylic acid cycle as the core to intervene in lipid metabolism and amino acid metabolism processes in T2DM. The protective effect of YQC on blood lipid balance and liver function in T2DM rats was also verified. In conclusion, our study identified key targets and pathways related to YQC in the treatment of T2DM using pharmacological and metabolomic experimental analysis.

Materials and Methods

Instruments and Reagents

An Acquity ultra high-performance liquid chromatograph and a Synapt™ G2Si High-definition mass spectrometer (HDMS) from Waters, USA were used in these experiments. A Hitachi 3100 automatic biochemical analyzer (Nanning Precision Instrument Co., Ltd.) was used to assess the biochemical indicators. Blood glucose test strips and an Accu-Chek Performa blood glucose meter were purchased from Roche (Roche, Germany).

HPLC-grade methanol and acetonitrile were purchased from Thermo Fisher Scientific (Massachusetts, USA). HPLC grade formic acid was from Merck (Darmstadt, Germany). The distilled water was purchased from Guangzhou Watsons Food & Beverage Co., Ltd (Guangzhou, China). The assay kits for Alanine aminotransferase (ALT, catalog number: 20220912), Aspartate aminotransferase (AST, catalog number: 20210912), Alkaline phosphatase (ALP, catalog number: 20210712), High-density lipoprotein (HDL, catalog number: 20210612), Low-density lipoprotein (LDL, catalog number: 20210412), Triglycerides (TG, catalog number: 2020121) and Total cholesterol (TC, catalog number: 20201212) were supplied by Kehua Bio-Engineering Co., Ltd. (Shanghai, China). The insulin assay kit (INS, catalog number: 2021070) was purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). 20% glucose solution was purchased from Beijing Coolpop Technology Co. (Beijing, China); Metformin hydrochloride extended-release tablets were purchased from Sino-American Shanghai Squibb Pharmaceuticals Ltd (ABR2339). Streptozotocin (STZ) was bought from Sigma Chemical (Sigma, USA), and a 45% high-fat diet (HFD, 12451M). The composition of the regular and HDL diets is shown in Supplementary Table 1. Trisodium citrate and citric acid were purchased from Boai Port Biotechnology Co., Ltd. (Beijing, China). YQC was procured from Increase Pharmaceutical (Yingkou) Co., Ltd. Before administration, 6 times the human clinical dose of YQC was dissolved in an appropriate amount of distilled water and mixed using ultrasound.

Animals

Experiments were performed with 8-week-old Sprague-Dawley (SD) rats weighing 180 ~ 200 g (Changsha Tianqin Biological Technology Co., Ltd., Permission No. SCXK (Hunan) 2022–0014, Changsha, China). The rats were housed under controlled conditions of 12/12 h light-dark cycles, at a relative humidity of 55–65%, and a temperature of 24 ± 2°C. They were provided

with water and their corresponding diet ad libitum. The animal welfare and experimental procedures complied with the regulations of the Guangxi Botanical Garden of Medicinal Plants (GXBGMP). The experiments were approved by the ethics committee of GXBGMP with a permit number of GXBGMP-2022010.

T2DM Rats' Model and Treatment

After one week of acclimatization, the animals were randomly divided into the following 4 groups with 8 rats in each group: control group (C); model group (M, 30 mg/kg); YQC group (YQC, 5.4 g/kg) and metformin group (Met, 200 mg/kg). The rats in the control group were fed with common pellet diets during the experiment, while the other three groups were fed with an HFD daily for 4 weeks. In weeks 5 and 6 of dietary intervention, rats in the experimental groups (M, YQC, and Met) were given intraperitoneal injections of 30 mg/kg STZ diluted in citrate buffer (0.1 M sodium citrate, pH = 4.5), while the control group was administered with the same dose of citrate buffer. The fasting blood glucose (FBG) of rats was measured from samples obtained from the tail vein, and when FBG ≥ 11.1 mmol/L, this was considered to be the model for T2DM.[13,14] From week 8 to week 10, rats in the C and M groups were administered saline (10 mL/kg), the YQC group received YQC (5.4 g/kg), and the Met group received metformin (200 mg/kg) by gavage. The dosages administered are converted from human doses.

Measurement of Body Weight, FBG, and Urine Output

During the experiment, the general condition of the rats was observed and recorded, including their feeding, drinking, mental state, hair condition, and activity. Body weight and FBG were measured weekly at weeks 8 and 10, rats were transferred to metabolic cages, fasted but allowed to drink water ad libitum, and urine output between 8 p.m. and 8 a.m. was collected and recorded.

HOMA-IR and Oral Glucose Tolerance Test (OGTT) Test

ELISA kit was used to detect fasting insulin levels in the serum of rats administered for 4 weeks. The homeostatic model assessment of insulin resistance (HOMA-IR) index was calculated by the following formula: HOMA-IR = [fasting blood insulin (mU/L) ×fasting blood glucose (mmol/L)] /22.5.[15] OGTT tests were performed on days 14 and 28 after dosing. After fasting for 12 h, the rats were given 20% glucose solution by gavage at a dose of 2 g/kg. Blood glucose levels were measured after 0, 0.5, 1, and 2 h, and the area under the OGTT curves were plotted and calculated.

Collection and Processing of Serum and Tissue Samples

After the last dose, the rats are anesthetized with 1% sodium pentobarbital until unconsciousness. Blood samples were collected from the abdominal aorta, and the samples were centrifuged after 30 min (at 4°C and 4000 rpm for 15 min), 3 volumes of methanol were quickly added to the supernatants and thoroughly mixed. The samples were centrifuged at 13000 rpm for 20 min, and the supernatant was dried by vacuum centrifugation at 40°C. After drying, the samples were reconstituted with 150 μL of methanol and centrifuged for UPLC-MS analysis. As a quality control (QC), 50 μL was taken from each sample, and the pre-treatment method was the same as above. Immediately after blood collection, the livers and kidneys were removed and weighed, and the pancreas and right lobe of the livers were removed and fixed in 4% paraformaldehyde solution for histopathological analysis (Guangxi Nanning Chenze Experimental Technology Co., Ltd.).

Measurement of Biochemical Indicators

The activities of ALT, AST, and ALP in rat serum were measured using commercial kits following the manufacturer's instructions to determine the effect of YQC on liver function. The concentrations of TC, TG, HDL, and LDL were also measured to evaluate the effect of YQC on lipid levels in T2DM rats.

Metabolomics Analysis

A UPLC-Q/TOF-MS system controlled by the Masslynx workstation (Waters, USA) was used for metabolomics analysis. The mobile phase consisted of solvent A (0.1% formic acid in water) and a mobile phase B (0.1% formic acid acetonitrile). The gradient elution conditions were as follows: 0–0.5 min, 2% B; 0.5–2.5 min, 40% B; 2.5–9 min, 75% B; 9–10 min, 100% B, 10–12 min 2% B. The chromatographic column used was a Waters

Acquity UPLC ® HSS T3 (2.1 mm × 100 mm, 1.8 μm) and the conditions were: column temperature, 30°C; sample room temperature, 10°C; flow rate, 0.4 mL/min; injection volume, 2 μL; capillary voltage, 2.2 kV; taper hole voltage, 40 V and ion source temperature, 105°C. The leucine-enkephalin, (ESI+: m/z = 556.2771, ESI−: m/z = 554.2615) was a locked mass solution at a concentration of 200 pg/mL and the mass scanning range was m/z = 50–1500 Da.

Statistical Analysis

Values are expressed as the means ± SD of 8 rats in each group. Statistical comparisons were performed with the SPSS 20.0 software (IBM Corp, Armonk, NY, USA) using one-way analysis of variance (ANOVA). A value of $p < 0.05$ was considered statistically significant. GraphPad Prism 8 was used to draw the graphs.

The collected data was imported into Progenesis QI software (Waters, USA)., and the software automatically performed peak identification, peak filtering, and peak alignment to screen compounds with a matching degree greater than 85%. Samples were grouped, extraction conditions, and retention times were set for peak extraction and unwinding. The Human Metabolome Database (HMDB) was selected for compound identification. After extraction and identification of the peaks, unsupervised principal component analysis (PCA) was performed on each group of data to analyze the differences between the groups due to the metabolic changes that occur in T2DM. Orthogonal partial least squares discriminant analysis (OPLS-DA) was performed to calculate the effects of the variables on the projection (VIP) values of T2DM rats. Unsupervised PCA was performed on each group of data using the nested Ezinfo 3.0 module, and a score plot reflecting the degree of clustering of each group of data was obtained. The data matrix containing mass-to-charge ratio, retention time, peak area, and differential compounds was derived, and 8667 compounds were obtained in positive ion mode and 9192 compounds in negative ion mode for further identification, and differential metabolite information was obtained, and the relative content of corresponding compounds was expressed by relative peak area. The exact mass values of potential markers were identified by using the human metabolome database. The metabolic pathways involved were constructed using MetaboAnalyst 3.0 (http://www.metaboanalyst.ca), which is a web-based metabolomics-based visualization tool.

Results

Effect of YQC on FBG and State

Normal rats have smooth, shiny and columnar formation in feces, and in normal drinking water, the urine color appeared light yellow and translucent; while diabetic rats show a debilitating state with coarse and lusterless coats, unresponsiveness, reduced activity, diarrhea, increased drinking water, urine color was clear and transparent and weight loss (Supplementary Figure 1). In weeks 2 and 4 (Figure 1A and B), FBG and urine volume were measured, and the FBG and urine volume in the T2DM group were significantly increased compared to the control group ($p < 0.01$). After 2 weeks of administration, the YQC and Met treatment groups significantly reduced FBG ($p < 0.01$). At weeks 2 and 4, the urine volume in the YQC and Met treatment groups was significantly reduced compared to the T2DM group ($p < 0.01$), with the YQC group returning to the level of the control group. The liver and kidney coefficients in the T2DM group were significantly higher than those in the control group (Figure 1C and D). Liver coefficient = (liver weight/body weight) × 100%. Kidney coefficient = (kidney weight/body weight) × 100%. The liver coefficients in the YQC group and the Met group were significantly lower than those in the T2DM group ($p < 0.05$). However, the renal coefficient was not statistically significant compared with the T2DM group.

The Effects of YQC on HOMA-IR and OGTT in T2DM Rats

Relative to the model group, YQC also attenuates symptoms of insulin resistance (HOMA-IR) in T2DM (Figure 2). It can be seen that the HOMA-IR Index of the YQC and Met groups was lower than that of the model group. However, there was no significant difference. We conducted an oral glucose tolerance test (OGTT) trial to verify that the capsules had a stabilizing effect in the treatment of T2DM (Figure 2). At weeks 2 and 4, the OGTT-AUC was significantly higher

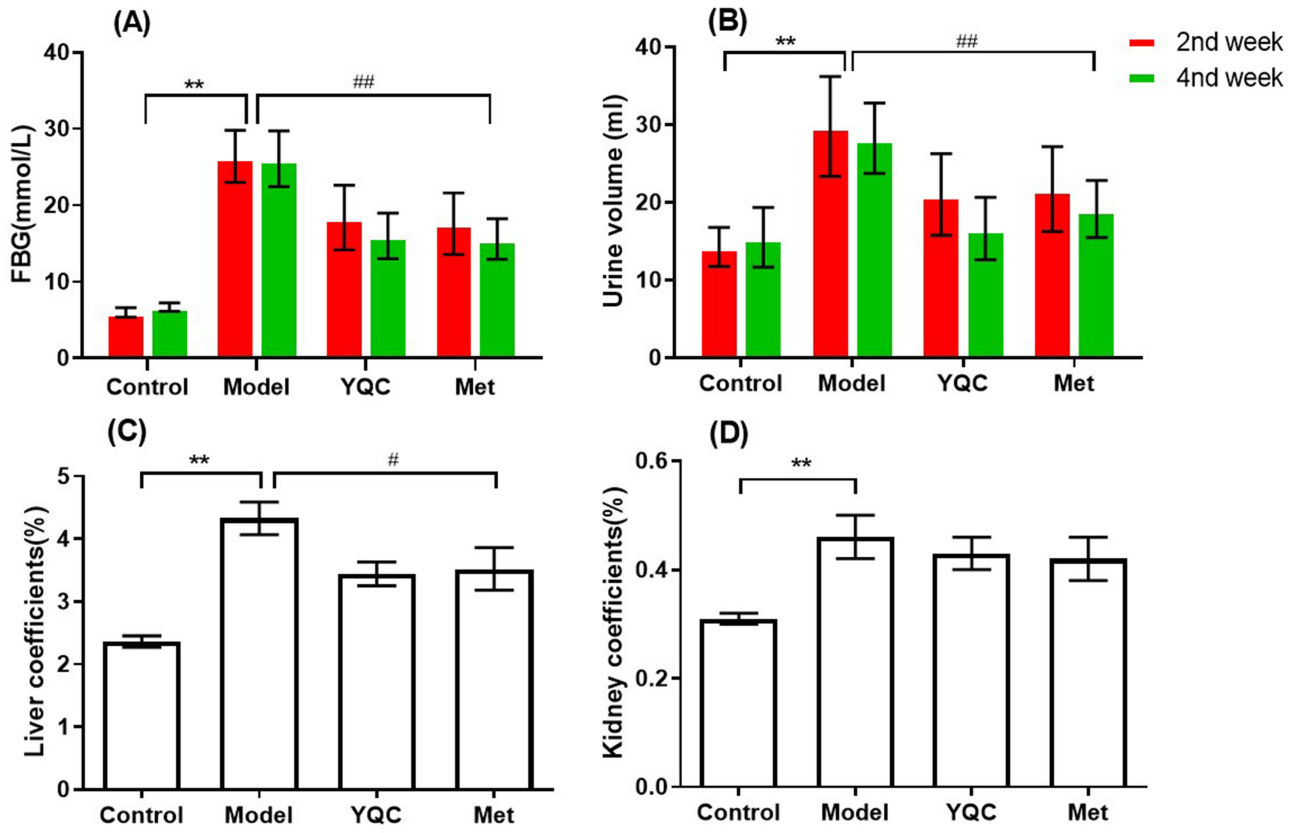

Figure 1 The effects of treatment with YQC on FBG levels and urine volume as well as on the liver and kidney coefficients in T2DM rats. (**A**) FBG (mmol/L); (**B**) Urine volume (mL); (**C**) liver coefficients (%); (**D**) kidney coefficients (%). **p < 0.01 vs control. # p < 0.05, ## p < 0.01 vs model.

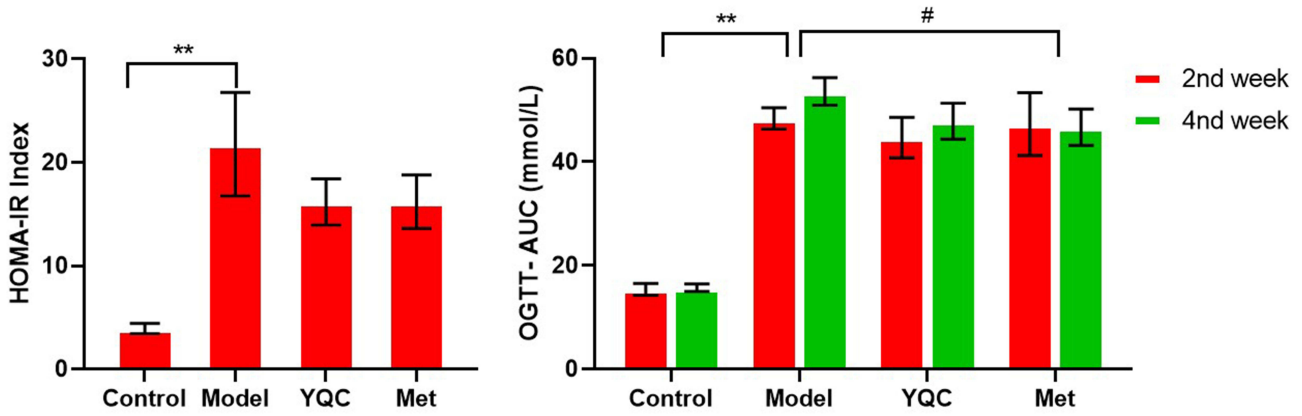

Figure 2 Effect of YQC on insulin resistance index and OGTT-AUC in T2DM. **p < 0.01vs Control; # p < 0.05 vs Model.

in the T2DM group when compared with the control group. The OGTT-AUC was significantly lower in the YQC and Met groups compared to the T2DM group.

Analysis of the Protective Effect of YQC on the Pancreas and Liver in T2DM

The effect of YQC on the biochemical indices was measured in serum (Figure 3). Compared to the control group, the ALT, AST, and ALP were significantly elevated in the T2DM group (p <0.01). ALT, AST, and ALP are important indicators of liver function. TC, TG and LDL levels were significantly higher in T2DM compared to the control group,

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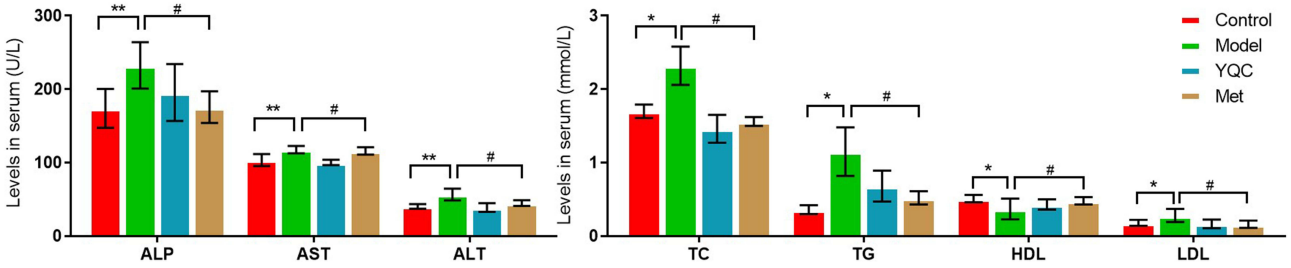

Figure 3 Analysis of the biochemical indexes with regards to the therapeutic effects of YQC on T2DM. *p < 0.05, **p < 0.01vs Control; # p < 0.05vs Model.

and there was a reduction in HDL ($p < 0.01$). The results showed that YQC could improve dyslipidemia and liver function impairment in T2DM.[16,17] The results of liver H&E staining of rats in each group are shown in Figure 4A–C. The hepatocytes in the C group were closely arranged and structurally intact, and Kupffer cells (KCs) were scattered among them. However, the hepatocytes in the M group were structurally disorganized, with obvious hepatocyte necrosis and fibrosis, the nuclei were fragmented and appeared to be lysed. The central lobular and interlobular veins were filled with inflammatory factors, and the number of KCs was reduced and stacked up around the central veins. The YQC group had significantly alleviated features such as liver tissue fibrosis and necrosis, with significantly reduced inflammatory factors. In Figure 4D–F, the islets in the control group had intact morphology with the boundaries of the exocrine glands appearing intact and clear, and the cells appeared homogeneous. Compared with the control group, the islets in the T2DM group had irregular morphology, with blurred boundaries between the islets and exocrine glands. The islet cells also had reduced cytoplasm and some showed a degeneration with vacuolation. After YQC treatment, there was a marked improvement in the morphology of islets, with increased cytoplasm in the cells and a reduction in vacuolation.

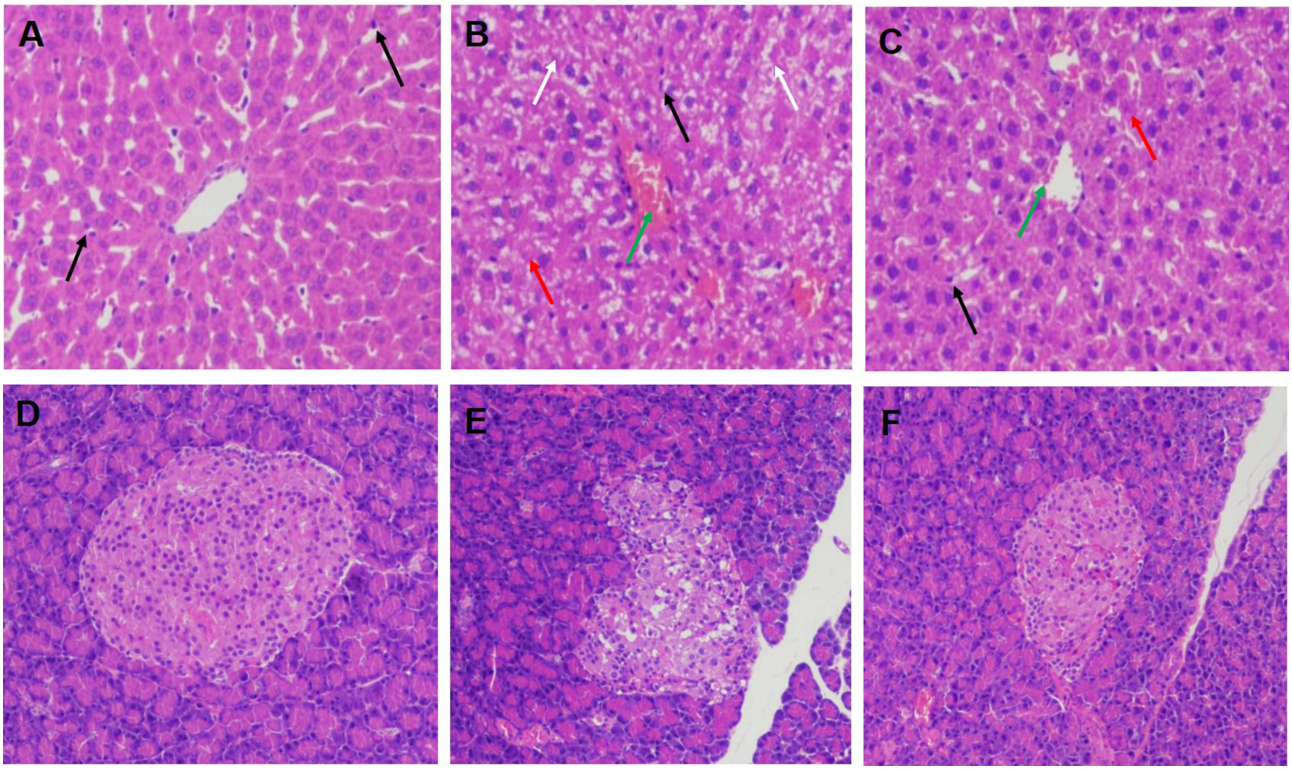

Figure 4 Histopathological analysis of the liver and pancreas in Control, Model, and YQC groups (×100). (**A–C**) liver; (**D–F**) pancreas. Black arrows: Kupffer cells; green arrows: inflammatory factors; red arrows: hepatocyte necrosis; white arrows: Hepatocellular fibrosis.

Multivariate Statistical Analysis

The serum data were collected using UPLC-G2Si-HMDS and analyzed using Progenesis QI software (Supplementary Figure 2). The OPLS-DA score plots showed a clear clustering of the presence of C and M in the sample sera (Figure 5A and B). The OPLS-DA substitution test showed that the values of R2X, R2Y, and Q2 for T2DM and control sera were 0.503, 0.914, 0.77 and 0.77, 0.994, 0.915, respectively, with each value greater than 0.5, indicating that the proposed model is reliable (Figure 5C and D). An unsupervised method of multivariate analysis showed well-separated control and T2DM groups in both the positive and negative ion modes. To determine whether YQC affected the metabolic pattern of T2DM rats, a PCA model was used to assess the changes between the C, M, and YQC groups (Figure 5E and F). The improvement of T2DM by YQC may be due to the modulation of T2DM metabolism by these capsules. Using nested Ezinfo 3.0 module software, score plots of VIP > 1 was screened out between the control group and the T2DM group (Figure 5G and H).

Identification of Potential Biomarkers and Pathway Analysis

The ions with significant intensity $p < 0.05$ and VIP > 1 between the control group and the T2DM group were selected as differential ions for fragment analysis to identify important candidate metabolites (Table 1) (the mass

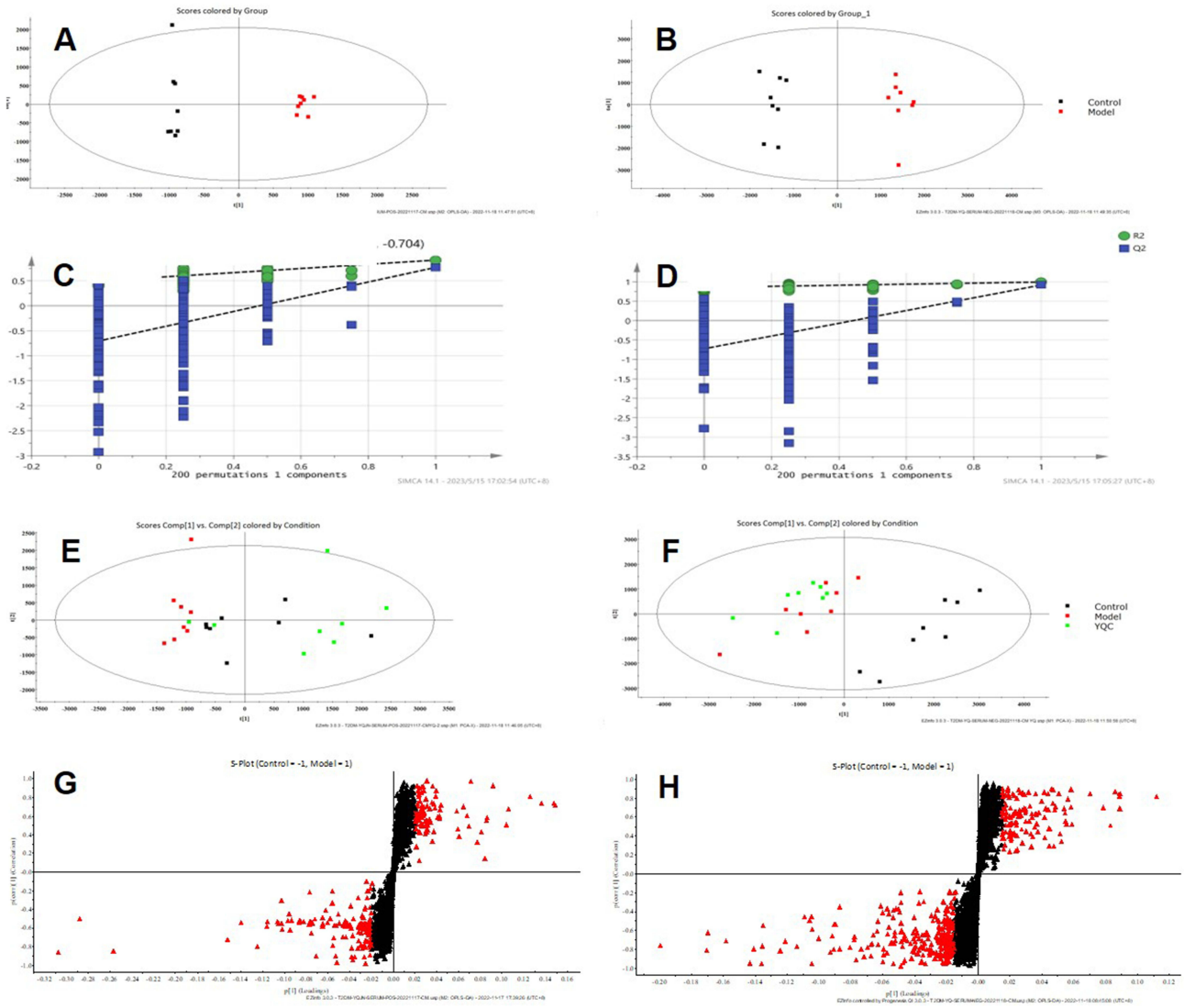

Figure 5 OPLS-DA score plots of rat blood in the Control and Model groups. The permutation test for OPLS-DA. PCA score plots of rat blood samples in the Control and Model groups and rats treated with YQC. VIP scatter plots of rat blood in the Control and Model groups. (**A, C, E** and **G**) Positive ion pattern; (**B, D, F** and **H**) Negative ion pattern.

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Table 1 Effects of YQC on Serum Metabolic Markers of T2DM

NO.	Compounds	Formula	Mass Error (ppm)	VIP	M vs C	YQC vs M
1	12-Hydroxydodecanoic acid	C12H24O3	−3.13	1.13	↓*	↑#
2	Ubiquinone-2	C19H26O4	−0.47	1.08	↑**	↓
3	Hexyl glucoside	C12H24O6	−1.56	1.32	↓*	↑#
4	Presqualene diphosphate	C30H52O7P2	4.60	2.93	↑**	↓#
5	Phenylpyruvic acid	C9H8O3	−1.01	1.08	↑**	↓#
6	Cinncassiol D4 2-glucoside	C26H42O10	2.24	1.11	↑*	↓
7	Chenodeoxycholic acid	C24H40O4	−1.91	8.43	↑**	↓##
8	Tetrahydrocortisol	C21H34O5	−3.67	1.28	↑*	↓#
9	3a,7a,12b-Trihydroxy-5b-cholanoic acid	C24H40O5	−3.170	4.49	↑**	↓#
10	Arachidonic acid	C20H32O2	−2.60	1.65	↑*	↓#
11	Valerenic acid	C15H22O2	−5.30	1.62	↑**	↓#
12	Palmitic acid	C16H32O2	−3.600	1.22	↑**	↓#
13	Ricinoleic acid	C18H34O3	−3.768	1.14	↓**	↑
14	7-Ketodeoxycholic acid	C24H38O5	−3.41	2.43	↑*	↓#
15	LysoPC(O-18:0/0:0)	C26H56NO6P	1.48	1.04	↓**	↑#
16	Furanodiene	C15H20O	9.79	1.13	↓*	↑
17	12S-HHT	C17H28O3	0.45	1.46	↑*	↓#
18	Glucose-1,3-mannose oligosaccharide	C12H22O11	−2.05	1.32	↑*	↓
19	Beta-D-Galactose	C6H12O6	−1.37	1.08	↑*	↓#
20	LysoPC (18:1/0:0)	C26H52NO7P	−0.89	2.41	↓**	↑#
21	N-Acetylleucine	C8H15NO3	−7.69	1.38	↑**	↓#

Notes: *$p < 0.05$, **$p < 0.01$ vs Control; #$p < 0.05$, ##$p < 0.01$ vs Model (↑refers up-regulation and ↓refers down-regulation).

tolerance was ± 10 ppm). Compared to the model group, the trend of metabolic markers after administration was observed. The results showed that 16 out of the 21 metabolic markers associated with T2DM were significantly different, with 4 up-regulated and 12 down-regulated. The identified metabolites were: 12-hydroxydodecanoic acid, hexyl glucoside, presqualene diphosphate, phenylpyruvic acid, chenodeoxycholic acid, tetrahydrocortisol, 3a,7a,12b-trihydroxy-5b-cholanoic acid, arachidonic acid, valerenic acid, palmitic acid, 7-ketodeoxycholic acid, lysopc(O-18:0/0:0), 12S-HHT, beta-d-galactose, lysopc (18:1/0:0) and n-acetylleucine.

21 target metabolites were uploaded to METPA (https://www.metaboanalyst.ca/) for online pathway analysis, which was related to 10 metabolic pathways. These included the biosynthesis of unsaturated fatty acids, phenylalanine, tyrosine and tryptophan biosynthesis, phenylalanine metabolism, ether lipid metabolism, glycerophospholipid metabolism, fatty acid elongation and degradation, steroid biosynthesis, arachidonic acid metabolism, primary bile acid biosynthesis and fatty acid biosynthesis, mainly lipid and amino acid metabolites (Table 2). Among these pathways, phenylalanine metabolism, ether lipid metabolism, glycerophospholipid metabolism, steroid biosynthesis, arachidonic acid metabolism, fatty acid biosynthesis was found to have greater effects on metabolic disorders such as polyphagia, polydipsia and polyuria in T2DM. Serum metabolic pathway analysis

Table 2 Specific Information on the Metabolism Pathway with 21 Potential Biomarkers Relevant to T2DM

NO.	Pathway	Class	Biomarkers	Total	Expected	Hits	Raw p	FDR	Impact
1	Biosynthesis of unsaturated fatty acids	Lipid metabolism	Palmitic acid; Arachidonic acid	36	0.1645	2	0.0105	0.7268	0
2	Phenylalanine, tyrosine and tryptophan biosynthesis	Amino acid metabolism	Phenylpyruvic acid	4	0.0157	1	0.0156	0.7268	0
3	Phenylalanine metabolism	Amino acid metabolism	Phenylpyruvic acid	10	0.0457	1	0.0449	1	0.2619
4	Ether lipid metabolism	Lipid metabolism	LysoPC(O-18:0/0:0)	20	0.0914	1	0.0880	1	0.0818
5	Glycerophospholipid metabolism	Lipid metabolism	LysoPC (18:1/0:0)	36	0.1655	1	0.1536	1	0.0174
6	Fatty acid elongation and degradation	Lipid metabolism	Palmitic acid	39	0.1782	1	0.1655	1	0
7	Steroid biosynthesis	Lipid metabolism	Presqualene diphosphate	41	0.1873	1	0.1733	1	0.0145
8	Arachidonic acid metabolism	Lipid metabolism	Arachidonic acid	44	0.2010	1	0.1849	1	0.2893
9	Primary bile acid biosynthesis	Lipid metabolism	Chenodeoxycholic acid	46	0.2102	1	0.1925	1	0
10	Fatty acid biosynthesis	Lipid metabolism	Palmitic acid	47	0.2148	1	0.1963	1	0.0147

Notes: Total is the number of metabolites in the metabolic pathway; Hits is the number of differential metabolite hits in the pathway; Raw P represents the P value of pathway enrichment analysis; P-value corrected by Holm-Bonferroni method; FDR is the P-value corrected by false discovery rate method; Impact is the Impact value of metabolic pathway topology analysis.

results and metabolic pathways associated with all potential biomarkers in KEGG in Figure 6. By analyzing the correlation between the pathways, we integrated 10 pathways, with a total of 7 core associated metabolites, centered on acetyl-coA, to form a homeostatic regulatory cycle of lipid metabolism and amino acid metabolism.

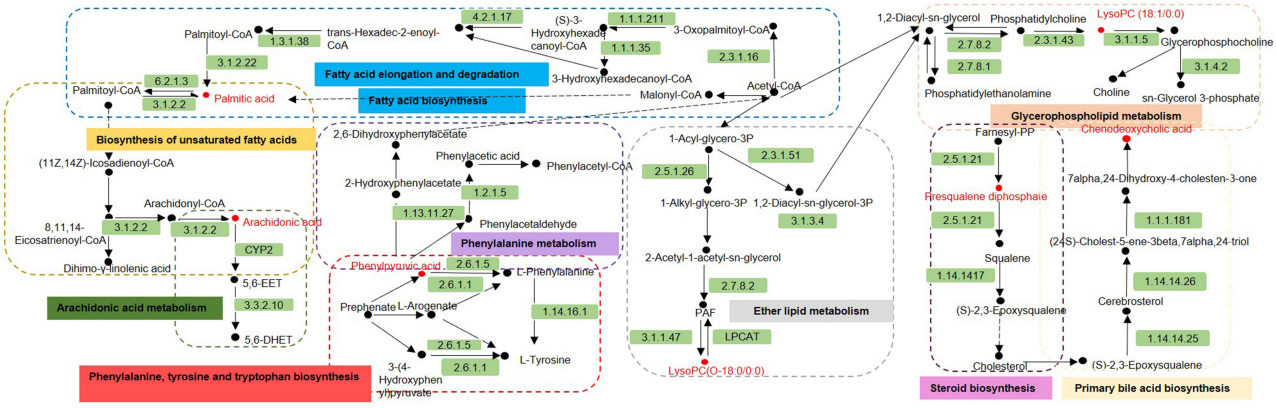

Figure 6 Metabolic pathways associated with all potential biomarkers in KEGG. The red color represents the biomarkers detected in our study.

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Discussion

YQC had a significant effect on the metabolism of phenylalanine metabolism and arachidonic acid (AA) metabolism as the key pathways (IF > 0.1). In our earlier study, we detected a total of 32 components of Yuquan capsule into the blood, including 17 prototypical components and 15 metabolized components, of which 12 were prototypical, but the relationship with the metabolized components of T2DM needs to be analyzed in further studies.[18] These two pathways were regulated by phenylpyruvic acid and AA. AA had the greatest effect on the lipid metabolic pathway in diabetic rats. It is a ω-6 polyunsaturated fatty acids that mainly exists on the cell membrane in the form of phospholipids and plays an important biological role in the liver.[19] AA is believed to have anti-inflammatory effects and regulate liver homeostasis due to abnormal glucose and lipid metabolism in the liver.[20] AA is also an important inflammatory lipid mediator that regulates oxidative stress in hepatocyte mitochondria, leading to massive fatty acid oxidation through three metabolic pathways involving cyclooxygenase, lipoxygenase and cytochrome P450. These metabolic changes can exacerbate hepatocyte damage and activation, ultimately accelerate injury to the liver.[21,22] AA content in the blood of the T2DM was significantly higher than that of the control group.[23] It indicates abnormal AA metabolism, and disruption of its metabolism pathway. A large number of inflammatory cell infiltration can be found in the pathological tissue section, further supporting the abnormal AA metabolism. After YQC treatment, the content of AA in the blood decreased significantly, tending towards the levels in the control group. This proves that YQC has a significant effect in the treatment of inflammation. Combined with biochemical and histopathological data, YQC can effectively inhibit weight loss by reducing urine output in rats. It also increases the level of HDL in the serum of diabetic rats, while reducing AST, ALP, ALT, TC, TG and LDL levels. Additionally, YQC reduces inflammatory factors in the liver and pancreatic tissue, enables cholesterol transport to the liver, accelerates lipid degradation in the liver, leading to a comprehensive decrease in lipid levels.[24]

The liver is the main site of phenylalanine metabolism. When the liver is diseased, phenylalanine metabolism is disordered, resulting in abnormal tyrosine metabolism. As some hepatocytes are damaged, substances within them, including aspartate aminotransferase, are released into the bloodstream, leading to the clinical manifestation of phenylketonuria (PKU).[25,26] We have innovatively discovered that amino acid metabolism plays a major role in T2DM rats. Phenylpyruvic acid is converted to l-phenylalanine under the catalysis of tyrosine aminotransferase and glutamate-oxaloacetate aminotransferase 1, and is involved in phenylalanine metabolism and biosynthesis and metabolism of phenylalanine, tyrosine, and tryptophan. Additionally, pyruvic acid, as a metabolite of phenyl pyruvic acid, is fermented by the gut microbiota into succinic acid, lactic acid, and acetyl-CoA, which are further metabolized into Short-chain fatty acid (SCFAs).[27] Acetyl-CoA connects the biosynthesis of unsaturated fatty acids, phenylalanine, tyrosine and tryptophan, valine, leucine and isoleucine, as well as phenylalanine metabolism, arachidonic acid metabolism, fatty acid elongation, degradation and biosynthesis, and aminoacyl-tRNA biosynthesis. This affects changes in glycolysis/gluconeogenesis and pyruvate metabolism levels. In recent studies, phenylalanine directly inhibits intracellular insulin signaling pathway transduction and glucose absorption.[28,29] We found that rats with diabetes mellitus had elevated phenylpyruvic acid levels and disorders of energy metabolism. After YQC treatment, phenylpyruvic acid levels returned to normal. As an alternative biochemical response of cells under nutrient overload, it provides a protective mechanism to inhibit excessive glucose uptake when intracellular amino acids are abundant. This implies that dysregulated amino acid metabolism is also a pathogenic risk factor for T2DM, providing new targets and strategies for intervening in the course of type 2 diabetes mellitus.[30,31] There is no YQC-related toxicology literature yet. Palmitic acid, one of the most common saturated fatty acids found in animals, plants, and microorganisms. Excess carbohydrates in the body are converted to palmitic acid. Studies have shown that elevated levels of palmitic acid in the blood of patients with T2DM can enhance plaque instability by activating macrophages and subsequent vascular smooth muscle cell (VSMC) senescence, mainly involves the TLR4/ERK/FOXC2 pathway.[32]

The increase in the amount of presqualene diphosphate in T2DM leads to an increase in cholesterol content. High cholesterol levels have been clinically shown to be a criterion for diagnosing early T2DM.[13] Cholesterol is connected to steroid biosynthesis, primary bile acid biosynthesis and steroid hormone biosynthesis metabolic pathways.[33,34] After YQC treatment, the content of presqualene diphosphate in the serum of T2DM rats decreased, which further regulated the primary bile acid biosynthesis and steroid hormone biosynthesis metabolic pathways by affecting the steroid biosynthesis metabolic pathway. Lysophosphatidylcholines (LysoPCs) are an important class of components in biological membranes

and provide most of the membrane lipids in cells. LysoPC (18:1(9Z)), LysoPC (O-18:0/0:0), and LysoPC (18:1/0:0) are all LysoPCs, and they were found to be the most abundant glycerophospholipids in mammals. Lower levels of LysoPC were found to be potential predictors of T2DM.[35,36] The results of this experiment showed that the content of LysoPC in the T2DM rats was significantly reduced, indicating that the abnormal expression of lysophosphatidylcholine acyltransferase (LPCAT) occurred during the cell proliferation of T2DM rats due to the hypermetabolic state, resulting in a decrease in LysoPC content.[37]

Conclusion

In this study, UPLC-Q/TOF-MS was used to explore the metabolism of T2DM rat sera after treatment with YQC. We investigated the effects and actions of YQC on T2DM using multivariate statistical analysis combined with biochemical measurements. The results showed that YQC inhibited hyperglycemia in T2DM rats by decreasing cholesterol levels. The accelerated lipid degradation led to an overall decrease in lipid levels, which reduced inflammatory factors in liver and pancreatic tissues. Additionally, YQC modulated amino acid metabolism and lipid metabolism pathways in the serum of T2DM rats by regulating the expression of metabolic potential biomarkers. We found that amino acid metabolism and AA metabolism play important roles in T2DM, and phenylpyruvic acid and AA may be the markers of these two metabolic pathways, which may provide new ideas for the treatment of this disease. The pharmacodynamic and mechanistic approaches used suggest that the treatment of T2DM with YQC primarily acts through reducing inflammatory factors and modulating amino acid mechanisms.

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Disclosure

The authors report no conflicts of interest in this work.

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