Food & Function

ROYAL SOCIETY OF CHEMISTRY

PAPER

View Article Online
View Journal

Cite this: DOI: 10.1039/d5fo03089g

Multi-omics strategies combined with molecular docking to explore the regulatory effects of licochalcone A on glycolipid metabolism in db/db mice

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Licochalcone A (LicA) is a flavonoid compound extracted from licorice. LicA has the function of regulating blood sugar and has been proven to be related to the intestinal flora, but its mechanism remains unclear. This study investigated the effect of LicA on improving insulin resistance and glycolipid metabolism in type 2 diabetic mellitus (T2DM) mice. A T2DM model was established using db/db mice. We found that LicA could effectively reduce insulin resistance and fat deposition in T2DM mice, alleviate liver inflammation, improve the integrity of the intestinal barrier, and reduce endotoxins in the circulation. Further studies on 16S rRNA have shown that LicA regulates the composition of the intestinal flora in T2DM mice and reduces the abundance of the genus *p-75-a5*. The combined analysis of liver metabolomics and liver proteomics indicated that LicA altered the intestinal microbiota metabolite hypotaurine through p-75-a5, and hypotaurine played a bridging role between p-75-a5 and glycolipid metabolism in T2DM. Through multi-omics joint analysis and WB experimental verification, the results showed that LicA activates the PPAR signaling pathway to improve glucose lipid metabolism disorders in T2DM. More importantly, after treating db/db mice with antibiotics, the beneficial effects of LicA were blocked, indicating that the gut microbiota plays a key role in LicA's improvement of glucose and lipid metabolism in T2DM. In conclusion, this study revealed a new mechanism by which LicA improves T2DM glucose and lipid metabolism disorder, and the potential improvement of LicA's glucose and lipid metabolism in T2DM may be related to the change of p-75-a5 targeting hypotaurine.

Received 21st July 2025,
Accepted 21st October 2025

DOI: 10.1039/d5fo03089g

rsc.li/food-function

Introduction

Type 2 diabetes mellitus (T2DM) is a metabolic disease characterized by insulin resistance and imbalance of blood glucose homeostasis. It is often accompanied by disorders of liver glucose and lipid metabolism, manifested as reduced liver glycogen synthesis, hypergluconeogenesis and lipid accumulation, which further aggravate insulin resistance and disease progression.[1–3] With the global obesity rate rising and lifestyle changes, the incidence of T2DM has been increasing year by year, becoming a major public health problem threatening human health.[4,5] The liver is the core organ for glucose and lipid metabolism, and its abnormal metabolic function plays a key role in the occurrence and development of T2DM.[6,7] Therefore, exploring natural active ingredients targeting liver glycolipid metabolism has significant clinical significance.

Licorice is one of the earliest discovered and most popular herbs since ancient times. Licorice is widely used in food, pharmaceutical products, health supplements and cosmetics due to its safety and effectiveness.[8] Licorice is believed in traditional Chinese medicine to help regulate blood sugar and relieve the symptoms of people with diabetes.[9] Licorice is used as a sweetener in cookies, candies, and condiments.[10] Licorice or licorice extract can be added to beer for emulsification and foaming, regulating the color and concentration of beer.[11] Its safety has been recognized by the U.S. Food and Drug Administration and it is considered a safe food; it is also considered a health food in the Food Hygiene Law of the State Food and Drug Administration of China.[12] Licochalcone A (LicA), as one of the important components of licorice, has strong anti-inflammatory, blood sugar and lipid regulation effects, and can be used as a food in daily diet.[13] Recent studies have found that LicA can regulate metabolic disorders by regulating the structure of the intestinal microbiota, inhi-

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biting adipocyte differentiation, and improving liver lipid deposition. However, the specific regulatory mechanism of LicA on liver glucose and lipid metabolism under T2DM, especially its role in the microbiota–host metabolic interaction and key molecular targets, remains unclear.

Our in-depth study found that LicA has a good effect on T2DM, and can regulate intestinal flora disorder and reduce intestinal opportunistic pathogens.[14] However, further investigation is needed to determine whether LicA can target opportunistic pathogens to protect T2DM mice. Moreover, it is also unknown whether LicA participates in the glycolipid metabolism process through microbial metabolites. More experiments are needed to find out about these scientific questions. Multi-omics strategies (such as metabolomics, gut microbiota analysis, and proteomics) can systematically analyze the complex metabolic regulatory networks within organisms by integrating multi-level biological information, providing a panoramic perspective for revealing the mechanism of action of natural products.[15–17] Meanwhile, molecular docking technology can verify the binding specificity of key targets at the atomic level by simulating the interaction between small molecules and target proteins, providing direct evidence for mechanism analysis.[18,19]

This study intends to take db/db mice as the research subjects. Combining the multi-omics techniques of metabolomics, 16S rRNA analysis and proteomics, the relevant indicators of liver glucose and lipid metabolism in mice after LicA intervention, the intestinal mucosal barrier function and serum inflammatory factors of mice, the histopathology of the liver, pancreas and intestine, the changes of the intestinal flora structure and the metabolite spectrum were systematically analyzed, and the key regulatory targets and flora metabolites were screened. The interference of antibiotics verified that the intestinal flora plays a key role in the beneficial effect of LicA. Furthermore, the interaction between key microbiota metabolites and target proteins was verified through molecular docking technology, revealing the molecular mechanism by which LicA regulates liver glycolipid metabolism by targeting the microbiota metabolite–host molecular axis, providing the experimental basis and theoretical support for the application of LicA in the prevention and treatment of T2DM.

Materials and methods

Materials and reagents

LicA (purity ≥ 98%) was purchased from Chengdu Biochem Pure Biotechnology Technology Co., Ltd. Total cholesterol (TC), triglyceride (TG), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C) detection kits, serum insulin (FINS), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were supplied by Nanjing Jiancheng Biotechnology Co., Ltd. The mouse glycosylated hemoglobin A1c (GHbA1c) ELISA kit was purchased from Shanghai Jianglai Biotechnology Co., Ltd. Lipopolysaccharide (LPS), interleukin-1β (IL-1β), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), mouse D-lactic acid (D-LA) and mouse diamine oxidase (DAO) were provided by Shanghai Enzyme-Linked Biotechnology Co., Ltd. Insulin-like growth factor binding protein 7 (IGFBP7), peroxisome proliferator-activated receptor alpha (PPARα), p-PPARα, polyribonucleotide nucleotidyltransferase 1 (PNPT1), sterol regulatory element-binding protein 1 (SREBP-1), acetyl-CoA carboxylase (ACC), fatty acid synthase (FASN) and NADPH oxidase 4 (NOX4) were provided by Cell Signaling Technology.

Mice

Thirty-two 8-week-old SPF grade male db/db mice with a body weight of (35 ± 2) g and 8 db/m mice with a body weight of (23 ± 2) g were purchased from Beijing Huafukang Biotechnology Co., Ltd., License no. SCXK (Beijing) 2024-0003. The mice were raised in an SPF grade animal house of Shenyang Medical College. All mice were kept at a constant temperature of 22 ± 2 °C, under a relative humidity of 50% ± 5% and a day/night cycle of 12/12 h without specific pathogens. All mice were free to eat and drink. The experimental protocol was approved by the Experimental Animal Ethics Committee of Shenyang Medical College (Ethics batch no. SYYXY2023111601).

Animal experiment

After 1 week of adaptive feeding, all SPF db/db mice were randomly divided into 5 groups with 8 mice in each group, namely the model group (normal saline), antibiotic group (vancomycin at a dose of 100 mg kg^{-1}, neomycin sulfate at a dose of 200 mg kg^{-1}, metronidazole at a dose of 200 mg kg^{-1}, and ampicillin at a dose of 200 mg kg^{-1}, continuous gavage for 5 days),[20] LicA group (35 mg kg^{-1}),[12] antibiotic + LicA group, and control group which consisted of 8 SPF db/m mice of the same week age. LicA was gavaged once a day for 6 weeks. The activity, shape, mental state and hair of the mice were observed. The nutrition content of ordinary maintenance feed was 4.7% fat, 57% carbohydrate, and 20% protein. The body weight, water intake, food intake and fasting blood glucose of the mice were monitored weekly. To evaluate the human equivalent dose (HED) of LicA in the experiment, the body surface area normalization method (FDA guidelines) was used for the calculation.

$$\mathrm{HED}\left(\mathrm{mg\,kg^{-1}} = \text{animal dose} \times \frac{K_m(\text{mouse})}{K_m\,(\text{human})}\right)$$

The K_m of mice was 3 and the K_m of adults was 37.

Oral glucose tolerance test (OGTT)

After fasting for 12 h, mice in each group were given 2 g kg^{-1} glucose solution by gavage. Subsequently, the FBG levels of mice in each group were measured at 0, 30, 60 and 120 min by using an Accu-Chek Performa blood glucose meter. The area under the OGTT curve was calculated based on the method described in the literature.[21]

Collection of biological samples

The mice were anesthetized with isoflurane to obtain the serum, which was stored at −80 °C for later use. Parts of the liver, pancreas, colon and ileum were removed, washed with normal saline and stored in a freezer at −80 °C. Other parts of the liver, pancreas, colon and ileum of mice were fixed in 4% paraformaldehyde for the preparation of the subsequent pathological sections.

Determination of biochemical indexes and glycolipid metabolic factors

The serum levels of TC, TG, LDL-C, HDL-C, AST, ALT, LPS, IL-1β, IL-6, TNF-α, D-LA and DAO were determined according to the kit instructions. The liver glycogen was determined according to the instructions of the kit provided by Nanjing Jiancheng Biotechnology Co., Ltd. The fasting serum insulin (FINS) and GHbA1c were determined by enzyme-linked immunosorbent assay. After obtaining the insulin concentration, the IR index (HOMA-IR) was calculated according to the following formula: HOMA-IR = FINS (mIU L^{-1}) × FBG (mmol L^{-1})/22.5.

Histopathological observation

Liver, pancreatic, colon and ileal tissues were fixed in 4% paraformaldehyde for 48 h. After gradient dehydration, they were embedded in paraffin, sliced, dewaxed, covered with water, dyed with hematoxylin for 5 min and washed back to blue, dyed with eosin for 1 min and washed. Finally, the tablets were sealed with neutral gum, observed under a microscope and photographed. Lipid deposition in the liver tissue was detected by oil red O staining.

Immunofluorescence analysis

Paraffin sections of the colon and ileum were prepared. The sealing solution was used for 30 min at room temperature away from light. After rinsing with phosphate buffer (PBS), the primary antibodies against ZO-1, occludin and claudin-3 (1 : 500) were added and incubated at 4 °C overnight. The next day, they were washed three times with PBS, 5 min each time, covered with the corresponding secondary antibody, and kept away from light at room temperature for 50 min. Subsequently, DAPI staining and sealing were performed, and images were collected under a fluorescence microscope to analyze the expression of fluorescence.

16S rRNA gene sequencing

The collected cecal samples were transferred to Shanghai Piceno Biotechnology Co., Ltd for high-throughput sequencing. The sequencing data were clustered according to 97% similarity to obtain an operational taxonomic unit (OTU). The composition distribution of each sample at the classification level was visualized, and the analysis results were presented in a bar chart. The specific primers of the V3–V4 region of bacterial 16S rRNA were selected for PCR amplification. The structure and quantity of the microbiota were analyzed by using species composition analysis, α-diversity analysis, species difference analysis and β-diversity analysis.

Metabolomics of the liver

The liver tissues were packaged and stored in liquid nitrogen, and then sent to Shanghai Personal Biotechnology Co., Ltd for testing. The sample was added to a pre-cooled methanol/acetonitrile/aqueous solution (2 : 2 : 1, v/v), mixed in a vortexer, subjected to ultrasound at a low temperature for 30 min, stood at −20 °C for 10 min, centrifuged at 4 °C for 20 min, and dried in a vacuum with the supernatant. During mass spectrometry, 100 μL of acetonitrile solution (acetonitrile : water = 1 : 1, v/v) was added to redissolve, vortex and centrifuge at 4 °C for 15 min, and the supernatant was taken for analysis. The separation was performed by using an Agilent 1290 Infinity LC ultra-high performance liquid chromatographic column and mass spectrometry was performed using a Triple TOF 6600 mass spectrometer (AB Sciex). Positive and negative ion modes of electrospray ionization (ESI) were used for detection. ProteoWizard software was used to convert the original data into mzXML format, and then XCMS software was used for peak alignment, retention time calibration and peak area extraction. Firstly, the metabolite structure identification and data preprocessing were carried out for the data extracted by XCMS, and then the experimental data quality evaluation was carried out, and finally the data analysis was carried out.

Proteomics of the liver

The proteomics technology was commissioned by Shanghai Personal Biotechnology Co., Ltd. Three cases of the liver tissue were randomly selected from the control group, model group and LicA group, respectively, and the following steps were performed: sample preparation, protein extraction, peptide enzymolysis, chromatographic fractionation, liquid chromatography-tandem mass spectrometry (LC-MS/MS) data acquisition and database search. Subsequently, bioinformatics analysis was performed on the data, which mainly included identification analysis, expression difference analysis, and functional analysis to screen for significantly differentially expressed proteins. The differential proteins were enriched by gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses.

Molecular docking analysis

The molecular structure of the active compound hypotaurine was downloaded from the PubChem database in SDF format (https://pubchem.ncbi.nlm.nih.gov/). OpenBabel 2.4.1 software was used to convert from sdf format to pdb format as a docking ligand. The 3D structures of ACC (2YL2), FASN (7MHD), sREGBP-1 (5GPD), IGFBP7 (8IVD), PPARα (2ZNN), and PNPT1 (3U1K) proteins were downloaded from the PDB website (https://www.rcsb.org/) as docking receptors. In order to purify the protein structure, the pdb file was imported into PyMOL software to delete the ligands and water. Then, the small molecular ligands of the compounds were imported into AutoDock 4.2.6 software for hydrogenation and charge calcu-

lations, and the coordinates of the grid box were determined for molecular docking. The docking results were derived according to the binding energy, and the docking results were visualized using PyMOL software to observe the 3D binding of the receptor and the ligand. The receptor and the ligand binding files were imported into Discovery Studio 2019 for a 2D visual analysis.

Western blotting

The RIPA protein lysate-containing protease inhibitor and broad-spectrum phosphatase inhibitor were added into the mouse liver tissues, crushed in a grinder, cracked on ice and centrifuged, and the supernatant was extracted. The protein concentration was determined by the BCA method, the sample buffer was blown and mixed, and the total protein was extracted by denaturation on a metal bath. After gluing, sampling, electrophoresis and film transfer, 5% skim milk powder was blocked at room temperature, rinsed with TBST, and incubated overnight at 4 °C with IGFBP7 (1 : 10 000), PPARα (1 : 5000), p-PPARα (1 : 5000), PNPT1 (1 : 5000), SREBP-1 (1 : 5000), ACC (1 : 5000), FASN (1 : 5000) and NOX4 (1 : 5000), respectively. TBST was cleaned, the secondary antibody (1 : 5000) was incubated at room temperature, and the chemiluminescent reagent was developed after washing, with GAPDH as the internal reference. ImageJ software was used for gray value analysis to calculate the relative expression of the target protein.

Statistical analysis

SPSS 25.0 statistical software was used for statistical analysis. GraphPad Prism 9.5 software was used for charting. All data were expressed as mean ± standard deviation. One-way analysis of variance was used for multi-group comparison, and the Bonferroni method was used for multiple comparison. $p < 0.05$ was considered statistically significant.

Results

Effects of LicA on blood glucose, body weight, food and water intake and glucose tolerance in db/db mice

Fig. 1A shows the experimental scheme of this study. As shown in Fig. 1B–E, the db/db mice exhibited typical diabetes symptoms compared to the control group, including elevated blood sugar, weight gain, and increased consumption of food and water. Compared with the model group, the LicA group exhibited significant reduction of these symptoms. Compared with the model group, the antibiotic group and the antibiotic + LicA group also showed reduction in blood sugar, body weight and water intake of mice, but these effects were not as significant as shown by the LicA group. As shown in Fig. 1F and G, the db/db mice showed impaired glucose tolerance and increased AUC values in the area under the OGTT curve compared to the control group ($p < 0.01$). The FBG and AUC values of the LicA group, the antibiotic group and the antibiotic + LicA group were lower than those of the model group ($p < 0.05$). Compared with the antibiotic + LicA group, the AUC value of the OGTT in the LicA group was significantly reduced ($p < 0.01$). The results showed that LicA could improve glucose metabolism disorder and impair glucose tolerance in db/db mice. In order to convert the dose used by the mice into a dose based on the human surface area, the mouse's 35 mg kg^{-1} (Baur's mouse dose) was multiplied by the K_m factor and then divided by the human K_m factor. The result of this calculation was that the human equivalent dose of LicA was 2.84 mg kg^{-1}, which was equivalent to a dose of 170 mg for a 60 kg person.

Effects of LicA on IR and blood glucose-related indexes in db/db mice

As can be seen in Fig. 2A and B, the levels of FINS and HOMA-IR in db/db mice were higher than those in the control group ($p < 0.01$). Compared with the model group, the levels of FINS and HOMA-IR in the LicA group, the antibiotic group and the antibiotic + LicA group were reduced. Compared with the antibiotic + LicA group, the levels of FINS and HOMA-IR in the LicA group were significantly reduced, with statistically significant differences ($p < 0.05$). As shown in Fig. 2C, liver glycogen synthesis was significantly reduced in the model group compared with the control group ($p < 0.01$). Compared with the model group, the LicA group showed a significantly increased liver glycogen content ($p < 0.01$), but there was no significant difference between the antibiotic group and the antibiotic + LicA group. The results showed that LicA could improve IR and liver glycogen metabolism in db/db mice.

Effects of LicA on blood lipids and liver function in db/db mice

As shown in Fig. 2D–G, it was observed that compared with the control group, the model group had obvious lipid metabolism disorder, which was manifested as a significant increase in the levels of TC, TG and LDL-C ($p < 0.01$) and a significant decrease in the level of HDL-C ($p < 0.01$). Compared with the model group, the levels of TC, TG and LDL-C in the LicA group, the antibiotic group and the antibiotic + LicA group were decreased, and the levels of HDL-C were increased, and the changes in the LicA group were the most significant ($p < 0.05$). Compared with the antibiotic + LicA group, the changes of TC, TG, LDL-C and HDL-C levels in the LicA group were statistically different, showing a better therapeutic effect ($p < 0.01$). The results indicated that LicA could improve dyslipidemia and regulate lipid metabolism in db/db mice. As shown in Fig. 2H and I, the ALT and AST levels in the model group were significantly higher than those in the control group ($p < 0.01$). Compared with the model group, the ALT and AST levels in the LicA group, the antibiotic group and the antibiotic + LicA group were all decreased, and the effect was most significant in the LicA group. Compared with the antibiotic + LicA group, the ALT and AST levels in the LicA group were dramatically reduced ($p < 0.01$). The results showed that LicA could improve liver function impairment during T2DM.

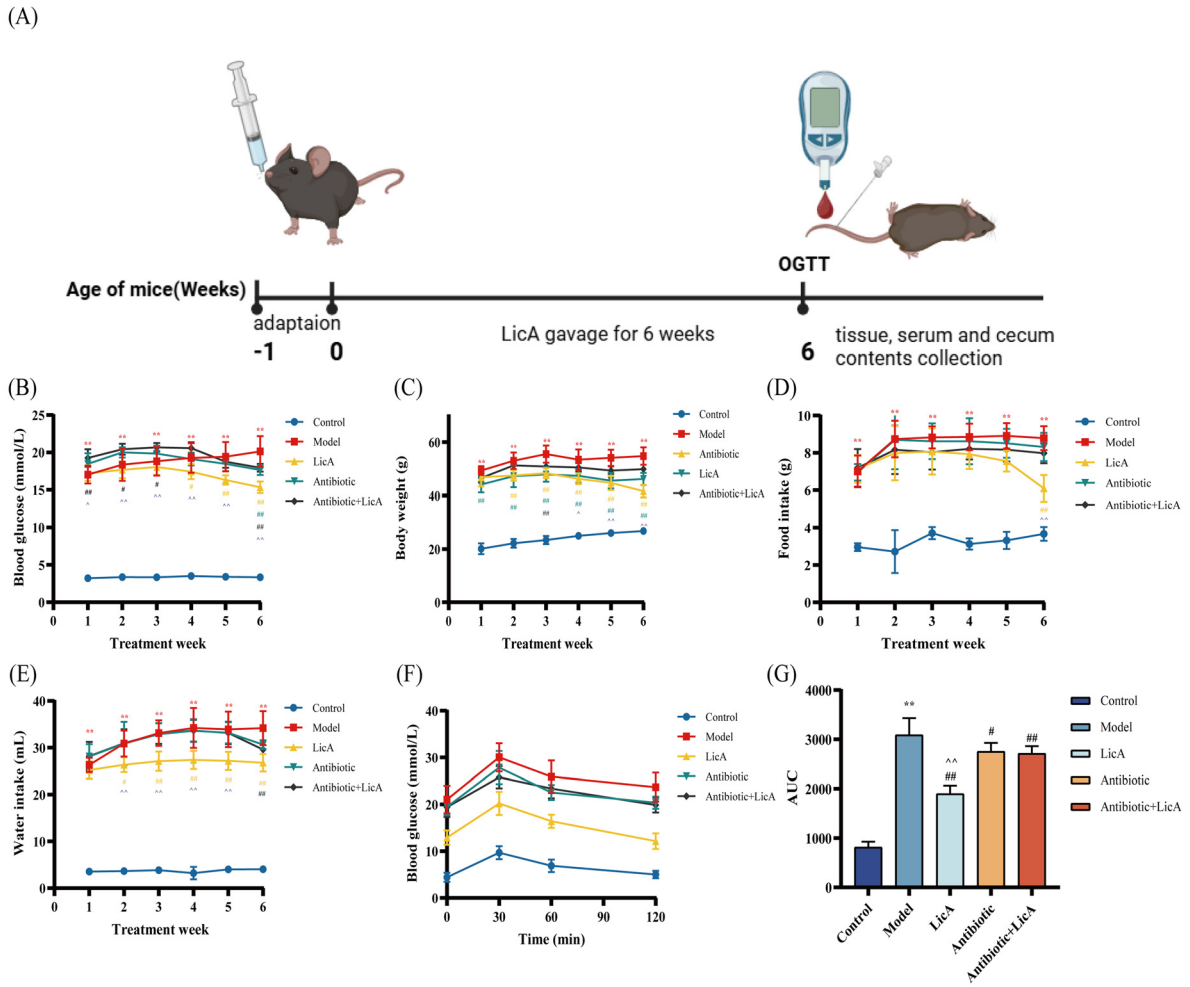

Fig. 1 The effects of LicA on blood glucose, body weight, food and water intake, and glucose tolerance in db/db mice. Research scheme of the experiment (A). Changes of FBG in each group (B). Changes of body weight in each group (C). Changes of food intake in each group (D). Changes of water intake in each group (E). Changes of glucose tolerance in each group (F). Changes of the AUC in each group (G). Data are expressed as the mean ± standard deviation (SD) (n = 6). *p < 0.05 and **p < 0.01 compared with the control group. $^{\#}p$ < 0.05 and $^{\#\#}p$ < 0.01 compared with the model group. $^{\wedge\wedge}p$ < 0.01 and $^{\wedge}p$ < 0.05 compared with the antibiotic + LicA group.

Effects of LicA on intestinal mucosal barrier function and serum inflammatory factors in db/db mice

As shown in Fig. 3A and B, compared with the control group, the expressions of D-LA and DAO in the model group were increased, with statistical significance (p < 0.01). Compared with the model group, the expressions of D-LA and DAO in the LicA group, the antibiotic group and the antibiotic + LicA group were decreased. Compared with the antibiotic + LicA group, the levels of D-LA and DAO in the LicA group were obviously decreased (p < 0.01). The results showed that LicA could improve the damaged intestinal mucosal barrier function and promote the recovery of intestinal barrier function in db/db mice. As shown in Fig. 3C–F, compared with the control group, serum levels of IL-1β, IL-6, TNF-α and LPS in the model group were significantly increased (p < 0.01). Compared with the model group, the levels of IL-1β, IL-6, TNF-α and LPS in the serum of mice in the antibiotic group, the LicA group and the antibiotic + LicA group were all decreased, but the reduction was most significant in the LicA group (p < 0.01). The levels of IL-1β, IL-6, TNF-α and LPS in the LicA group were evidently decreased compared with those in the antibiotic + LicA group (p < 0.01). The results showed that LicA could improve the inflammatory response in db/db mice, while reducing serum LPS levels.

Effects of LicA on the liver, pancreas and intestine of db/db mice

As shown in Fig. 4A, hepatocytes of mice in the control group had a regular structure and shape, an orderly arrangement, a uniform size, clear boundaries, and no steatosis and inflammatory cell infiltration. In the model group, the arrangement of liver cells was disordered, the cell volume was larger than normal, and liver steatosis and inflammatory cell infiltration existed. Compared with the model group, the LicA group signifi-

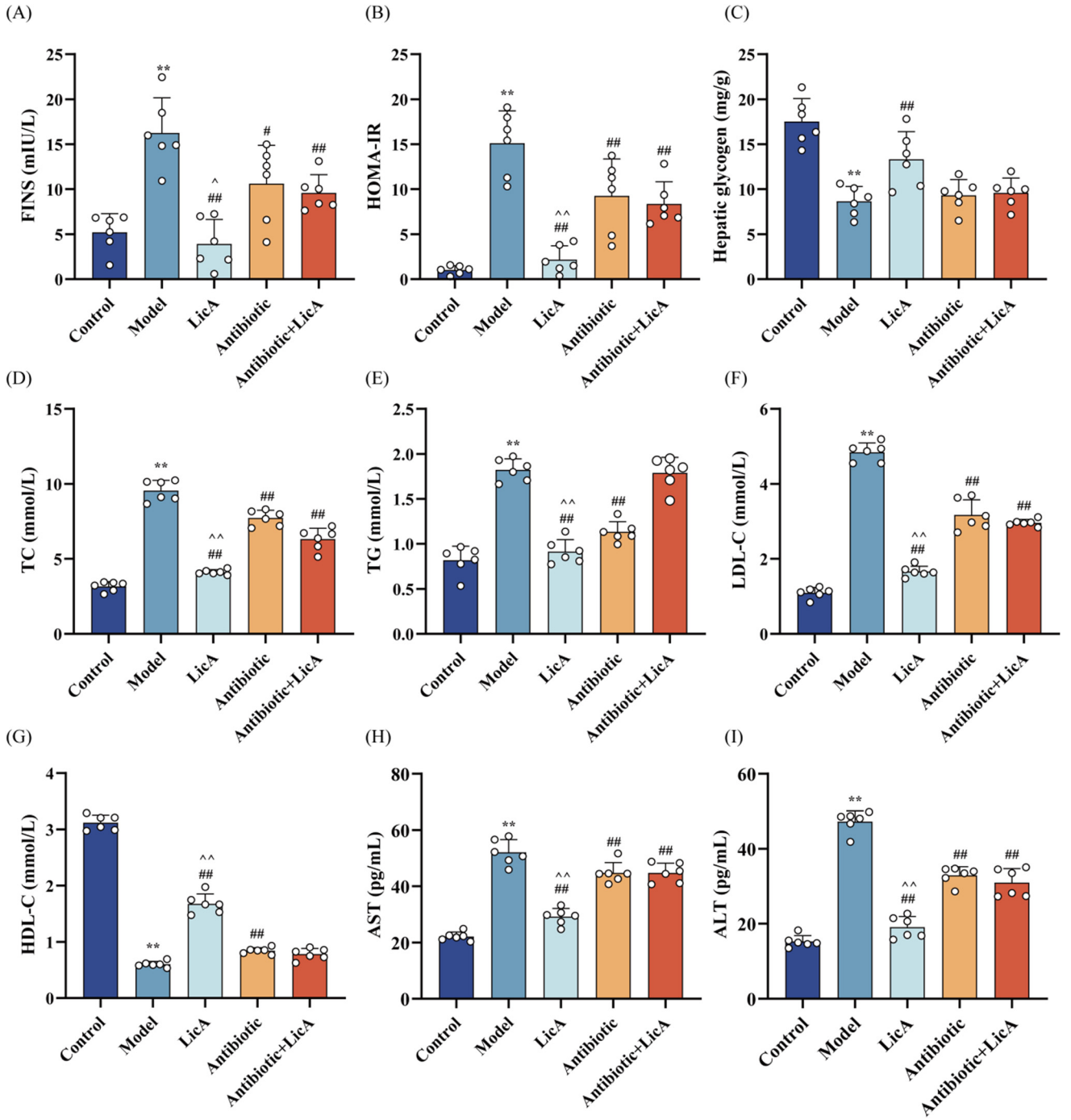

Fig. 2 Effects of LicA on FINS, HOMA-IR, liver glycogen, blood lipids and liver function in db/db mice. Changes in the FINS levels in different groups of mice (A). Changes in HOMA-IR of mice in each group (B). Changes in the liver glycogen levels in different groups of mice (C). Effect of LicA on TC in db/db mice (D). Effect of LicA on TG in db/db mice (E). Effect of LicA on LDL-C in db/db mice (F). Effect of LicA on HDL-C in db/db mice (G). Effect of LicA on AST in db/db mice (H). Effect of LicA on ALT in db/db mice (I). Data are expressed as the mean ± standard deviation (SD) (n = 6). $^{**}p$ < 0.01 compared with the control group. $^{\#}p$ < 0.05 and $^{\#\#}p$ < 0.01 compared with the model group. $^{\wedge\wedge}p$ < 0.01 compared with the antibiotic + LicA group.

cantly improved the phenomenon of fat vacuoles of different sizes in the liver, and the arrangement of liver cells was relatively uniform. The antibiotic + LicA group reversed the liver damage induced by LicA in diabetic mice. Fig. 4B shows the HE staining of the pancreas. In the control group, the pancreatic tissue structure of the mice was clear, and the islets were spherical, with a complete structure and full shape. In the model group, the morphology of the islet beta cells was changed, the islet edges were

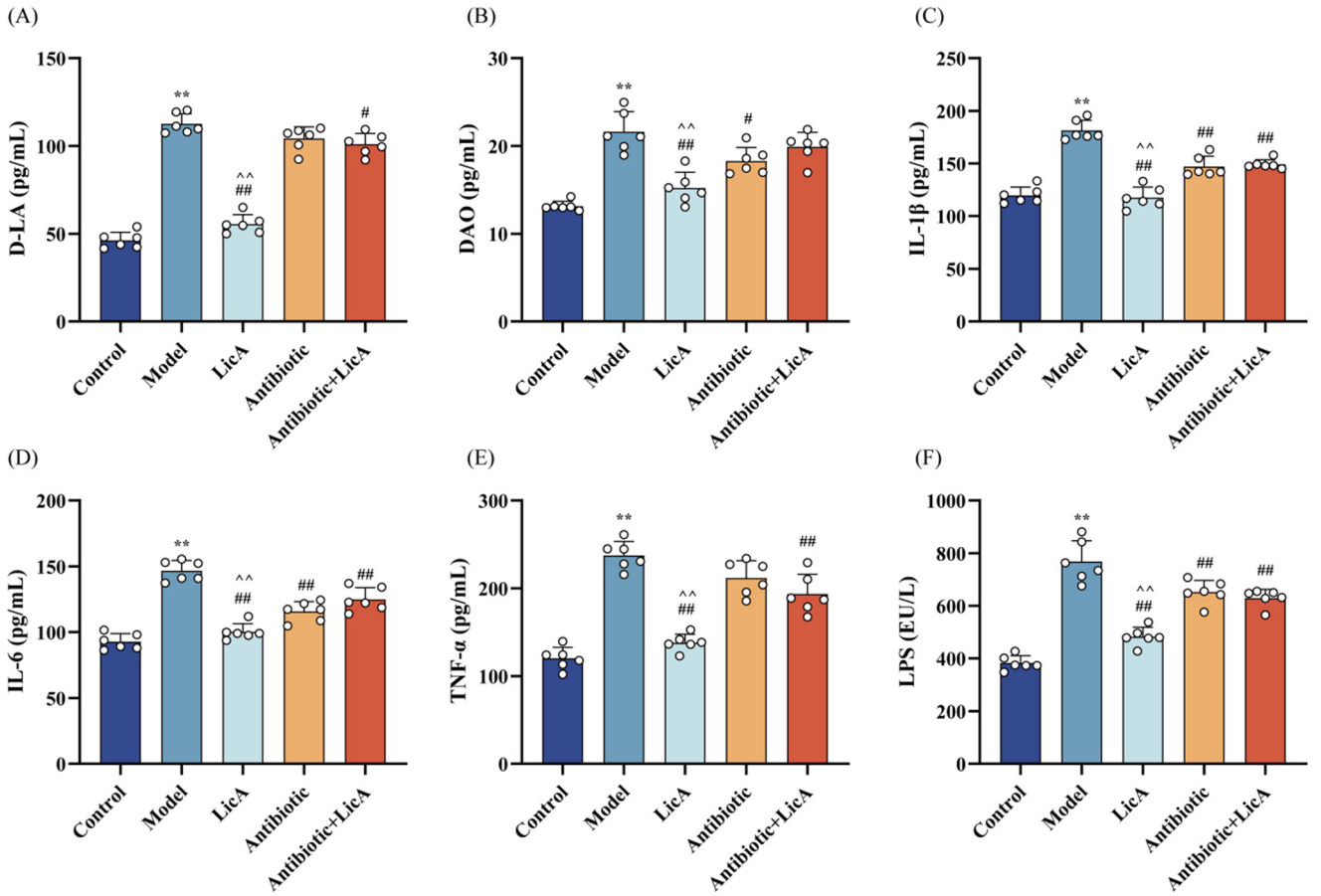

Fig. 3 Effects of LicA on intestinal mucosal barrier function and serum inflammatory factors in db/db mice. Changes of the serum D-LA level in db/db mice in each group (A). Changes in the serum DAO levels in db/db mice of different groups (B). Changes in the serum IL-1 β levels in db/db mice of different groups (C). Changes in the serum IL-6 levels in db/db mice of different groups (D). Changes in the serum TNF-α levels in db/db mice of different groups (E). Changes in the serum LPS levels in db/db mice of different groups (F). Data are expressed as the mean ± standard deviation (SD) (n = 6). **p < 0.01 compared with the control group. $^{\#}p$ < 0.05 and $^{\#\#}p$ < 0.01 compared with the model group. $^{\wedge\wedge}p$ < 0.01 compared with the antibiotic + LicA group.

not clear, the structure was disorganized, and the whole cell was swollen. Compared with the model group, the pathological damage of mice in the LicA intervention group was obviously alleviated, and its morphology was improved to varying degrees, the boundary of islet cells was clear, and the disorderly arrangement was reduced. The antibiotic + LicA group did not show improvement in the pathological injury of pancreas. Fig. 4C and D show that oil red O staining was lighter in the control group. In the model group, a large amount of lipid was accumulated in the liver, and the area ratio of oil red O staining was increased significantly (p < 0.01). Compared with the model group, the number of lipid droplets and the staining area of oil red O in the LicA group were evidently reduced (p < 0.01). Compared with the antibiotic + LicA group, the lipid droplets in the liver cells of the LicA group were reduced, and the staining area was also significantly reduced (p < 0.01). These results indicated that LicA could significantly improve and protect the liver and pancreas of db/db mice.

As shown in Fig. 4E, the ileal mucosa of mice in the control group was complete in structure, intestinal glands were abundant and neatly arranged, and goblet cells in the intestinal gland epithelium were clearly visible without obvious inflammatory cell infiltration. In the model group, the ileum showed damage of the mucosal structure, disordered villus arrangement, reduced villus height, significantly reduced crypt depth, reduced goblet cells, and infiltration of a large number of inflammatory cells in the mucosal layer and submucosa. Compared with the model group, the above-mentioned intestinal wall inflammation was alleviated and the mucosal structure was improved in the LicA group. HE staining of the colon of mice is shown in Fig. 4F. The crypt structure of the colon of mice in the control group was complete, and goblet cells were evenly distributed and abundant in the intestinal glands. Compared with the control group, the colonic crypt structure of the model group basically disappeared, the number of goblet cells was reduced, and there were obvious inflammatory cell infiltration and glandular destruction. The above pathological injuries in the LicA group were alleviated compared with those in the model group. The antibiotic and antibiotic + LicA groups showed no significant effect on the pathological injury of the ileum and colon.

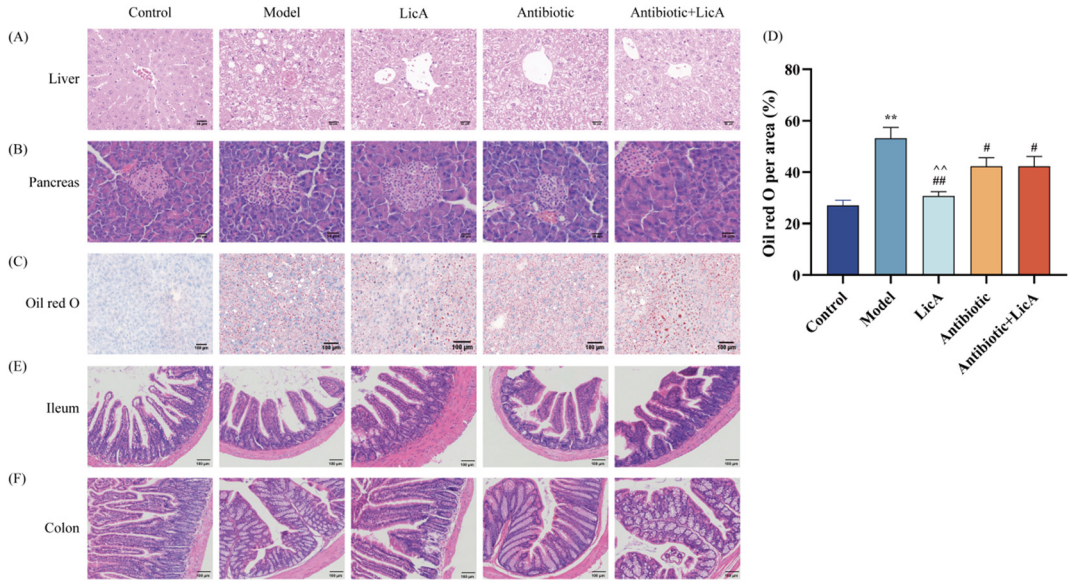

Fig. 4 Effects of LicA on the liver, pancreas and intestine of db/db mice. HE staining of the liver (A). HE staining of the pancreas (B). Oil red O staining of the liver (C). Quantitative results of oil red O staining (D). HE staining of the ileum (E). HE staining of the colon (F). $^{**}p < 0.01$ compared with the control group. $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ compared with the model group. $^{\wedge\wedge}p < 0.01$ compared with the antibiotic + LicA group.

Effect of LicA on tight junction protein expression in the ileum and colon of db/db mice

As shown in Fig. 5A–L, the fluorescence intensity of ZO-1, occludin and claudin-3 in the ileal and colon tissues of the model group was significantly decreased compared with that of the control group. Compared with the model group, the fluorescence intensity of ZO-1, occludin and claudin-3 in the ileal and colon tissues of the LicA group was significantly enhanced, and the fluorescence quantification showed a significant difference ($p < 0.01$). However, the fluorescence intensity and relative fluorescence expression of ZO-1, occludin and claudin-3 in the intestinal tissue of the antibiotic group and the antibiotic + LicA group were not significantly different from the LicA group. These results all suggest that the protective effect of LicA on the intestinal epithelial barrier may be mainly through up-regulating the mechanical barrier tight junction protein of the intestinal mucosa, improving the damaged tight junction between cells, and thereby reducing the permeability of the intestinal mucosal barrier.

Effect of LicA on the intestinal flora in db/db mice

The α-diversity results and β-diversity results are shown in Fig. S1 of the SI. The intestinal flora of the db/db mice was analyzed. The specific changes of the intestinal flora in Kingdom, Phylum, Class, Order, Family, Genus and Species were analyzed. It was found that g_unidentified_S24-7, g__Allobaculum, g_Sutterella, g__p-75-a5, g_Lactobacillus, p__Verrucomicrobia, p_TM7, c__Verrucomicrobiae, f__Verrucomicrobiaceae, f_S24-7 and o__Verrucomicrobiales were significantly increased in the model compared to the control group. After the LicA intervention, it was significantly reduced. Next, Spearman's correlation analysis was used to analyze the correlation between opportunistic pathogens and general physiological indicators of diabetes. As shown in Fig. 6A, these opportunistic pathogens were negatively correlated with the body weight and HDL-C level and positively correlated with FBG, glucose tolerance, HOMA-IR, TC and LDL-C ($p < 0.05$). This further suggested that LicA may improve T2DM by reducing these opportunistic pathogens. It was found that p-75-a5 was closely related to the occurrence and development of glucose and lipid metabolism. Therefore, we focused our research on p-75-a5. p-75-a5 is a member of Erysipelotrichaceae,[22] belonging to the phylum Firmicutes,[23] which is highly related to protein and fat digestion.

Differential microbiota–liver metabolome–physiological index association analysis to locate the key microbiota and metabolites

The basic non-targeted metabolomics data of LicA on db/db mice are shown in the SI (Fig. S2). The metabolites up-regulated in the model group and down-regulated in the LicA group were selected for the Venn diagram analysis. As shown in Fig. 6B, 34 intersecting metabolites were identified, with specific information provided in the SI (Table S1). Spearman's correlation analysis was performed between 34 different metabolites and opportunistic pathogens. As shown in Fig. 6C, p-75-a5 was found to be positively correlated with hypotaurine (M108T348.neg), L-gulono-1,4-lactone (M195T361.neg) and silydianin (M499T418.neg). G__allobaculum was positively correlated with l-cysteine sulfinic acid (M152T389.neg) and pyruvate (M87T284.neg). The chord diagram (Fig. 6D) was consistent with Spearman's correlation analysis. Notably, as shown in Fig. 7E, hypotaurine

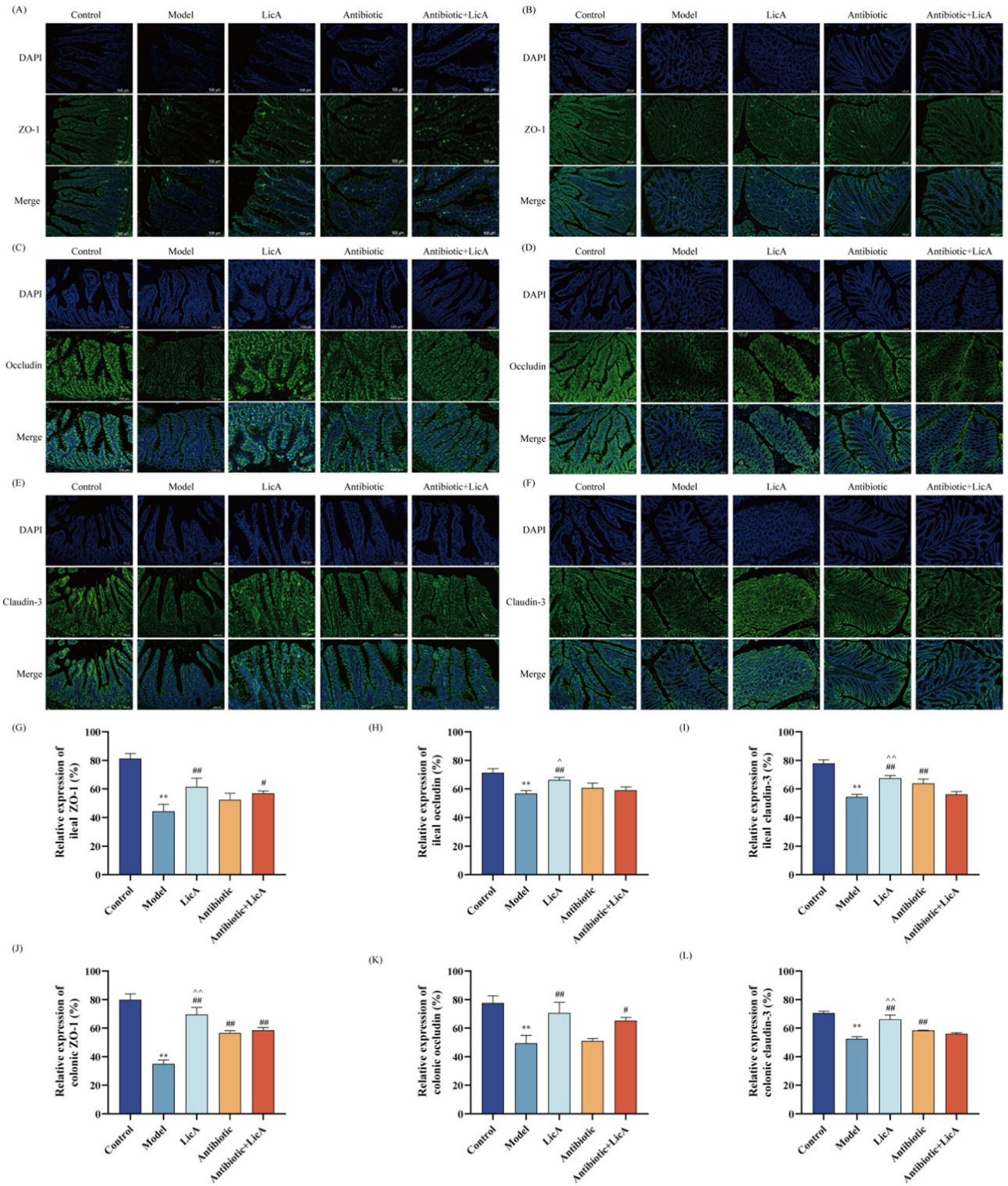

Fig. 5 Effect of LicA on the ileum and colonic tight junction protein in db/db mice. Fluorescence image of ZO-1, occludin and claudin-3 protein expression in the ileum and colon (A–F). Quantitative analysis of ZO-1, occludin and claudin-3 protein expression (G–L). $^{**}p < 0.01$ compared with the control group. $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ compared with the model group. $^{\wedge}p < 0.05$ and $^{\wedge\wedge}p < 0.01$ compared with the antibiotic + LicA group.

was increased in the model group compared with the control group ($p < 0.05$). Compared with the model group, the content of hypotaurine in the LicA group was significantly decreased ($p < 0.01$). As shown in Fig. 6F, hypotaurine could be identified as a metabolite of p-75-a5 bacteria and was closely related to T2DM.

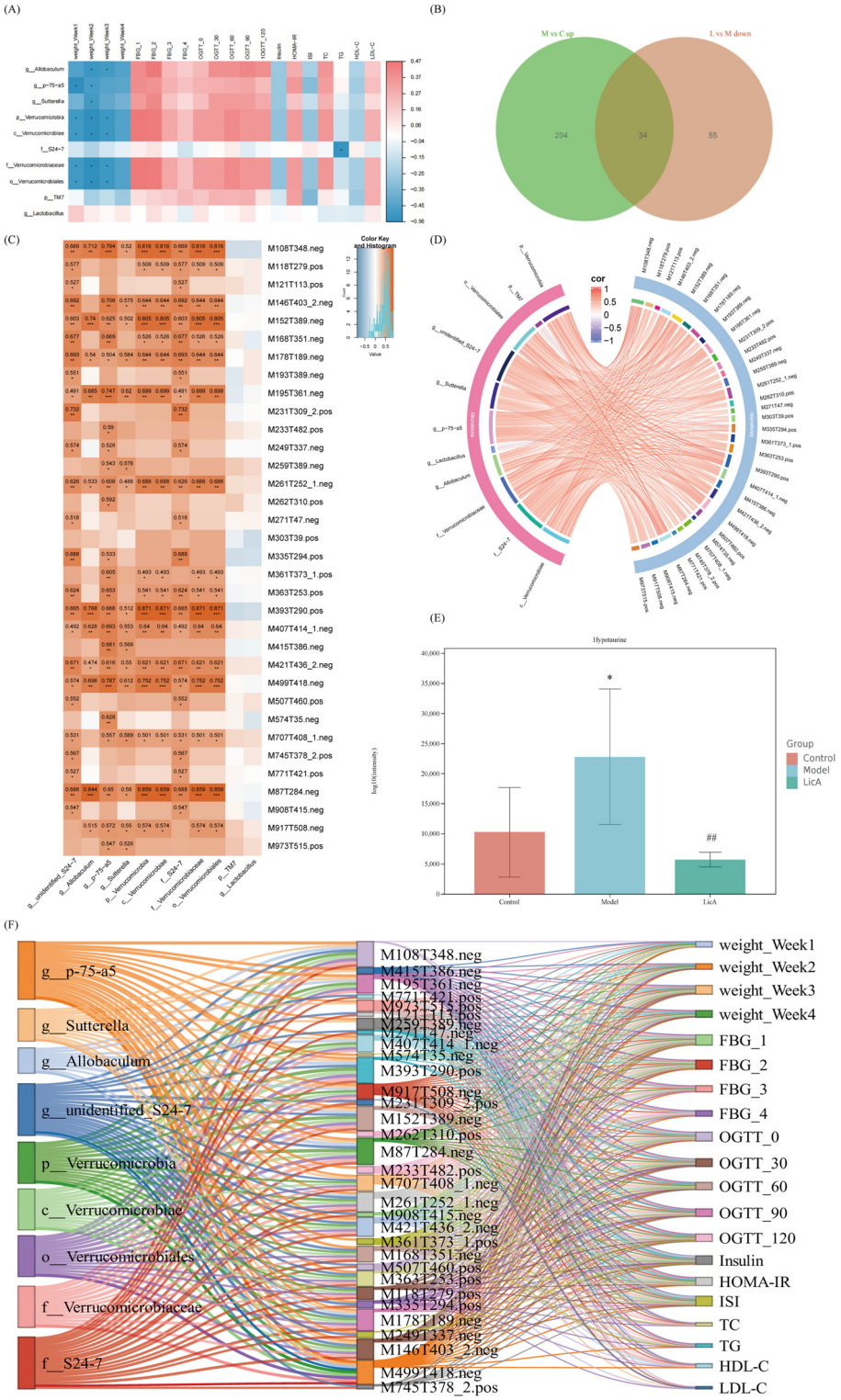

Fig. 6 Correlation analysis of the differential microbiota, liver metabolome and physiological indexes. Correlation between the opportunistic pathogens and the glycolipid metabolism parameters in diabetes (A). Venn diagram of the intersection of liver metabolites – Control *vs.* Model (up) and Model *vs.* LicA (down) (B). Heat map of the correlation analysis between the opportunistic pathogens and the differential metabolites of the liver (C). Chord diagram of the opportunistic pathogens and the differential metabolites of the liver (D). The content difference of hypotaurine among the control group, model group and LicA group (E). Sankey diagram of the correlation between the opportunistic pathogens, differential metabolites and general physiological indexes of T2DM (F). $p < 0.05$ was considered to have a significant difference.

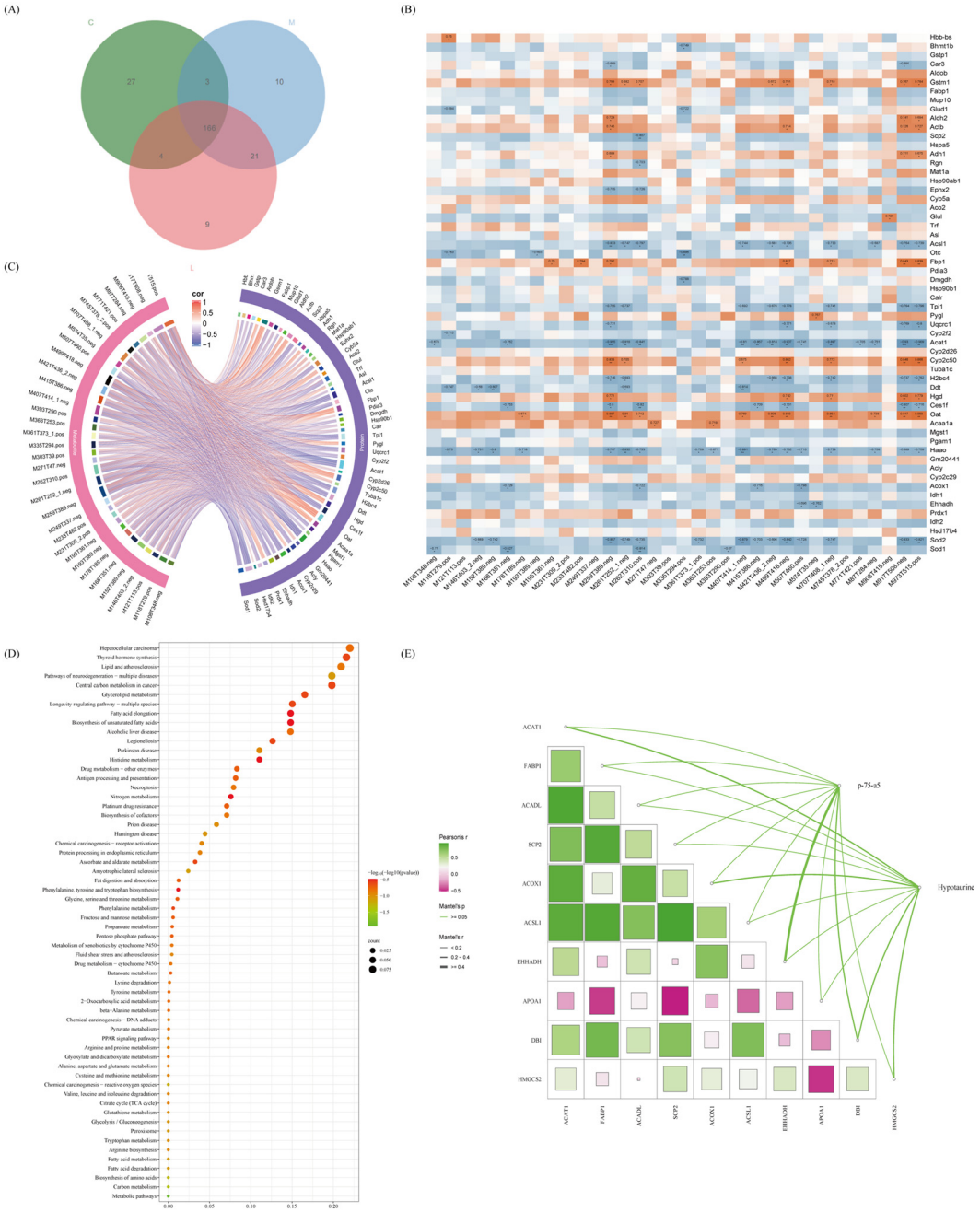

Fig. 7 Comprehensive analysis of the protein–metabolite–physiological indicators. Venn diagrams of the top 200 proteins in the control group, model group and LicA group (A). Heat map of the correlation between the differential metabolites in the liver and the intersected proteins (B). A chord chart of the differential metabolites and proteins in the liver (C). Bubble diagram of the KEGG enrichment analysis of intersection protein (D). Heat map of the correlation between p-75-a5–hypotaurine–ACAT1 and PPAR pathway proteins (E). *p*-value <0.05 was considered to have a significant difference.

Combined analysis of protein–metabolite–physiological indexes clarified the mechanism of LicA regulating T2DM

Differential proteins were screened through liver proteomics, and the relevant results are shown in Fig. S3 of the SI. The top 200 proteins expressed in the control group, the model group and the LicA group were selected for Venn diagram analysis, as shown in Fig. 7A, and 166 intersection proteins were identified. 34 different metabolites and intersection proteins were analyzed by using a chord chart and Spearman's correlation analysis. As shown in Fig. 7B and C, hypotaurine had a strong negative correlation with Acat1 and Sod1, while Acat1 was closely related to lipid metabolism. Liver protein was further enriched by the KEGG pathway, and the results showed that

Table 1 Molecular docking results of hypotaurine with ACC, FASN, SREBP-1, IGFBP7, PPARα and PNPT1

Compound	Protein	PDB ID	Binding energy (kcal mol^{-1})
Hypotaurine	ACC	2YL2	−4.32
Hypotaurine	FASN	7MHD	−4.47
Hypotaurine	SREBP-1	5GPD	−4.72
Hypotaurine	IGFBP7	8IVD	−4.25
Hypotaurine	PPARα	2ZNN	−4.01
Hypotaurine	PNPT1	3U1K	−3.36

the remission of db/db mice treated with LicA was related to changes in a variety of key signaling pathways, including PPAR, histidine metabolism, phenylalanine metabolism, and fatty acid metabolism (Fig. 7D). As shown in Fig. 7E, FABP1, ACADL, SCP2, ACOX1, ACSL1, EHHADH, APOA1, DBI, and HMGCS2 are PPAR signaling pathway-related proteins. The heatmap of the correlation between p-75-a5 bacteria and hypotaurine and protein indicated that both p-75-a5 bacteria and the metabolite hypotaurine had significant correlations with the Acat1 protein and the PPAR pathway. Therefore, LicA may reduce the liver metabolite hypotaurine by reducing the p-75-a5 bacteria, and then increase liver Acat1 protein expression through the PPAR signaling pathway, thereby regulating T2DM glucose and lipid metabolism.

Molecular docking analysis

When the docking score is <0 kcal mol^{-1}, the ligand can automatically bind to the receptor, and when the docking score is <−5.0 kcal mol^{-1}, better docking binding energy can be achieved between the two. In the results of molecular docking, the binding affinity of hypotaurine with ACC, FASN, SREBP-1, IGFBP7, PPARα and PNPT1 was <−3.0 kcal mol^{-1} (Table 1), indicating that hypotaurine was well bound to the six proteins. According to the 3D results shown in Fig. 8A–F, hypotaurine was bound in the cavity pockets of all six proteins. The two-dimensional results showed that the interaction between hypotaurine and the protein mainly included hydrogen bonding. Hydrogen bonding stabilizes molecular docking complexes and improves binding specificity and affinity. Hypotaurine formed hydrogen bonds with the ACC protein residues ASP-330 and ASP-333. Hypotaurine formed hydrogen bonds with the FASN residues GLU-251 and ILE-250, and formed a pi-sulfur bond with the sulfur atom in HIS-481. Hypotaurine formed hydrogen bonds with the SREBP-1 residues GLU-714 and GLU-724. Hypotaurine formed hydrogen bonds with the IGFBP7 residues ASP-249 and ASP-295. Hypotaurine formed hydrogen bonds with the PPARα residues MET-220, GLU-286 and THR-283. Hypotaurine formed hydrogen bonds with the PNPT1 residues ASP-301 and GLU-303.

Effects of LicA on the expression of lipid metabolism-related proteins and IGFBP7/PPARα/PNPT1 pathway proteins

In this study, western blotting was used to further verify the expression of related proteins. As shown in Fig. 9, compared with the control group, the expression levels of ACC, FASN, SREGBP-1C, NOX4 and IGFBP7 in the liver tissues of the model group were significantly increased ($p < 0.01$), and the expressions of PPARα, P-PPARα and PNPT1 were significantly decreased ($p < 0.01$). Compared with the model group, the expression of ACC, FASN, SREBP-1c, NOX4 and IGFBP7 in the LicA group was evidently reduced ($p < 0.01$), and the expression of PPARα, P-PPARα and PNPT1 was obviously up-regulated ($p < 0.01$). The regulatory effects of the antibiotics and antibiotic + LicA on ACC, FASN, SREGBP-1C, NOX4, IGFBP7, PPARα, P-PPARα and PNPT1 were not obvious.

Discussion

The occurrence of T2DM is mostly due to the disorder of glucose and lipid metabolism caused by excessive energy intake.[24] In recent years, with the improvement of living standards, the incidence of T2DM has increased year by year. The persistent hyperglycemia caused by diabetes will also affect the function of many tissues, leading to various serious complications, such as diabetic nephropathy and diabetic vascular disease.[25] Liver, as the main site of glucose and lipid metabolism, is also a major organ closely related to intestinal microbes, and its function is seriously affected in the pathogenesis of T2DM.[26,27] It is notable that the liver and intestinal microbiome form a bidirectional regulatory network through the "gut–liver axis".[26] On the one hand, the intestinal flora and its metabolic products (such as short-chain fatty acids and secondary bile acids) directly act on the liver through the portal vein system.[27] On the other hand, the liver regulates the composition and function of the intestinal flora by secreting substances such as bile acids and immune factors.[27] Given the significant role of the "gut–liver axis", current research is dedicated to identifying safe and effective bioactive substances that can target this regulatory network. It is recorded in *Compendium of Materia Medica* that licorice can alleviate type 2 diabetes.[28] It has also been found that licorice can reduce IR caused by high-fat feeding in mice.[29] We found that licorice flavonoids improve blood glucose in T2DM mice by establishing a T2DM mouse model in the early stage.[12] Further research found that LicA, the monomer component of flavonoids, can regulate glucose and lipid metabolism and antioxidants, and improve the liver tissue damage caused by diabetes.[14] These studies provide a theoretical basis for the development of hypoglycemic health care products.

LicA is a flavonoid, which is one of the active ingredients in licorice. Its extraction and purification mainly depend on licorice. In addition, the daily dosage of licorice as food is 3–10 g for adults weighing 50–70 kg.[30] Institutions such as the European Union have supported the food safety limit of 10 g per day.[31] Dinteren *et al.*'s study showed that the average content of LicA in licorice was 16.96 mg g^{-1}.[32] That is, the proportion of LicA in daily consumption of licorice is 51–170 mg for adults weighing 50–70 kg. In this study, the LicA dose in mice was converted into the human equivalent dose (HED), which was about 170 mg day^{-1} based on 60 kg adult body

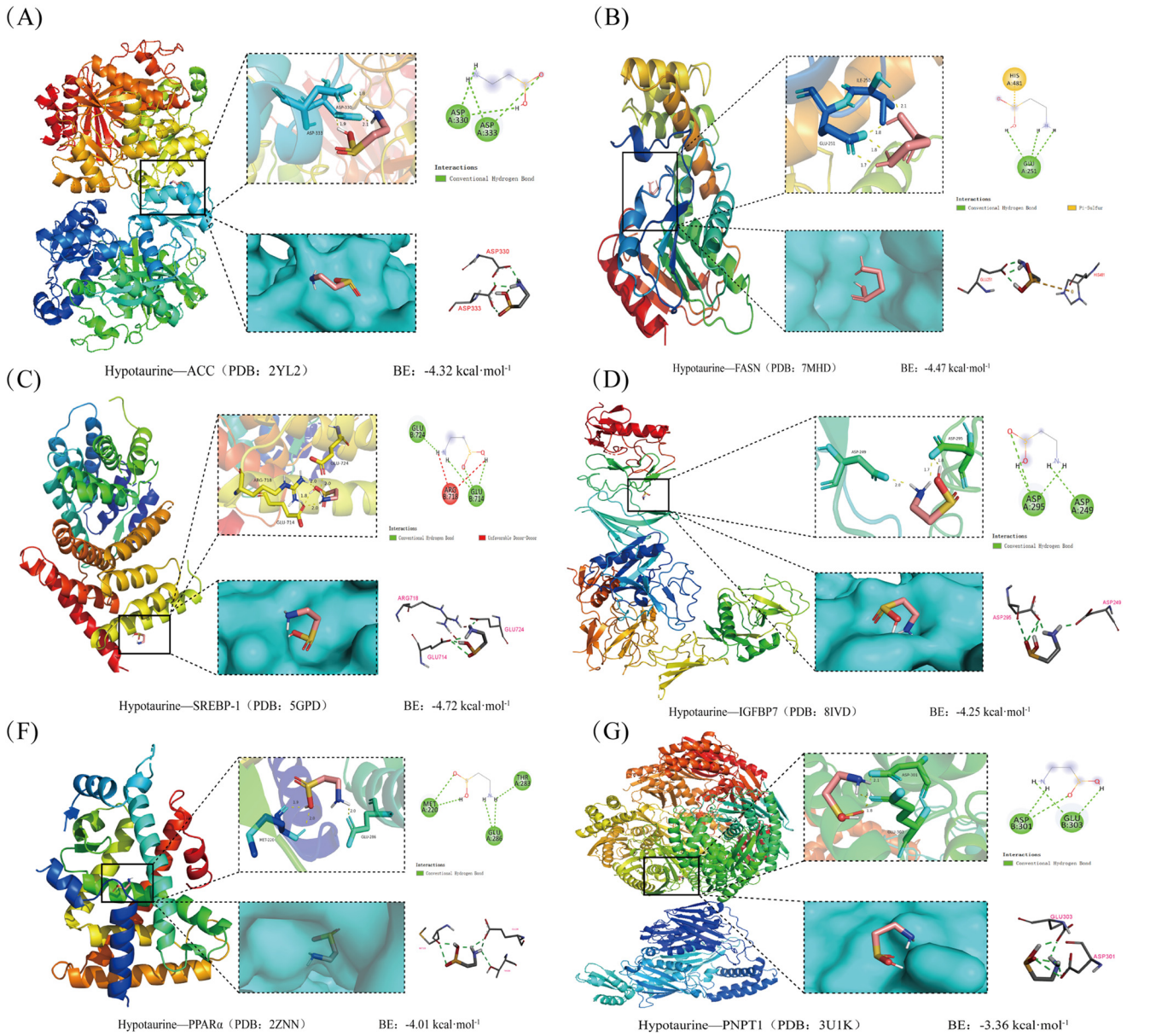

Fig. 8 Molecular docking results of hypotaurine binding with key proteins. Molecular docking results of hypotaurine binding with ACC, FASN, SREBP-1, IGFBP7, PPARα and PNPT1 proteins (A–G).

weight. After a comparative analysis, it was found that the dosage of LicA in this study was safe. LicA, as a common food, is embodied in the use of licorice for tea, soup and stew. The total human equivalent dose of LicA fed to mice was 10 g of licorice boiled in soup.

Our previous experiments also confirmed that LicA supplementation could reverse T2DM induced by HFD and STZ, and improve the disorder of liver glucose and lipid metabolism.[12] Intestinal flora detection showed that LicA could reduce the abundance of a variety of opportunistic pathogens, indicating that LicA has a therapeutic prospect for the remission of T2DM.[14] However, it is still unknown whether LicA improves glucose and lipid metabolism disorders in T2DM through the gut microbiota. Therefore, a pseudo-sterile mouse model was constructed by using broad-spectrum antibiotics to clarify the key role of intestinal flora in the regulation of glucose and lipid metabolism activities of LicA in this study. It provides a new scientific research idea for the prevention and treatment of T2DM by LicA, and provides a theoretical basis for the development of dietary maintenance of glucose and lipid metabolism homeostasis based on the intestinal flora.

T2DM mice need to take in a large amount of water to excrete glucose from the body, resulting in an increase in daily drinking water volume, and a decrease in drinking water volume after LicA intervention ($p < 0.05$). In addition, there was significant recovery in diet and body weight, indicating

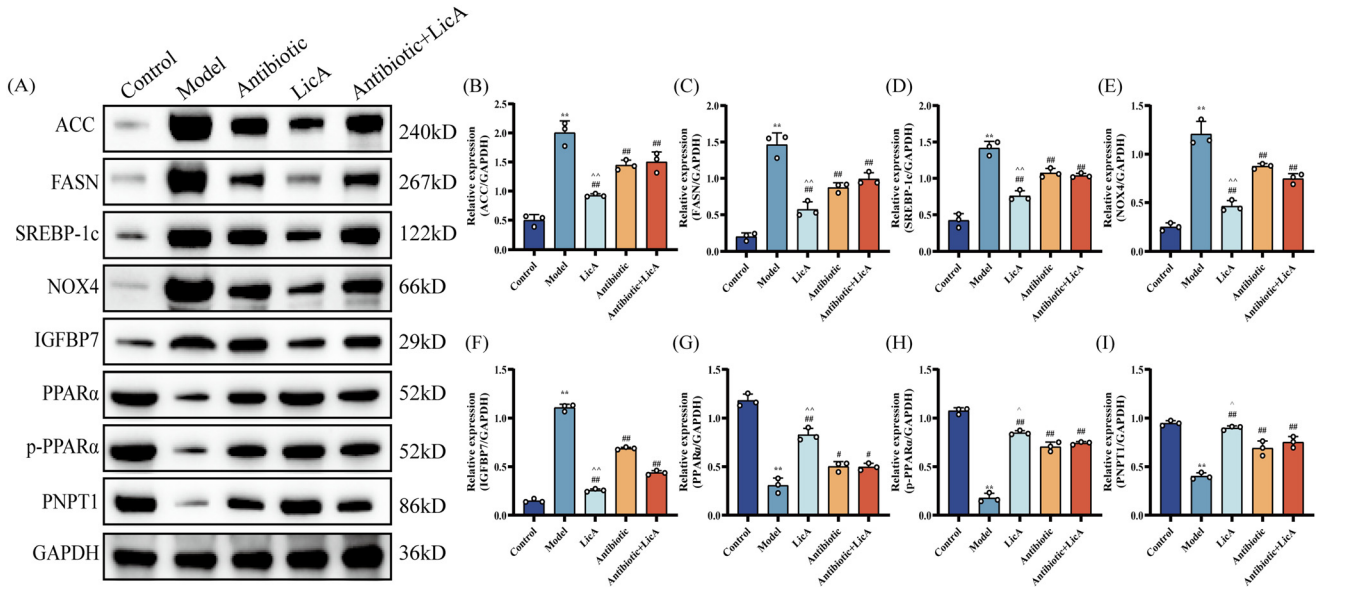

Fig. 9 Effect of LicA on protein expression in liver of db/db mice. Images and quantitative analysis of protein expression of ACC, FASN, SREBP-1C, NOX4, IGFBP7, PPARα, P-PPARα, and PNPT1 (A–G). The protein levels (ACC, FASN, SREBP-1C, NOX4, IGFBP7, PPARα, P-PPARα and PNPT1) were normalized to GAPDH. Data are expressed as means ± S.D of triplicate experiments performed independently. $^{**}p < 0.01$ compared with the control group. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared with the model group. $^{\wedge\wedge}p < 0.01$, $^{\wedge}p < 0.05$ compared with the antibiotic +LicA group.

that LicA improved the physiological parameters of db/db mice. Diabetic patients are prone to abnormal blood lipid levels. Li *et al.* found that red ginseng extracts can significantly reduce lipid levels and IR in mice,[33] which is consistent with the study on blood lipid levels in this study. The blood lipid level of T2DM mice was several times higher than that of the control group and significantly decreased after LicA intervention. The increase of FBG and GHbA1c is an important symptom of T2DM and is closely related to the prognosis of T2DM.[34] GHbA1c can reflect the body's long-term blood sugar level.[35] Therefore, the focus of diabetes treatment is to control the rise of blood sugar. This study observed that LicA can reduce the levels of FBG and GHbA1c in T2DM mice. In addition, eukaryotic cells in the liver will store excess glucose to synthesize glycogen to combat the abnormal increase of blood sugar.[36] When the body is in a state of diabetes, blood sugar homeostasis is destroyed and blood sugar continues to rise, but the liver cannot consume excessive glucose in the blood through glycogen synthesis.[37] Xu *et al.* also reported that the liver glycogen content was reduced in T2DM mice.[38] In this study, we found that LicA reversed the reduction of liver glycogen in db/db mice, indicating that LicA had the ability to enhance liver glycogen storage. However, LicA cannot effectively improve the glucose metabolism and glycogen synthesis of pseudo-sterile db/db mice, which confirms the role of intestinal flora in improving glucose metabolism by LicA.

The blood glucose control of diabetic patients can be evaluated through the OGTT.[39] Normal body intake of glucose leads to rapid secretion of insulin, promoting hepatic glycogen synthesis and inhibiting breakdown, while enhancing peripheral tissue utilization of glucose to maintain blood sugar stability.[40] However, IR can lead to a decrease in insulin sensitivity of target tissues, as well as disorders in glucose uptake and utilization, manifested as elevated FBG and impaired glucose tolerance. Our results showed that T2DM mice showed significant glucose intolerance in the OGTT experiment, and the area under the blood glucose curve (AUC) decreased significantly after LicA intervention. This is consistent with the research results of Wang *et al.*[41] Furthermore, the improving effect of glucose tolerance in the LicA + antibiotic group was weakened, indicating the significance of the gut microbiota. It is worth noting that FINS and HOMA-IR are important indicators for evaluating pancreatic islet function and IR.[42] Their changes can directly reflect the functional status of pancreatic β cells and the body's sensitivity to insulin. FINS increases due to the decrease of insulin receptors or sensitivity, resulting in an increase in HOMA-IR.[43] Our results showed that the levels of FINS and HOMA-IR in T2DM mice were significantly increased ($p < 0.01$), indicating that there was obvious IR. This is consistent with the clinical manifestations of T2DM patients.[44] After LicA intervention, the levels of FINS and HOMA-IR were significantly improved. Furthermore, the pathological changes in the islet structure further supported the vicious cycle of IR and β-cell dysfunction.[45] Long-term hyperglycemia and IR can aggravate β-cell damage, and the decline of β-cell function will further intensify the disorder of glucose metabolism.[46] The protective effect of LicA on pancreatic islet morphology may indirectly promote the recovery of insulin secretion function, thereby showing improvement effects in both glucose tolerance and IR indicators. More importantly, the beneficial effect of concurrent use of antibiotics + LicA on pancreatic injury in db/db mice was weakened. This result strongly suggests that

the gut microbiota plays a key role in LicA's improvement of pancreatic injury in mice. The broad spectrum of antibiotics disturbs the intestinal flora, resulting in a significant reduction in the number of targets of LicA. At this point, LicA can still exert some antibacterial effects, but due to the significant reduction in the number of regulated microbiota in the intestinal tract, its effect of protecting the pancreas by improving the structure of the microbiota becomes severely weakened.

Dysfunction of glucose metabolism leads to liver damage and affects the repair of liver cells.[47] The liver is one of the main sites for sugar and lipid metabolism, and also the main site for the metabolism of toxic substances.[48] Liver inflammation, degeneration of hepatic adipose tissue and fat accumulation are often observed in the livers of T2DM mice.[49] Multiple studies have shown that the function of liver tissue is severely affected during the development of T2DM.[50,51] Abnormal lipid accumulation in liver cells leads to elevated levels of ALT and AST.[52] In this experiment, the levels of serum ALT and AST in the model group mice were significantly increased ($p < 0.01$), and severe inflammatory cell infiltration, steatosis and fat accumulation occurred in the liver tissue. This is consistent with the research results of Tsai *et al.*[53] After LicA intervention, the levels of ALT and AST were significantly reduced ($p < 0.01$), and LicA had a significant improvement effect on liver lipid accumulation and tissue damage. Consistent with the reduction of fat accumulation, we observed that LicA treatment significantly reduced the expression levels of ACC, FASN and SREBP1c key proteins related to fat production in the liver of db/db mice. In the state of exhausted gut microbiota, the effect of LicA on reducing liver tissue damage and fat accumulation was weakened, confirming the role of gut microbiota in LicA improving liver tissue damage and fat accumulation. The anti-aging gene Sirtuin 1 (*Sirt 1*) is crucial for preventing insulin resistance and liver glucose and lipid metabolism.[54,55] It has been proved that LicA can act as an activator of Sirt 1 and is associated with metabolic liver dysfunction and non-alcoholic fatty liver disease (NAFLD).[56] Therefore, the role of LicA in T2DM mice may be mainly mediated by *Sirt 1*.

Intestinal integrity is the ability of the body to maintain a good regulatory barrier function.[57] Multiple studies have shown that during the period of dysbiosis characterized by metabolic syndrome or obesity, the increased abundance of opportunistic pathogenic bacteria causes intestinal inflammatory responses, thereby increasing LPS levels and leading to damage of intestinal epithelial cells and an increase in intestinal permeability.[58,59] In addition, many traditional Chinese medicines with hypoglycemic effects can also regulate the intestinal flora. Berberine can reduce the abundance of opportunistic bacteria in the intestine of diabetic mice and reduce the level of body inflammation.[60,61] In this study, compared with the model group, LicA significantly reduced the contents of serum LPS, IL-6, TNF-α and IL-1β, and decreased the expression of DAO and D-LA, serum markers of intestinal mucosal injury. The possible reason was that the intestinal barrier was enhanced after LicA intervention, which showed increased expression of occludin, claudin-3 and ZO-1, and the intestinal flora composition was adjusted. HE staining of the ileum and colon also indicated that LicA had a protective effect on the intestinal mucosal barrier. Compared with the antibiotic + LicA group, the LicA group had more significant regulatory effects on the intestinal mucosal barrier function and serum inflammatory factors in db/db mice ($p < 0.01$), suggesting that the intestinal flora played an important role.

After noticing the beneficial effects of LicA, 16S rRNA high-throughput sequencing further revealed the influence of LicA on the structure of intestinal flora. LicA can significantly affect the composition of the intestinal microbiota and reduce the content of LPS. LPS released from bacteria can inhibit the expression of Sirt 1, interrupt the metabolism of liver glycogen, lipoprotein and cholesterol, and aggravate insulin resistance and the development of NAFLD.[62,63] LicA may enhance intestinal integrity by reshaping the composition of the intestinal microbiota and reduce the number of Gram-negative bacteria, thereby decreasing the release of LPS. Spearman's correlation analysis indicated that LicA could significantly reduce the abundance of the opportunistic pathogen p-75-a5. Previous studies on the intervention of natural Chinese herbs in diabetic mice have also shown similar results. After the intervention of polysaccharides from *Rosa roxburghii* tratt fruit in db/db mice, it not only improved the parameters of diabetic mice, but also reduced the proportion of Firmicutes and Bacteroidetes.[64] Mulberry leaves, as a traditional Chinese herbal medicine for treating T2DM, alleviate inflammation and insulin resistance in T2DM mice by reducing the abundance of *Acetatifactor* and *Clostridiales_unclassified* and increasing the abundance of *Akkermansia* and *Bifidobacterium*.[65] These studies further confirm that reducing opportunistic pathogenic bacteria is a key factor in the treatment of T2DM with traditional Chinese medicine.

Wei and Zhu *et al.* have shown that there is a significant correlat hypoglycemic effect of ginsenoside Rg5 mediated partly by modulating gut microbiota dysbiosis in diabetic db/db mice on between the gut flora and the body's circulating metabolites.[66,67] By studying the small molecule metabolites of the microbiome, we can discover the potential mechanism of the interaction between the microbe, metabolite and host.[68] Zhang *et al.* reported that metabolites of the intestinal flora, such as lipopolysaccharides and secondary bile acids, could enter the liver through portal vein circulation, triggering liver inflammation and immune response, leading to liver damage.[69–71] It is worth noting that studies have shown that LicA can be absorbed into the blood circulation through the intestine, and its distribution in the body is targeted, especially in the liver.[72,73] This provides a structural basis for it to directly act on the liver and participate in the regulation of glucose and lipid metabolism.

Therefore, this study identified specific metabolites related to T2DM glucose and lipid metabolism through the analysis of liver metabolites, thereby revealing the relationship between intestinal flora metabolism and T2DM. Based on the Sankey

map, the correlation analysis of harmful flora, harmful metabolites and physiological indexes was constructed, which showed that p-75-a5 may play a leading role in the disturbance of glucose and lipid metabolism through hypotaurine. The abnormal accumulation of hypotaurine will hinder its conversion to taurine, thus interfering with the normal physiological function of taurine.[74] Studies have shown that taurine has multiple roles in metabolic processes, including reducing hepatic lipid accumulation, alleviating oxidative damage, and regulating Ca^{2+} homeostasis.[75] In addition, animal experiments further confirmed that taurine supplementation can improve hyperglycemia and IR in non-insulin-dependent diabetic rats.[76]

However, the mechanism by which the changes of microbiota metabolites affect glycolipid metabolism in T2DM remains unclear and requires further study. In this study, based on the multi-omics joint analysis of the intestinal microbiota, metabolites and proteome, we speculated that LicA may reduce the accumulation of hypotaurine in the liver by reducing the abundance of p-75-a5 bacteria, and then up-regulate liver Acat1 protein expression by activating the PPAR signaling pathway, and ultimately improve the glucose and lipid metabolism disorders in T2DM. Previous studies have shown that in diabetic mouse models, the activation of the PPAR signaling pathway can not only significantly reduce fasting blood glucose and IR, but also reduce liver fat accumulation and improve lipid metabolism disorders.[77] Acat1 is a key enzyme in lipid metabolism. It uses free cholesterol as a substrate to catalyze the synthesis of cholesterol ester, thus promoting the storage of cholesterol ester in intracellular lipid droplets and maintaining the homeostasis of intracellular lipid metabolism such as cholesterol and fatty acids.[78] Molecular docking and western blotting further confirmed that LicA could regulate the expression of ACC, FASN, SREBP-1, IGFBP7, PPARα, PNPT1 and Acat1 proteins.

Conclusions

In summary, our results suggested that LicA could significantly improve the disorder of glucose and lipid metabolism and liver damage in db/db mice. Based on the joint analysis of multi-omics strategies and molecular docking, it was found that LicA played a therapeutic role in T2DM glycolipid metabolism by targeting the intestinal opportunistic pathogen p-75-a5, improving the intestinal barrier, affecting the level of the metabolite hypotaurine, and then regulating the PPAR signaling pathway. This provides a theoretical and practical basis for the development and application of LicA.

Author contributions

Xinyi Han: conceptualization, formal analysis, and writing – original draft. Yaoyi Zheng: investigation and data curation. Yi Li: methodology and visualization. Qingqing Gao: validation and investigation. Changci Tong: methodology and resources. Yunen Liu: project administration, funding acquisition and supervision. Zhonghua Luo: funding acquisition, supervision, and writing – review & editing. All authors contributed to the article and approved the submitted version.

Conflicts of interest

There are no conflicts to declare.

Data availability

The data supporting the findings of this study are available within the article and its supplementary information. Supplementary information (SI) is available. SI includes gut microbiome analysis, basic data of liver non-targeted metabolomics, basic data of liver proteomics, images from WB experiments, as well as the intersecting metabolite IDs and names. See DOI: https://doi.org/10.1039/d5fo03089g.

Acknowledgements

This research was supported by the Basic Scientific Research Project of Liaoning Provincial Department of Education (JYTMS20231399) and Liaoning Province Science and Technology Department Joint Project (2024011888-JH3/4700).

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