Drug Design, Development and Therapy

Dovepress
open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

Unveiling the Pharmacological Mechanisms of Davidiin's Anti-Diabetic Efficacy in Streptozotocin-Treated Rats: A Comprehensive Analysis of Serum Metabolome

Mingming Li[1,2,*], Xin Zhou[1,*], Doudou Huang[1,*], Yingkui Zhao[2], Jiani Chen[2], Zhiying Dong[1], Wansheng Chen[1,2], Feng Zhang[2], Lianna Sun[1]

[1]School of Pharmacy, Shanghai University of Traditional Chinese Medicine, Shanghai, 201203, People's Republic of China; [2]Department of Pharmacy, Changzheng Hospital, Second Military Medical University, Shanghai, 200003, People's Republic of China

*These authors contributed equally to this work

Correspondence: Lianna Sun; Wansheng Chen, School of Pharmacy, Shanghai University of Traditional Chinese Medicine, 1200 Cailun Road, Shanghai, 201203, People's Republic of China, Tel +86-13818946096; +86-15901818646, Email sssnmr@163.com; chenwansheng@shutcm.edu.cn

Background: *Polygonum capitatum* Buch.-Ham. ex D. Don (*P. capitatum*), a traditional herb used in Miao medicine, is renowned for its heart-clearing properties. Davidiin, the primary bioactive component (approximately 1%), has been used to treat various conditions, including diabetes. Given its wide range of effects and the diverse biomolecular pathways involved in diabetes, there is a crucial need to study how davidiin interacts with these pathways to better understand its anti-diabetic properties.

Materials and Methods: Diabetic rats were induced using a high-fat diet and streptozotocin (STZ) administered intraperitoneally at 35 mg/kg. Out of these, 24 rats with blood glucose levels ≥ 11.1 mmol/L and fasting blood glucose levels ≥ 7.0 mmol/L were selected for three experimental groups. These groups were then treated with either metformin (gavage, 140 mg/kg) or davidiin (gavage, 90 mg/kg) for four weeks. After the treatment period, we measured body weight, blood glucose levels, and conducted untargeted metabolic profiling using UPLC-QTOF-MS.

Results: Davidiin has been shown to effectively treat diabetes by reducing blood glucose levels from 30.2 ± 2.6 mmol/L to 25.1 ± 2.4 mmol/L ($P < 0.05$). This effect appears stronger than that of metformin, which lowered glucose levels to 26.5 ± 2.6 mmol/L. The primary outcomes of serum metabolomics are significant changes in lipid and lipid-like molecular profiles. Firstly, davidiin may affect phosphatide metabolism by increasing levels of phosphatidylinositol and sphingosine-1-phosphate. Secondly, davidiin could influence cholesterol metabolism by reducing levels of glycocholic acid and glycochenodeoxycholic acid. Lastly, davidiin might impact steroid hormone metabolism by increasing hepoxilin B3 levels and decreasing prostaglandins.

Conclusion: Our study demonstrates that davidiin modulates various lipid-related metabolic pathways to exert its anti-diabetic effects. These findings offer the first detailed metabolic profile of davidiin's action mechanism, contributing valuable insights to the field of Traditional Chinese Medicine in the context of diabetes treatment.

Keywords: diabetes mellitus, streptozotocin, metformin, davidiin, metabolomics

Introduction

Diabetes mellitus (DM) is a chronic metabolic disease characterized by sustained hyperglycemia, which could damage the endothelial blood vessels, thus leading a range of organ lesions such as cardiovascular disease, retinopathy, diabetic foot, and nephropathy. It is also one of the leading causes of death worldwide.[1–3] Since long-term effective control of blood glucose is essential, exploration of potential anti-diabetic drugs is required for better efficacy and safety. Traditional Chinese medicine (TCM) has always served as a treasure for drug development because of its long-term-practice-proved efficacy and safety.

Received: 23 February 2024
Accepted: 27 May 2024
Published: 5 June 2024

Polygonum capitatum Buch.-Ham. ex D. Don (*P. capitatum*, named Tou Hua Liao in Chinese) is a Miao herb belonging to the family of *Polygonaceae*. It is a heart-clearing drug, and mainly distributes in the southwest of China. Based on the perspective of TCM, DM is an "emaciation-thirst disease", which is caused by internal heat due to deficiency of Yin.[4,5] This condition is traditionally treated by heart-clearing drugs such as *P. capitatum*. Consistently, previous pharmacological studies have shown that the extract of *P. capitatum* has anti-oxidant, hypoglycemic, anti-inflammatory and analgesic effects. And it could inhibit α-glucosidase (α-Gase) in a dose-dependent manner.[6,7] It has been reported that the hypoglycemic activity of *P. capitatum* was associated with the significant improvement of lipid metabolism and antioxidant capacity, as well as the up-regulation of gene expression of Adenosine 5' monophosphate activated protein kinase (AMPK) and Glucose transporter protein (GLUT4).[8] Therefore, *P. capitatum* is a potential TCM for treating DM.

Davidiin, the primary constituent of tannins, has demonstrated a range of biological activities,[9] The percentage of davidiin in Polygonum capitatum was determined using the heating reflux method, the content of davidiin in the plant sample ranges from 1.69 to 10.93 mg/kg, with an approximate concentration of 1%.[10] It is believed to be responsible for the antioxidant and hypoglycemic effects of Polygonum capitatum. Beyond these, davidiin has shown the ability to suppress hepatocellular carcinoma, inhibit SUMO protein modification, and modulate the activity of various receptors, including opioid receptors, dopamine 2 receptors, α2 adrenergic receptors, and β adrenergic receptors.[11–13] These varied effects suggest that davidiin can target multiple biological pathways. Given this, there is a crucial need to study how these various targets are influenced in relation to its anti-diabetic properties. Similarly, our previous experiments showed that both *P. capitatum* extract and davidiin compound had significant hypoglycemic activity in vitro and in vivo. Based on our previous patented invention,[14] it has been confirmed that davidiin is the main active ingredient of *P. capitatum*. This study aims to investigate these mechanisms, contributing to a deeper understanding of davidiin's therapeutic potential. The chemical structure of davidiin is shown in Figure 1A.

The metabolome is an essential tool for studying the causes of DM, and more importantly, for implementing preventive measures against diabetes. DM is a metabolic syndrome, which is accompanied by changes in molecules related to glucose metabolism, lipid metabolism, and protein metabolism[9]. Consistently, the most widely accepted model for DM is induced by STZ injection, which can trigger a series effect to initiate DM. STZ is an antibiotic derived from colorless Streptomyces species, known for its broad spectrum of antimicrobial properties. STZ specifically targets pancreatic islet β cells, initiating a signaling cascade that results in the production of pro-inflammatory cytokines. Ultimately, this process leads to the development of diabetes.[15,16] Furthermore, a growing body of evidence strongly suggests that STZ impacts multiple key enzymes within the energy metabolic pathway, directly contributing to the onset and progression of DM.[17] On the other hand, both metformin and davidiin also have multiple targets along the energy metabolism pathway against DM.[4,18]

Metabolomics, which can offer a comprehensive analysis of the metabolite differences between pathological state and normal state, can be used to reveal the metabolites and molecular pathways associated with the pathological state including DM.[19] This study aimed to investigate the effects of davidiin on endogenous metabolites by non-targeted metabolomics and explore its potential hypoglycemic mechanisms. Our findings provide the first plasma metabolic profile of davidiin's potential anti-diabetic mechanism, and lay a foundation on further TCM studies of DM.

Materials and Methods

Experimental Animals and Reagents

Male Sprague-Dawley (SD) rats were purchased from Shanghai Slack Laboratory Animal Co., Ltd. Shanghai, China, animal license number: SCXK (Shanghai) 2022–0009. *P. capitatum* was purchased from Guizhou Warmen Pharmaceutical Co., Ltd. (Guizhou, China), and it was verified by the corresponding author of this article. Davidiin was extracted from *P. capitatum* using a reported method, and its purity was 83.60%. Metformin and Streptozotocin (STZ) (purity ≥ 99%), high-fat feed, and MAJOR Blood Glucose Monitoring System were purchased from Dalian Meilun Biotechnology Co., Ltd. (Dalian, China), Jieming Department Store, MAJOR Beijing Taierlong Economic and Trade Co., Ltd. (Beijing, China), respectively. All experimental protocols were designed according to the Guide for the

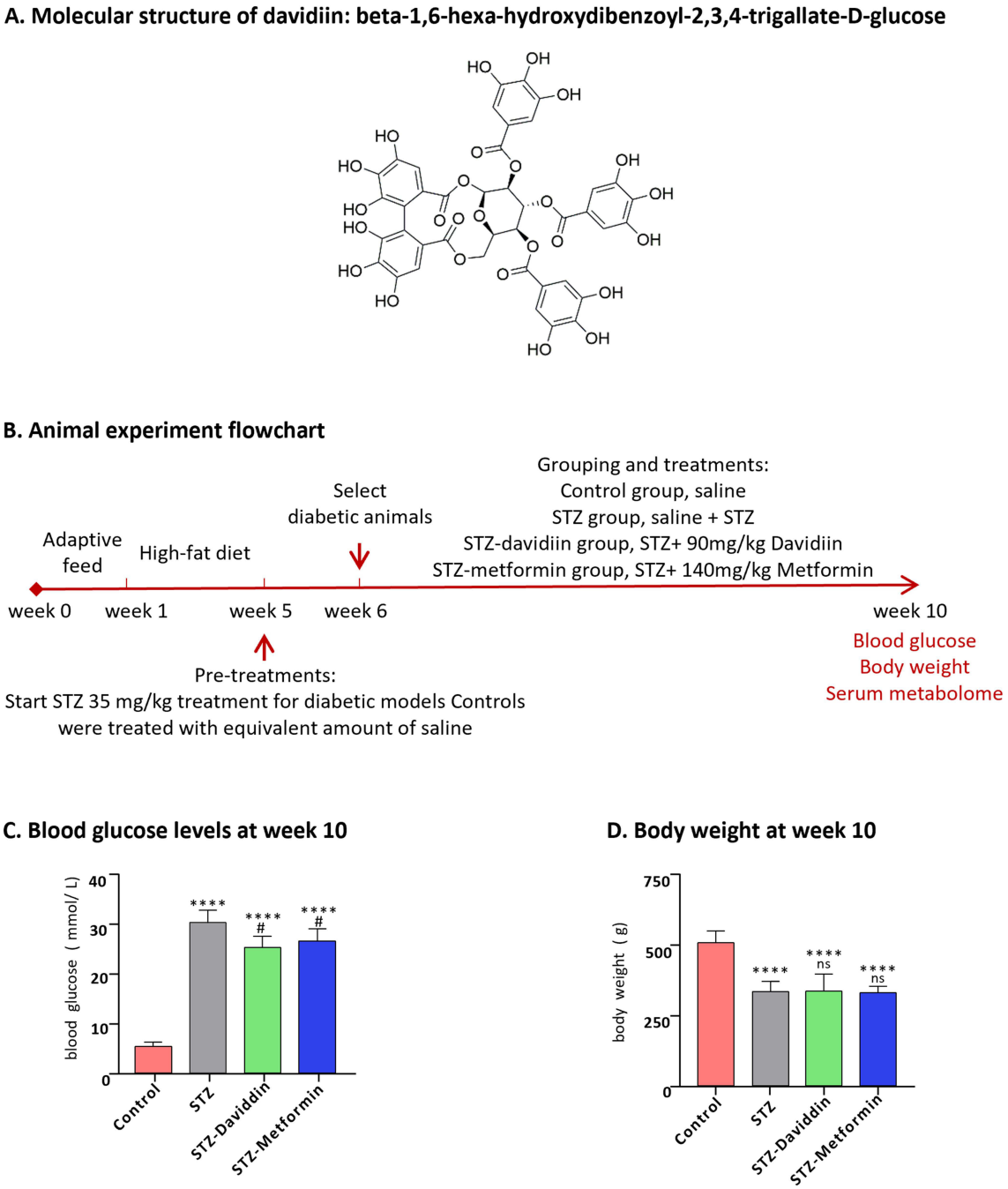

Figure 1 Chemical structure of davidiin and change of blood glucose and body weight. (**A**) Molecular structure of davidiin: Beta-1,6-hexa-hydroxydibenzoyl-2,3,4-trigallate-D-glucose. (**B**) Animal experiment flowchart. (**C**) Blood glucose at week 10 (**D**) Body weight at week 10. The Student's t-test was utilized to compare the control group with the other three groups (denoted by $^{\#}P < 0.05$; and $^{\#\#}P < 0.01$). Additionally, comparisons between the STZ-treated group and the remaining three groups were conducted, with significance levels indicated as $^{*}P < 0.05$ and $^{**}P < 0.01$. Notably, statistical significance at the 0.05 level was marked as $^{*}p < 0.05$ vs Control, and $^{\#}p < 0.05$ vs STZ.

Care and Use of Laboratory Animals (NIH Publication, 8th edition, 2011) and approved by the Ethics Committee of Changzheng Hospital of Shanghai (Permit Number: PZSHUTCM2309070008).

Animal Preparation and Blood Collection

The project refers to the previous modeling method of the research group and the literature report to establish the diabetes model: high-fat feed plus small dose intraperitoneal injection of STZ.[20–23] The overall flowchart of the animal experiment is shown in Figure 1B. A total of 22 male SD rats were raised to 180 ± 2 g. After 1 week of adaptation, 5 rats were randomly

selected to be fed with a normal diet as the control group; the remaining 17 rats were fed with a high-fat diet (18% lard, 1% cholesterol, 0.2% bile salt, 16% sugar, and high protein basic diet) for 4weeks. After another week, the 17 high-fat-fed rats received an intraperitoneal injection of STZ (35 mg/kg) after 12-hour fasting. Subsequently, rats with random blood glucose value ≥ 11.1 mmol/L and fasting blood glucose value ≥ 7.0 mmol/L were selected. Then, 15 rats with the highest fasting blood glucose levels were randomly divided into three treatment groups. These were diabetic model group (STZ group, n=5), STZ-davidiin group (90 mg/kg/d, n=5) and STZ-metformin group (140 mg/kg/d, n=6). The dosage of davidiin was determined based on our prior studies, which tested a series of concentrations (from 5 to 90 mg/kg/d) of Polygonum capitatum (equivalent to davidiin ranging from 3.8 mg/kg/d to 120.27 mg/kg/d) for diabetes treatment.[14,20,24] Dosages of 90 mg/kg/d or higher demonstrated significant and consistent therapeutic effects without any notable side effects, including any damage to the pancreas by histopathological analysis.[14,18] The dosage for metformin was established by both information from published literature and our own previous experimental experience.[25,26] The control group and STZ groups were given an equal volume of physiological saline by gavage. All treatments were carried out at 8:30 am every day for 4 weeks. Fasting blood glucose and body weight were measured and blood samples were collected from the orbital venous plexus after 12-hour fasting at 9:30 am at week 10 after drug administration. The blood samples were centrifuged at 1200 g for 10 min, and the supernatant was collected and stored at −80°C until subsequent analysis.

UHPLC-Q-TOF-MS Analysis

The blood supernatant was spiked with 1 ug/mL puerarin as an internal standard and mixed with methanol (1:3, V/V) to precipitate protein. The mixture was then vortexed for 30s and centrifuged at 13,000 r/min for 15min. A volume of 5 μL of the supernatant was loaded on the Accurate-Mass Q-TOF LC/MS system (Agilent Technologies, Santa Clara, US) in positive Dual Agilent Jet Stream Electrospray Ionization (Dual AJS ESI) mode (Agilent Technologies, Santa Clara, US).

The column was an ACQUITY UPLC®HSS T3 column (2.1 mm × 100 mm, 1.8 μm), kept at 45 °C. Briefly, the mobile phase consisted of 0.2% formic acid (A) (V/V) and acetonitrile (B) with a gradient started with 45% B, kept steady for 9 min then increased to 60% at 9.5 min, 60% at 18 min, 95% at 20 min, followed by a post-run of 5 min. The flow rate was kept at 0.3 mL/min constantly. The quality control (QC) sample was injected after every 10 non-QC sample. The other parameters were set as follows: desolvation gas temperature, 350 °C; desolvation gas flow, 11 L/min, sheath gas temperature, 350 °C, sheath gas flow, 11 L/min; capillary voltage, 2000 V.

Data Analysis

The acquired MS data was analyzed by the Profinder program (Version b8.0, Agilent Technologies, Santa Clara, US). After integration and alignment, a list of spectral features was obtained with retention time (RT), m/z, and spectral area by recursive feature extraction. The spectral features generated by the internal standard, noise and column bleed were removed from the data set. Then, the integration results were manually checked before they were transferred to the Mass Profiler Professional program (Agilent Technologies, Santa Clara, US) for subsequent analysis. The background, nonbiologically relevant information, and mask effect were eliminated according to the 80% rule.[27]

The online statistical tool Metaboanalyst 3.0 (https://www.metaboanalyst.ca) was used for further analyses.[28] P-value < 0.05 was considered statistically significant. Principal component analysis (PCA) was applied to exam data distribution, and to get a comprehensive and complete understanding of the metabolic profile. Partial least squares discriminant analysis (PLS-DA) was carried out to focus on clustering information and visualize the metabolic alterations in all groups at week 3 and 5. Student's *t*-test was performed between two groups to select biomarker candidates. Spectral features with a low P-value (< 0.05) and a high fold change (FC) (≥ 2) in Student's *t*-test, or with a high value of variable importance in the projection scores (> 1) based on PLS-DA model were added to the candidate list for further metabolite identification. These metabolites were identified by an integrated method which includes comparing to commercially approached standards and web-based spectrum databases such as Human Metabolome Database (https://www.hmdb.ca) and METLIN (https://metlin.scripps.edu).[29,30]

Results

Weight and Blood Glucose

In our experiment, the mortality rate was 0% in the normal group. In contrast, the STZ group experienced a 20.0% mortality rate (one out of five rats died). The STZ-davidiin group had a slightly lower mortality rate of 16.7% (one out of six rats died), while the STZ-metformin group recorded no deaths (0% mortality rate among six rats). These mortality rates fall within the expected range for STZ-induced type 2 diabetes in rats.[21,22,31]

After five weeks of treatment with STZ, blood glucose levels in rats significantly increased from 5.3 ± 0.9 to 30.2 ± 2.6 mmol/L compared to the control group, success-fully establishing the STZ-induced diabetes model (Figure 1C). This method is commonly used to create experimental diabetes models in animals. These findings align with those reported in the existing literature.[22,32] In this model, both metformin and davidiin significantly reduced blood glucose levels, demonstrating their effectiveness in managing hyperglycemia. Despite the reductions, blood glucose levels in treated rats remained above normal levels (25.1 ± 2.4 mmol/L for davidiin and 26.5 ± 2.6 mmol/L for metformin), indicating that while these treatments effectively lower high blood glucose, they do not completely restore normal levels. Notably, davidiin was found to be more effective than metformin in reducing blood glucose levels.

Regarding body weight, the STZ group experienced a significant decrease from 504.4 ± 42.7 to 327.8 ± 40.9 g ($P < 0.0001$). The body weight in the STZ-metformin and STZ-davidiin groups also decreased, reaching 343.75 ± 65.29 g and 328.83 ± 22.30 g ($P < 0.0001$), respectively. Although both the STZ-metformin and STZ-davidiin groups had lower body weights compared to the control, their body weights were not significantly different from the STZ group (Figure 1D).

Serum Metabolic Profiles

The typical metabolic profiles for the control group, STZ group, STZ-davidiin group, and STZ-metformin group are illustrated in Figure 2 with peaks include a mix of endogenous metabolites and drug-derived metabolites. Detailed data tables listing all the positive and negative ions detected in each sample are available in Supplementary Tables 1 and 2. Additionally, summary tables that visually repre-sent the key TIC peaks in Figure 2 for both positive and negative ions can be found in Supplementary Tables 3 and 4.

In the total ion chromatogram, the positive ionization mode yielded significantly more ions than the negative mode, recording 20,922 versus 3491 ions respectively. Among the treatment groups, the general profile of the total ion chromatograms displayed similar overall shapes but with notable differences, especially in the number of peaks observed. Specifically, the STZ, STZ-davidiin, and STZ-metformin groups exhibited more peaks compared to the control group, predominantly appearing between 10 to 16 minutes retention time. This observation indicates that the serum metabolism of rats was altered following STZ-induced type 2 diabetes, evidenced by a significant disturbance in the serum metabolome. The variation in the number and height of peaks among these groups highlights the broader physiological impacts of the treatments. These metabolic changes emphasize the need for a comprehensive metabolomic analysis to delve deeper into the broader implications of these treatments on metabolic pathways and biological processes.

Differential Analysis of Serum Metabolomics Amongst the Four Rat Groups

Compounds were identified and endogenous metabolites filtered based on their chemical composition. Lipids and lipid-like molecules emerged as the most prevalent chemical class, totaling 270 compounds (Figure 3A). Among these, 48 decreased and 222 increased when comparing the control group with the STZ group. In the comparisons of STZ-metformin versus STZ, 23 compounds decreased and 138 increased, while in STZ-davidiin versus STZ, 32 decreased and 104 increased. This pattern of change was consistent across the control, STZ-metformin, and STZ-davidiin groups in comparison to the STZ group, which served as the diabetic model. Thus, both metformin and davidiin treatments appear to significantly counteract the biomolecular effects induced by STZ.

PCA and PLS-DA based on these differential metabolites demonstrated relative-ly strong separation among groups (Figure 3B and C). The control group and the STZ group showed the least intra-group variation and were distinctly separate, with no overlapping prediction areas in both PCA and PLS-DA. However, the STZ-metformin and STZ-davidiin groups

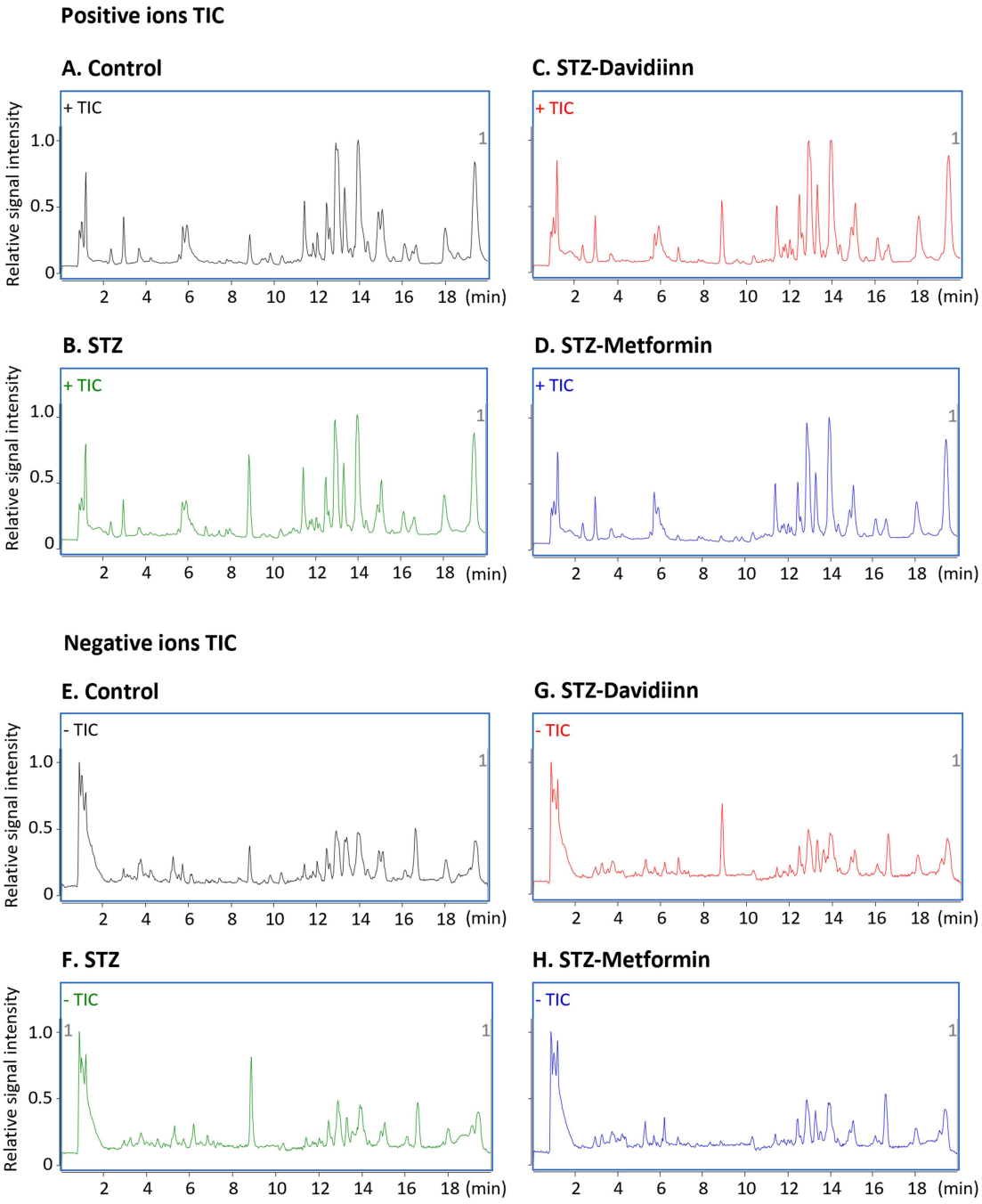

Figure 2 Typical total ion chromatograms (TIC) via UHPLC-QTOF-MS. The x-axis represents retention time (minutes), and the y-axis represents intensity (arbitrary units). The groups depicted are: (**A** and **E**) control group; (**B** and **F**) STZ-treated group; (**C** and **G**) STZ-davidiin group; and (**D** and **H**) STZ-metformin group.

exhibited higher intra-group variation. Despite this, the center of their prediction areas was located between the control and STZ groups, with this trend being more pronounced in PLS-DA.

Functional Analysis of Differential Serum Metabolomics Amongst the Four Rat Groups

Pathway analysis reveals that Transport of Small Molecules, Metabolism of Lipids and Immune System are the 3 major enriched pathway categories exhibiting significant changes among the four rat groups in Figure 4A. Within the pathways showing significant changes, in comparison to the STZ group, the STZ-davidiin group showed downregulated Synthesis of Prostaglandins (PG) and Thromboxanes (TX), Eicosanoid ligand-binding receptors, Acyl chain remodeling of CL. The

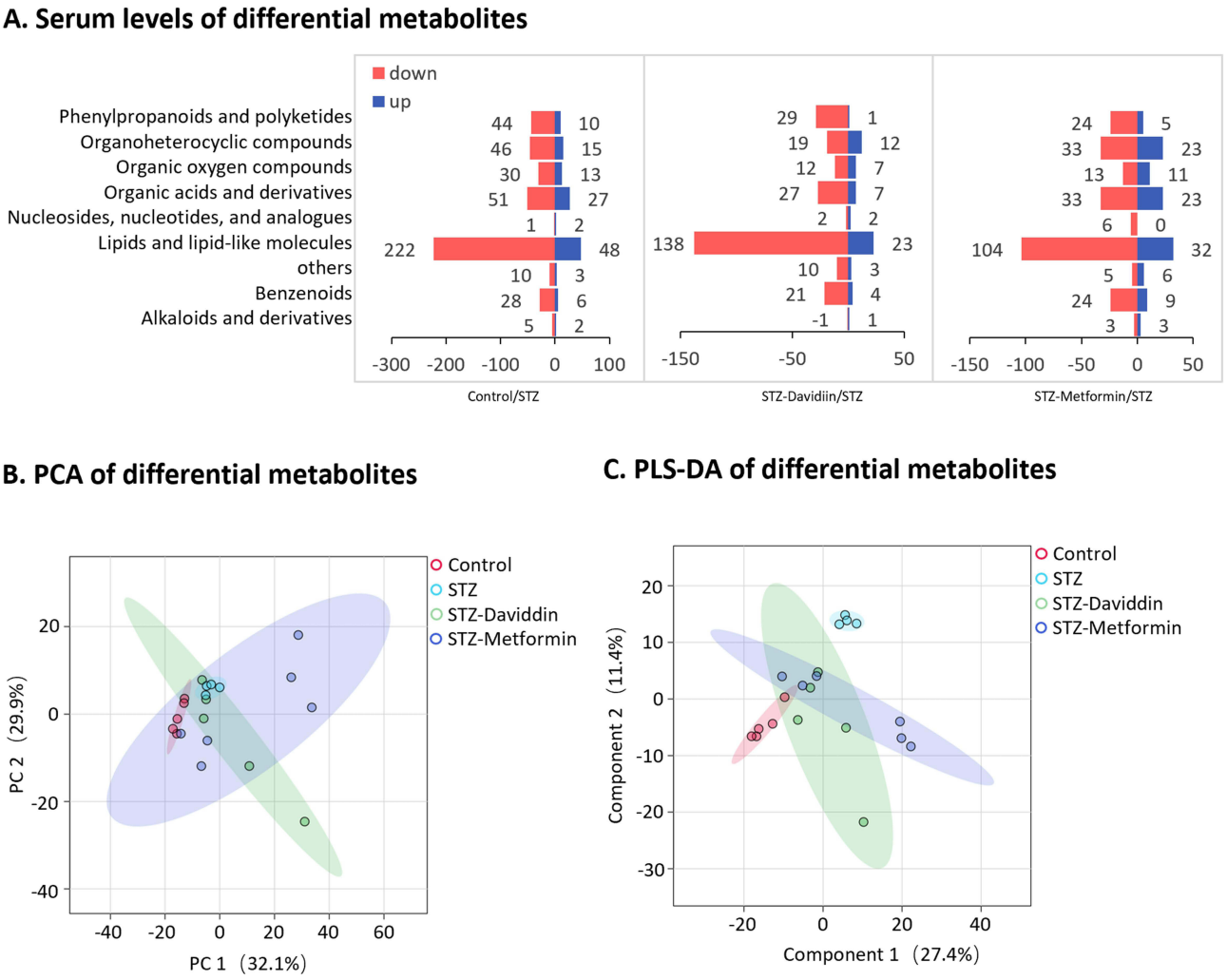

Figure 3 Serum levels of differential metabolites among four groups. (**A**) Serum levels of differential metabolites; (**B**) PCA of differential metabolites; (**C**) PLS-DA of differential metabolites.

STZ-metformin group showed downregulated Transport of organic anions, Arachidonic acid metabolism, Synthesis of PG and TX.

Both of metformin and davidiin seems not directly affect the phosphatide abundant, On the other hand, they showed significant influence on lysophosphatidic acid (LPA) and its metabolites (Figure 4B and C). Elevated LPA does not contribute to recovery of insulin resistance, it might be a side effect of metformin.

Next, we tried to reveal the underline mechanism of pathological and pharmacological mechanisms by STZ, metformin and davidiin based on those metabolome changes we have identified. Firstly, differential compounds from those significantly altered metabolome pathways and key compounds selected through PLS-DA were adopted for bioinformatic analysis. Subsequently, literature on how the selected compounds were involved in diabetes-related molecule mechanism, such as glucose metabolism and lipid metabolism were reviewed and studied. At last, based on the current knowledge of this field and the selected molecules, we produced a metabolic network described the most likely mechanism how STZ induced diabetes and how metformin and davidiin prevented it (Figure 5). This detail of this pathway network was discussed in the discussion section.

Discussion

STZ damages rat through multiple targets, disrupting normal glucose and lipid metabolism, thereby promoting the onset of diabetes. The anti-diabetic effects of metformin and davidiin are supported by both lowered glucose levels and

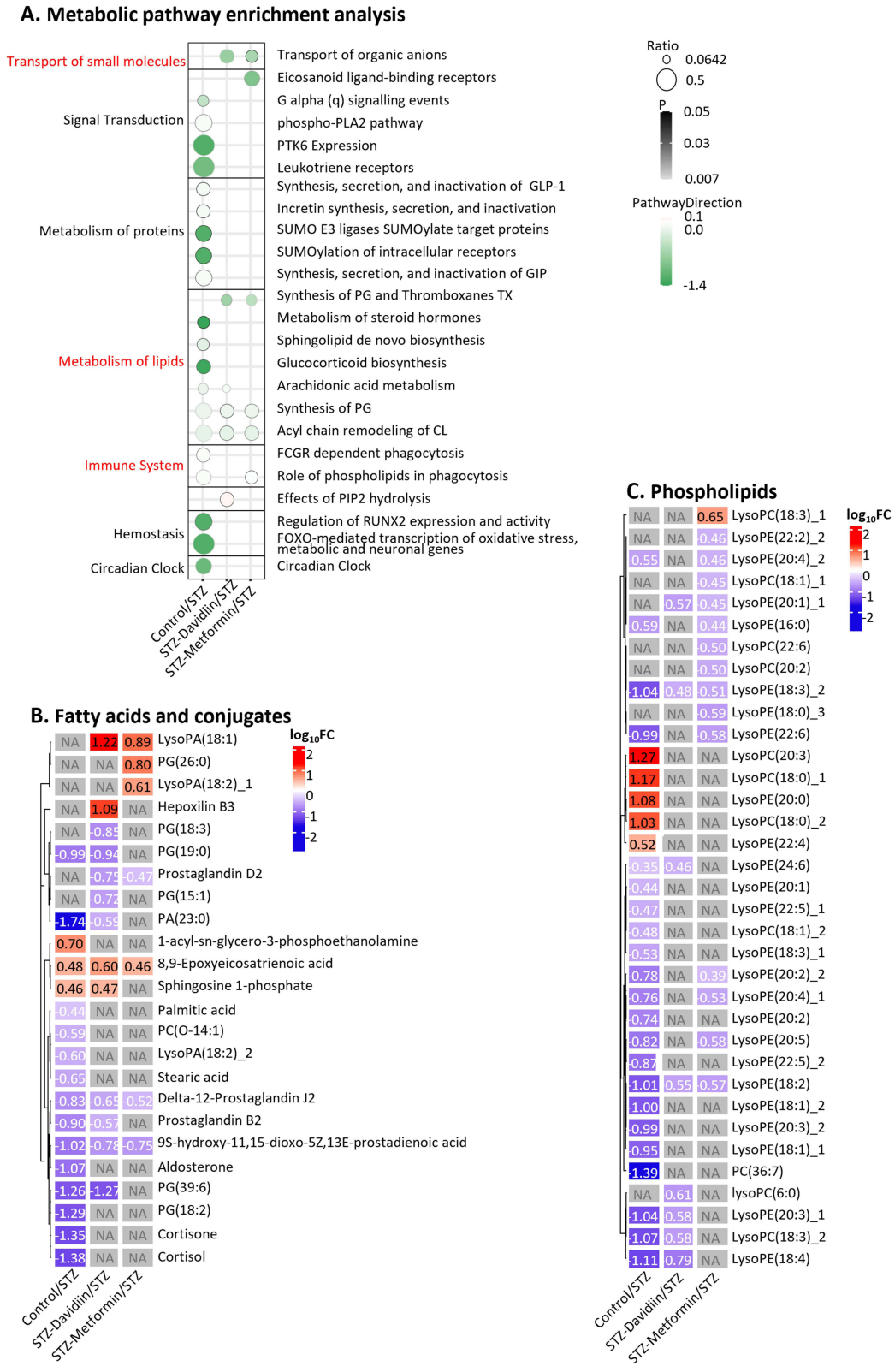

Figure 4 Metabolic pathway enrichment analysis and Heatmap and clustering analysis of metabolites among four rat groups. (**A**) Metabolic pathway enrichment analysis; (**B**) Heatmap of fatty acids and conjugates; (**C**) Heatmap of phospholipids.

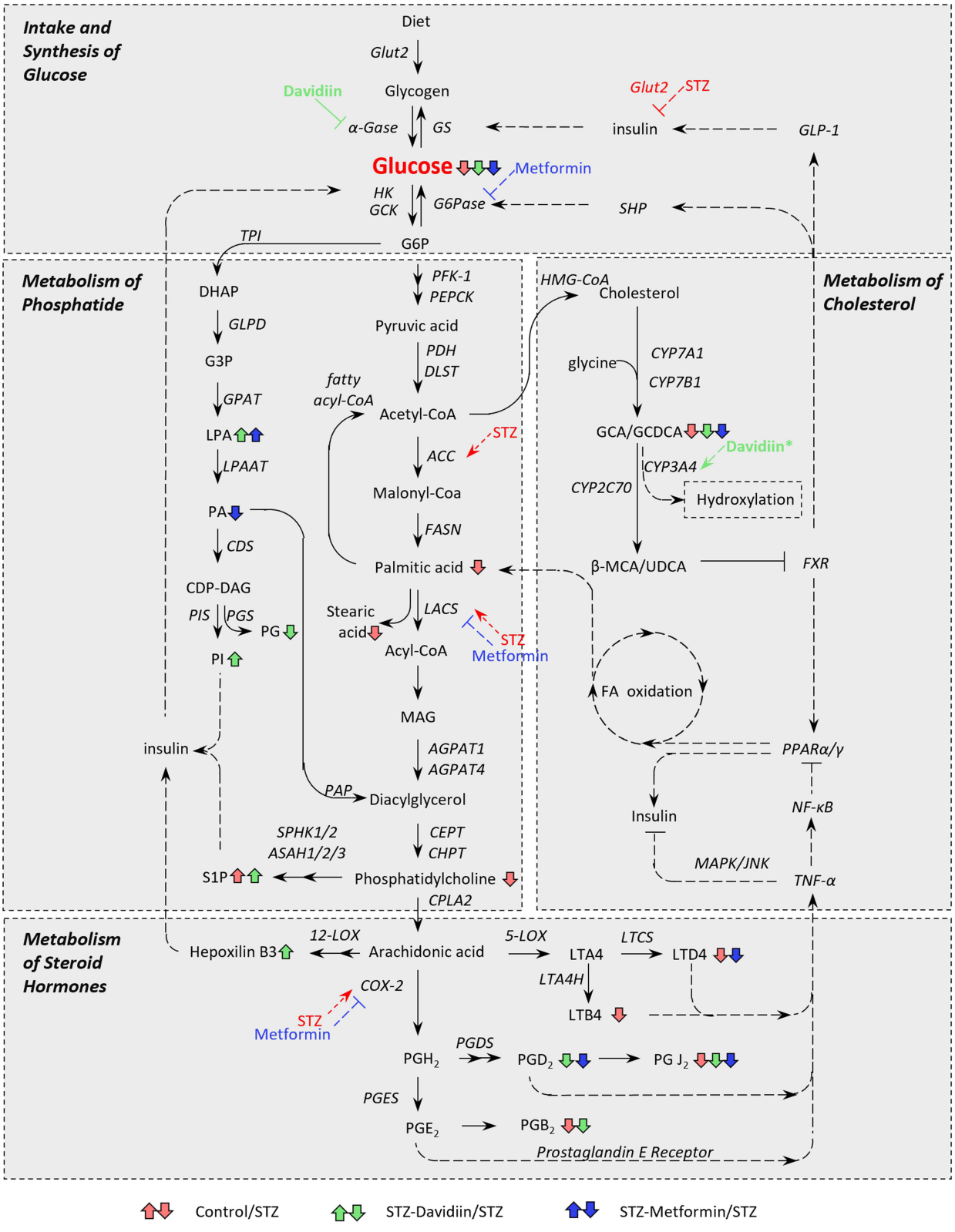

Figure 5 Metformin and davidiin promoting anti-Diabetic effects through altered lipid metabolism. A metabolic network based on intake and synthesis of Glucose, metabolism of phosphatide, cholesterol and steroid hormones was constructed to describe the most likely mechanism how STZ induced diabetes and how metformin and davidiin prevented it.

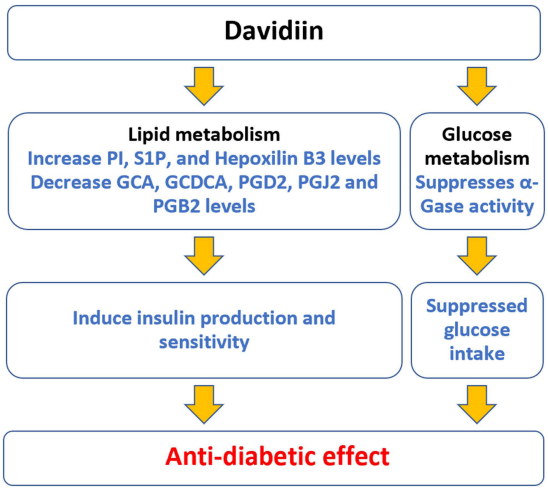

Figure 6 A proposed mechanism of davidiin's anti-diabetic action. Davidiin exerts itshypoglycemic effect by inhibiting α-Gase to reduce glucose uptake and affecting multiple products in the lipid metabolism pathway to increase insulin synthesis and sensitivity.

metabolomic analyses. In our experiment, both metformin and davidiin significantly reduced the rise in blood glucose induced by STZ, with davidiin demonstrating a greater hypoglycemic effect compared to metformin. At the same time, both davidiin and metformin are multi-target drugs, which can counteract the negative effects of STZ by influencing various genes involved in glucose and lipid metabolism.[15,16,33,34] The following discussion will primarily focus on the functional effects of the STZ-induced metabolome on the diabetes phenotype. This is followed by a discussion of the beneficial functional effects of the davidiin-induced metabolome on the diabetes phenotype and a comparison with the effects of metformin. And the discussion is summarized graphically in Figure 5 and succinctly summarized in Figure 6, highlighting Davidiin's proposed anti-diabetic mechanism and its potential therapeutic implications.

Intake and Synthesis of Glucose

The accumulation of glucose in the body is not only a consequence of various factors contributing to diabetes, but it also stands as a primary instigator of symptoms and complications associated with different types of diabetes. Carbohydrate nutrients from food are converted into glycogen via the Glut2 transporter protein. Glycogen is enzymatically converted into glucose by α-Gase. Subsequently, it undergoes metabolism through the glycolytic pathway, resulting in the generation of pyruvic acid and acetyl coenzyme A, which are then utilized in lipid synthesis. The changes in blood glucose levels following treatments align with these expectations in this study. These results also correspond with previous literature, where STZ is known to inhibit the expression of the Glut2 transporter protein, metformin can decrease the expression of glucose 6-phosphatase (G6Pase) in the gluconeogenesis pathway.[17,18] For the suppression of glucose intake, α-Gase inhibition may play a key role. Davidiin, the primary active compound in polygonum capitatum, makes up about 1% of the plant's composition.[14,20] Research has shown that an 80% ethanol extract of Polygonum capitatum has α-Gase inhibitory activity.[6] Polygonum capitatum can mitigate lipid metabolism disorders by upregulating the expression of AMPK and GLUT4 genes in liver tissues, promoting glucose uptake.[8] It has been shown to influence the metabolism of phosphatides, arachidonic acid, steroid hormones, and cholesterol.[7] These changes might indirectly contribute to a hypoglycemic effect. These factors directly influence the synthesis and metabolism of glucose.

Metabolism of Phosphatide

The body's most abundant glycerophospholipids are phosphatidylcholine and phosphatidylethanolamine, comprising over 75% of the total phospholipids. Changes in PC and/or PE content in various tissues have been reported to be associated with insulin resistance and obesity.[35] Fatty acids and 3-phosphoglycerat, the basic raw materials of glycerol phospholipid synthesis, primarily derived from glucose metabolism.[36] Glycerophospholipids is a metabolic intersection of energy metabolism, which link glucose metabolism and types of lipid metabolisms.

It was reported that STZ can increase acetyl-CoA carboxylase (ACC), and long-chain acyl-coenzyme A synthetase (LACS),[17] key enzymes on the phospholipid metabolism pathway. Consistently, in the STZ-group, we have found that important products on the ACC and LACS pathways, palmitic acid, stearic acid and phosphatidylcholine were upregulated. Stearate is obtained in the diet or through the extended synthesis of palmitate, the main product of the fatty acid synthase system in animal cells. Like palmitate, stearate is the primary substrate of stearyl-CoA desaturase that catalyzes the conversion of stearate to oleate, which is the preferred substrate for the synthesis of triglyceride and other complex lipids. In rat, targeted disruption of the stearyl-CoA desaturase-1 gene results in the production of a lean mouse that is resistant to diet-induced obesity and insulin resistance.[37] Thus, the downregulation of palmitic acid, stearic acid, and phosphatidylcholine may indicate a diminished metabolic capacity of phosphate.

Both of metformin and davidiin seems not directly affect the phosphatide abundant, which is consistent with the observation that they also showed no significant influence on the overall body weight. On the other hand, they showed significant influence on LPA and its metabolites. Elevated LPA does not contribute to recovery of insulin resistance,[38,39] it might be a side effect of metformin.

On top of this, davidiin's influence on metabolism of phosphatide seems stronger than metformin, where the STZ-davidiin group up-regulated phosphatidylinositol (PI) and sphingosine-1-phosphate (S1P), downregulated PG. Both PI and PG are derived from CDP-DAG synthesized under the action of different enzymes. It has been reported that adipocyte PI biosynthesis through the gonad circulation can prevent insulin resistance.[40] PG is a mitochondrial phospholipid involved in various metabolic diseases, and it has been reported that loss of the remodeling acyltransferase LPGAT1 of phosphatidylglycerol (PG) significantly impairs insulin signaling in the liver.[41] S1P is a multifunctional bioactive lipid mediator, S1P can promote β cell proliferation and antagonize its apoptosis and insulin resistance in muscle tissue, playing a positive role in preventing and delaying the development of T2DM.[42] We are the first to report that davidiin can raise S1P. Therefore, we speculate that davidiin enhanced phospholipid metabolism promotes PI and S1P expression to play a hypoglycemic effect.

Metabolism of Arachidonic Acid and Steroid Hormones

Phosphatide can be utilized to synthesis Arachidonic Acid (AA) through phosphatidylcholine. AA has anti-inflammatory and anti-diabetic actions by enhancing the production of its anti-inflammatory metabolite.[43] AA can be metabolized in the body through three different pathways, regulated by cyclooxygenases (COX), lipoxygenases (LOX), and cytochrome P450 (CYP450), leading to synthesis of Steroid Hormones.[44] The metabolic alterations associated with davidiin exhibit potential anti-diabetic effects on downstream metabolites of both COX and LOX, as suggested by findings in published literature.

AA could be converted into hepoxilins and leukotrienes (LTs) through the enzymatic activity of 12-LOX and 5-LOX respectively. Hepoxilin, particularly hepoxilin B3, has been shown to directly stimulate insulin release.[45] Notably, davidiin appears to positively influence 12-LOX activity, as evidenced by an increase in hepoxilin B3 levels. Additionally, AA can be transformed into LT A4 by 5-LOX, subsequently leading to the generation of LT D4 and LT B4. These LTs have the potential to induce the production of inflammatory factors such as TNF-α, ultimately contributing to impaired insulin signaling.[45–47] While our data revealed a downregulation LTs with metformin treatment, such an effect was not observed with davidiin.

AA could be converted into prostaglandin H2 (PGH2) through the enzymatic activity COX2, which can be further converted to all the other prostaglandins, such as PGD2, PGJ2, PGE2, PGB2 with a series of enzymatic reactions. It is known that STZ could increase the expression of inflammatory mediators such as TNF- α and COX2 in rat spinal cord lysates.[48] And this negative effect could be reversed by metformin by suppression of COX2.[17] The PGs have distinct influence on the insulin production and sensitivity. Inhibition of PGD2 receptor GPR44 can reduce plasma TNF-α level, increasing insulin sensitivity; PGE, can promote TNF- α and IL-6, leading to inflammation in adipose tissue and ultimately insulin resistance.[49–51] In line with the prevailing understanding of PGs and their impact on insulin sensitivity, our observations indicate a relatively lower level of PGB2, a direct product of PGE2, in the control group compared to the STZ-treated group. Metformin exhibits downregulation of PGD2, while davidiin demonstrates downregulation of both PGB2 and PGD2. Moreover, PGJ2, recognized as a PPAR-γ agonist, attenuates TNF-α-induced glycerol release, thereby reducing serum free fatty acids and enhancing insulin sensitivity.[52–56] It was also found that *P. capitatum* can inhibit TNF- α generation though PGE2 in the body.[55] These results indicated that both metformin and davidiin

effectively downregulate PGJ2, counteracting the impact of STZ. This suggests that the beneficial effect of PGD2 (or PGE2) downregulation on insulin sensitivity outweighs the potentially adverse effects associated with PGJ2 downregulation.

Our study reveals, for the first time, showed that the anti-diabetic mechanisms of davidiin may involve increased insulin sensitivity through the upregulation of hepoxilin B3, as well as the regulation of steroid hormones to inhibit TNF-α levels and promote PPAR expression.

Metabolism of Cholesterol

Phosphatides can be utilized for the synthesis of cholesterol via acetyl-CoA, which, in turn, contributes to the production of bile acids.[36] Under the influence of CYP2C70, glycocholic acid (GCA) and glycochenodeoxycholic acid (GCDCA) can be converted into β-MCA and UDCA. Our findings indicate that STZ increases GCA and GCDCA levels, a phenomenon effectively mitigated by both davidiin and metformin. The impact of davidiin may be attributed to the observed induction of CYP3A4-mediated hydroxylation of GCA and GCDCA, as suggested by previous studies on polygonum.[4,57]

These findings align well with the potential pro-diabetic effects of β-MCA and UDCA through FXR suppression.[58] Firstly, the intestinal FXR signaling pathway directly stimulates the secretion of GLP-1 and insulin.[59] Secondly, FXR promotes fatty acid consumption by activating fatty acid β-oxidation.[60,61] Thirdly, the FXR/SHP pathway inhibits PEPCK and G6Pase, thereby suppressing gluconeogenesis and enhancing glucose tolerance and insulin sensitivity.[62] The results suggest that davidiin may modulate the bile acid pool by promoting CYP3A4 expression, leading to a hypoglycemic effect through the FXR-GLP-1/PPAR/SHP pathway.

Here, we have discovered that metformin and davidiin target different molecular pathways, a finding supported by three key factors. First, metformin and davidiin possess distinct molecular structures, leading to different interactions with biomolecules and hence, unique mechanisms of action. This structural variance not only explains their distinct therapeutic effects but also why each can treat different conditions. For instance, metformin is known to target multiple pathways associated with type 2 diabetes management.[63–65] Second, DM is a complex and heterogeneous metabolic disorder with diverse underlying causes, suggesting that targeting multiple pathways may be beneficial for anti-diabetic effects. Both metformin and davidiin impact lipid metabolism, though through different mechanisms.[66–68] Finally, advancements in metabolomics have enhanced our ability to investigate these mechanisms in greater detail, as demonstrated in this study.

Limitation and Future Direction Research

Our study, while comprehensive in its analysis of davidiin's anti-diabetic effects through serum metabolomic profiling, encounters several limitations. Firstly, the translation of results from animal models to human patients remains a significant challenge, as physiological responses in humans can differ considerably. Furthermore, while we have demonstrated notable effects on certain metabolic pathways, our study has not fully explored the mechanistic bases of these changes at the molecular level. The interactions between davidiin and specific metabolic enzymes or receptors were inferred but not directly confirmed. Also, the metabolic pathways were analyzed at specific time points, which may not fully capture the dynamic changes over time or the potential delayed effects of davidiin treatment.

Despite its limitations, the current study has unveiled several potential anti-diabetic mechanisms of davidiin. Future research should aim to translate these findings into clinical settings to evaluate davidiin's efficacy and safety in human subjects. A more detailed investigation into the molecular interactions between davidiin and key metabolic enzymes or receptors, using both in vitro and in vivo methods, would enhance our understanding of its mechanisms. Longitudinal studies are also essential to monitor the long-term effects and stability of metabolic changes induced by davidiin. Broadening the scope of research to include transcriptomic and proteomic analyses could deepen our insight into the metabolic shifts and aid in identifying new therapeutic targets. Additionally, the design of our study could be applied to investigate the potential pharmacological mechanisms of other TCMs beyond davidiin. By utilizing advanced metabolomic techniques and other analytical tools, our understanding and application of TCM could be significantly expanded, ultimately providing more options for disease treatment.

Conclusion

Davidiin has demonstrated effectiveness in treating diabetes by reducing blood glucose levels from 30.2 ± 2.6 mmol/L to 25.1 ± 2.4 mmol/L ($P < 0.05$). This effect is also seemingly more potent than metformin, which lowered glucose levels to 26.5 ± 2.6 mmol/L. This indicates davidiin's potential as a viable anti-diabetic treatment option.

Serum metabolomics analysis has revealed potential metabolic pathways that contribute to the anti-diabetic effects of davidiin and metformin. The primary effects are notable changes in lipid and lipid-like molecular profiles, which can be grouped into three categories. First, davidiin may alter phosphatide metabolism by increasing levels of PI and S1P, possibly through the induction of LACS or ACC. Second, davidiin seems to impact cholesterol metabolism by reducing levels of GCA and GCDCA, potentially by inducing CYP3A4. Finally, davidiin may affect steroid hormone metabolism by increasing hepoxilin B3 and decreasing prostaglandins, which could be related to the induction of COX-2. In phosphatide metabolism and steroid hormone metabolism, davidiin exhibited a more substantial impact than metformin, with a greater number of affected metabolites. These findings offer new insights into davidiin's anti-diabetic mechanisms, providing a solid foundation for further studies in TCM for DM.

Abbreviations

AA, arachidonic acid; ACC, acetyl-coA carboxylase; AMPK, adenosine 5' monophosphate activated protein kinase; CDP-DAG, cytidine diphosphate diacylglycerol; COX, cyclooxygenases; CYP450, cytochrome P450; CYP2C70, cytochrome P450 family 2 subfamily c polypeptide 70; CYP3A4, cytochrome P450 family 3 subfamily A member 4; DM, diabetes mellitus; FXR, farnesoid x activated receptor; G6Pase, glucose 6-phosphatase; GCA, glycocholic acid; GCDCA, glycochenodeoxycholic Acid; GLP-1, glucagon-like peptide-1; Glut4, glucose transporter protein 4; LACS, long-chain acyl-coenzyme A synthetase; LOX, lipoxygenases; LPA, lysophosphatidic acid; LT, leukotriene; LT B4, leukotriene B4; LT D4, leukotriene D4; PGB2, prostaglandin B2; PC, phosphatidylcholine; PCA, principal component analysis; PEPCK, phosphoenolpyruvate carboxykinase; PG, phosphatidylglycerol; PI, phosphatidylinositol; PGD2, prostaglandin D2; PGE2, prostaglandin E2; PGH2, prostaglandin H2; PGJ2, prostaglandins J2; PLS-DA, partial least squares discriminant analysis; PPAR, peroxisome proliferator-activated receptors; QC, quality control; S1P, sphingosine-1-phosphate; SD, Sprague-Dawley; SHP, small heterodimer partner; STZ, streptozotocin; TCM, traditional Chinese medicine; TCA, taurocholic acid, TX, thromboxanes, P. capitatum, Polygonum capitatum; UDCA, ursodeoxycholic acid, α -Gase, α -glucosidase; β-MCA, β-Muricholic acid.

Data Sharing Statement

The data generated in this study are available from the corresponding author upon request.

Ethics Approval and Consent to Participate

The animal study was reviewed and approved by the Animal Ethic Committee of Second Military Medical University.

Consent for Publication

Written informed consent was obtained from all researchers before writing this manuscript.

Acknowledgments

We want to express our gratitude to the current and former members of our laboratories and collaborators for their contributions to this article.

Author Contributions

Mingming Li, Xin Zhou, Doudou Huang are the first authors. Lianna Sun and Zhiying Dong are the corresponding author. All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

This study was supported by National Key Research and Development Program of China (2022YFC3501700), National Natural Science Foundation of China (82274059), Science and Technology Commission of Shanghai Municipality (22S21901900), and the Shanghai Municipal Health Commission (ZYCC2019018).

Disclosure

The authors declare that they have no competing interests in this work.

References

1. Hu J, Ye M, Zhou Z. Aptamers: novel diagnostic and therapeutic tools for diabetes mellitus and metabolic diseases. *J Mol Med*. 2017;95:249–256. doi:10.1007/s00109-016-1485-1
2. Wang X, Lv S, Huang D, Chen W, Sun L. Hypoglycemic activity of extracts from leaves of Terminalia catappa. *Chinese Traditional Patent Med*. 2018;40:2531–2535. doi:10.3969/j.issn.1001-1528.2018.11.032
3. Zhou B, Lu Y, Hajifathalian K; NCD Risk Factor Collaboration (NCD-RisC). Worldwide trends in diabetes since 1980: a pooled analysis of 751 population-based studies with 4.4 million participants. *Lancet*. 2016;387:1513–1530. doi:10.1016/S0140-6736(16)00618-8
4. Zheng L, Lu Y, Cao X, et al. Evaluation of the impact of Polygonum capitatum, a traditional Chinese herbal medicine, on rat hepatic cytochrome P450 enzymes by using a cocktail of probe drugs. *J Ethnopharmacol*. 2014;158:Pt A:276–82. PMID: 25446640. doi:10.1016/j.jep.2014.10.031
5. Zhou F, Chen X. Status of Traditional Chinese Medicine Constitutional Theory in Diabetes Mellitus. *China J Chin Med*. 2015;30:343–345+348. doi:10.16368/j.issn.1674-8999.2015.03.115
6. Chen B, Li C, Chang X, Kang W. Inhibitory activity of polygonum capitatum to α-Glucosidase. *Chin J Exp Traditional Med Formulae*. 2010;16:151–153. doi:10.13422/j.cnki.syfjx.2010.08.055
7. Tong N, Wu M, Wang J, Chen P, Huang S. Study on in vitro hypoglycemic effect and mechanism of Polygonum capitatum. *Chin Traditional Herbal Drugs*. 2017;48:3401–3407. doi:10.7501/j.issn.0253-2670.2017.16.024
8. Liu B, Tong N, Li Y, Huang S. Hypoglycemic mechanism of polygonum capitatum extract on spontaneous model of type 2 Diabetic db/db Mice. *Chin Pharm J*. 2017;52:384–390. doi:10.11669/cpj.2017.05.011
9. Ma J. *Studies on Metabolism of FR429, a Bioactive Ellagitannin from Miao Herb Polygonum Capitatum*. Peking Union Medical College; 2013.
10. Yang Y, Hong Q, Zhu B, Zhou Z, Yang J. Quality standard for Polygonum capitatum. *Chinese Traditional Patent Med*. 2020;42(2):408–415.
11. Wang Y, Ma J, Chow SC, et al. A potential antitumor ellagitannin, davidiin, inhib-ited hepatocellular tumor growth by targeting EZH2. *Tumour Biol*. 2014;35(1):205–212. doi:10.1007/s13277-013-1025-3
12. Takemoto M, Kawamura Y, Hirohama M, et al. Inhibition of protein SUMOylation by davidiin, an ellagitannin from Davidia involucrata. *J Antibiot*. 2014;67(4):335–338. doi:10.1038/ja.2013.142
13. Zhu M, Phillipson JD, Greengrass PM, Bowery NE, Cai Y. Plant polyphenols: biologically active compounds or non-selective binders to protein? *Phytochemistry*. 1997;44(3):441–447. doi:10.1016/s0031-9422(96)00598-5
14. Sun L, Huang D, Han J, et al. Application of beta-1,6-hexa-hydroxydibenzoyl-2,3,4-trigallate-D-glucose compound in medicine preparation, CN201610663093.5; 2018.
15. Wu J, Yan LJ. Streptozotocin-induced type 1 diabetes in rodents as a model for studying mitochondrial mechanisms of diabetic β cell glucotoxicity. *Diabetes Metab Syndr Obes*. 2015;8:181–188. PMID: 25897251; PMCID: PMC4396517. doi:10.2147/DMSO.S82272
16. Guo Y, Xiao Z, Wang Y, et al. Sodium Butyrate Ameliorates Streptozotocin-Induced Type 1 Diabetes in Mice by Inhibiting the HMGB1 Expression. *Front Endocrinol*. 2018;9:630. PMID: 30410469; PMCID: PMC6209660. doi:10.3389/fendo.2018.00630
17. Ghadge A, Harsulkar A, Karandikar M, Pandit V, Kuvalekar A. Comparative anti-inflammatory and lipid-normalizing effects of metformin and omega-3 fatty acids through modulation of transcription factors in diabetic rats. *Genes Nutr*. 2016;11:10. PMID: 27551311; PMCID: PMC4968436. doi:10.1186/s12263-016-0518-4
18. Xuan W. *Study on Metabolomics of Davidiin Based on Diabetic Rat Model*. Fujian University of Traditional Chinese Medicine; 2019.
19. Han H, Song F, Shu Z, Liu Z, Ren Y, Pi Z. An untargeted urinary metabolomics strategy for investigation of therapeutical mechanism of schisandra chinensis on complications of diabetes rats. *Chin J Anal Chem*. 2017;45:389–399. doi:10.11895/j.issn.0253-3820.160753
20. Zhao Y. Study on the preparation process of polyphenols from Polygonum capitatum and its metabolite identification in diabetes model. *Naval Medical University*. 2021. doi:10.26998/d.cnki.gjuyu.2020.000272
21. Zhang C, Xie G, Jiang Y, et al. Studying ofstreptozotocin in-ducing type2 diabetes rat mode. *Anhui Med Pharm J*. 2012;16(9):1241–1244.
22. Zhang B, Quan A, Fei M, et al. Studying ofstreptozotocin inducing type2 diabetesratmode. *J Hubei Univer Sci Technol*. 2012;26(6):468–469.
23. Srinivasan K, Viswanad B, Asrat L, Kaul CL, Ramarao P. Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: a model for type 2 diabetes and pharmacological screening. *Pharmacol Res*. 2005;52(4):313–320. doi:10.1016/j.phrs.2005.05.004
24. Yang Y, Huang G, Wu Z, Han J, Liang F, Cheng W. *Effect of Polygonum Capitatum Extract on Hypoglycemic Drugs*. China: Inventor; Naval Medical University, assignee; 2015.
25. Xiang L, Wu Q, Osada H, Yoshida M, Pan W, Qi J. Peanut skin extract ameliorates the symptoms of type 2 diabetes mellitus in mice by alleviating inflammation and maintaining gut microbiota homeosta-sis. *Aging*. 2020;12(14):13991–14018. doi:10.18632/aging.103521
26. Xiang L, Li J, Wang Y, et al. Tetradecyl 2,3-dihydroxybenzoate improves the symptoms of diabetic mice by modulation of insulin and adiponectin signaling pathways. *Front Pharmacol*. 2017;8:806. doi:10.3389/fphar.2017.00806
27. Yang J, Zhao X, Lu X, Lin X, Xu G. A data preprocessing strategy for metabolomics to reduce the mask effect in data analysis. *Front Mol Biosci*. 2015;2. doi:10.3389/fmolb.2015.00004
28. Xia J, Sinelnikov IV, Han B, Wishart DS. MetaboAnalyst 3.0--making metabolomics more meaningful. *Nucleic Acids Res*. 2015;43:W251–257. doi:10.1093/nar/gkv380

29. Gotoh N, Nagao K, Ishida H, et al. Metabolism of natural highly unsaturated fatty acid, tetracosahexaenoic acid (24:6n-3), in C57BL/KsJ-db/db Mice. *J Oleo Sci*. 2018;67:1597–1607. doi:10.5650/jos.ess18167
30. Jia W, Xie G, Jia W. Bile acid-microbiota crosstalk in gastrointestinal inflammation and carcinogenesis. *Nat Rev Gastroenterol Hepatol*. 2018;15:111–128. doi:10.1038/nrgastro.2017.119
31. Tianyue Xu. Research progress on the rat model of experimental type 2 diabetes induced by high-fat and high-sugar diet combined with streptozotocin. *Diet Science*. 2019;1(18):205.
32. Wang Z, Yang Y, Xiang X, Zhu Y, Men J, He M. Estimation of the normal range of blood glucose in rats. *Wei Sheng Yan Jiu*. 2010;39(2):133–7, 142.
33. Li Y, Lu Zhang Q, Hu W, Qu G, Hong Y. Roles and regulation of phosphatidic acid phosphatase in lipid metabolism and signaling. *Plant Physiol J*. 2017;53:897–904. doi:10.13592/j.cnki.ppj.2017.1010
34. Schlame M, Greenberg ML. Biosynthesis, remodeling and turnover of mitochondrial cardiolipin. *Biochim Biophys Acta Mol Cell Biol Lipids*. 2017;1862(1):3–7. PMID: 27556952; PMCID: PMC5125896. doi:10.1016/j.bbalip.2016.08.010
35. van der Veen JN, Kennelly JP, Wan S, Vance JE, Vance DE, Jacobs RL. The critical role of phosphatidylcholine and phosphatidylethanolamine metabolism in health and disease. *Biochim Biophys Acta Biomembr*. 2017;1859(9):1558–1572. PMID: 28411170. doi:10.1016/j.bbamem.2017.04.006
36. Xiaoke Z. *Biochemistry*. Beijing: People ‘s Health Publishing House; 2020:178–179.
37. Sampath H, Ntambi JM. The fate and intermediary metabolism of stearic acid. *Lipids*. 2005;40(12):1187–1191. PMID: 16477801. doi:10.1007/s11745-005-1484-z
38. D’Souza K, Paramel GV, Kienesberger PC. Lysophosphatidic acid signaling in obesity and insulin resistance. *Nutrients*. 2018;10(4):399. PMID: 29570618; PMCID: PMC5946184. doi:10.3390/nu10040399
39. El-Magd NF A, Ramadan NM, Eraky SM. The ameliorative effect of bromelain on STZ-induced type 1 diabetes in rats through Oxi-LDL/LPA/LPAR1 pathway. *Life Sci*. 2021;285:119982. PMID: 34592232. doi:10.1016/j.lfs.2021.119982
40. Bornfeldt KE. Adipocyte phosphatidylinositol biosynthesis via the Lands cycle protects against insulin resistance. *J Lipid Res*. 2023;64(6):100383. PMID: 37127068; PMCID: PMC10239062. doi:10.1016/j.jlr.2023.100383
41. Zhang X, Zhang J, Sun H, et al. Defective phosphatidylglycerol remodeling causes hepatopathy, linking mitochondrial dysfunction to hepatosteatosis. *Cell Mol Gastroenterol Hepatol*. 2019;7(4):763–781. PMID: 30831319; PMCID: PMC6463126. doi:10.1016/j.jcmgh.2019.02.002
42. He Q, Bo J, Shen R, et al. S1P Signaling Pathways in Pathogenesis of Type 2 Diabetes. *J Diabetes Res*. 2021;2021:1341750. PMID: 34751249; PMCID: PMC8571914. doi:10.1155/2021/1341750
43. Gundala NKV, Naidu VGM, Das UN. Amelioration of streptozotocin-induced type 2 diabetes mellitus in Wistar rats by arachidonic acid. *Biochem Biophys Res Commun*. 2018;496(1):105–113. PMID: 29309791. doi:10.1016/j.bbrc.2018.01.007
44. Wei C, Wang M, Wang X-J. Evolutionary conservation analysis of human arachidonic acid metabolism pathway genesJ. *Life Medicine*. 2023;2(2):9. doi:10.1093/lifemedi/lnad004
45. Pace-Asciak CR, Martin JM, Corey EJ. Hepoxilins, potential endogenous mediators of insulin release. *Prog Lipid Res*. 1986;25(1–4):625–628. PMID: 3321096. doi:10.1016/0163-7827(86)90127-x
46. Khan H, Gupta A, Singh TG, Kaur A. Mechanistic insight on the role of leukotriene receptors in ischemic-reperfusion injury. *Pharmacol Rep*. 2021;73(5):1240–1254. PMID: 33818747. doi:10.1007/s43440-021-00258-8
47. Chakrabarti SK, Cole BK, Wen Y, Keller SR, Nadler JL. 12/15-lipoxygenase products induce inflammation and impair insulin signaling in 3T3-L1 adipocytes. *Obesity (Silver Spring)*. 2009;17(9):1657–1663. PMID: 19521344; PMCID: PMC2887741. doi:10.1038/oby.2009.192
48. Qureshi S, Ali G, Muhammad T, et al. Thiadiazine-thione derivatives ameliorate STZ-induced diabetic neuropathy by regulating insulin and neuroinflammatory signaling. *Int Immunopharmacol*. 2022;113(Pt B):109421. PMID: 36403520. doi:10.1016/j.intimp.2022.109421
49. Han PC, Hsiao FC, Chang HM, Wabitsch M, Hsieh PS. Importance of adipocyte cyclooxygenase-2 and prostaglandin E2-prostaglandin E receptor 3 signaling in the development of obesity-induced adipose tissue inflammation and insulin resistance. *FASEB J*. 2016;30(6):2282–2297. PMID: 26932930. doi:10.1096/fj.201500127
50. Chan PC, Liao MT, Hsieh PS. The Dualistic Effect of COX-2-mediated signaling in obesity and insulin resistance. *Int J Mol Sci*. 2019;20(13):3115. PMID: 31247902; PMCID: PMC6651192. doi:10.3390/ijms20133115
51. Abadpour S, Tyrberg B, Schive SW, et al. Inhibition of the prostaglandin D2-GPR44/DP2 axis improves human islet survival and function. *Diabetologia*. 2020;63(7):1355–1367. PMID: 32350565; PMCID: PMC7286861. doi:10.1007/s00125-020-05138-z
52. Maggi LB, Sadeghi H, Weigand C, Scarim AL, Heitmeier MR, Corbett JA. Anti-inflammatory actions of 15-deoxy-delta 12,14-prostaglandin J2 and troglitazone: evidence for heat shock-dependent and -independent inhibition of cytokine-induced inducible nitric oxide synthase expression. *Diabetes*. 2000;49(3):346–355. PMID: 10868955. doi:10.2337/diabetes.49.3.346
53. Souza SC, Yamamoto MT, Franciosa MD, Lien P, Greenberg AS. BRL 49653 blocks the lipolytic actions of tumor necrosis factor-alpha: a potential new insulin-sensitizing mechanism for thiazolidinediones. *Diabetes*. 1998;47(4):691–695. PMID: 9568706. doi:10.2337/diabetes.47.4.691
54. Thieringer R, Fenyk-Melody JE, Le Grand CB, et al. Activation of peroxisome proliferator-activated receptor gamma does not inhibit IL-6 or TNF-alpha responses of macrophages to lipopolysaccharide in vitro or in vivo. *J Immunol*. 2000;164(2):1046–1054. PMID: 10623855. doi:10.4049/jimmunol.164.2.1046
55. Lu L, Fan Z, Chai Y. Antipyretic effect and mechanism of relinqing granules on rats induced by dry yeast. *J Hubei Minzu University*. 2022. doi:10.13501/j.cnki.42-1590/r.2022.04.016
56. Chi PL, Liu CJ, Lee IT, Chen YW, Hsiao LD, Yang CM. HO-1 induction by CO-RM2 attenuates TNF-α-induced cytosolic phospholipase A2 expression via inhibition of PKCα-dependent NADPH oxidase/ROS and NF-κB. *Mediators Inflamm*. 2014;2014:279171. PMID: 24616552; PMCID: PMC3927740. doi:10.1155/2014/279171
57. Chen J, Zhao KN, Chen C. The role of CYP3A4 in the biotransformation of bile acids and therapeutic implication for cholestasis. *Ann Transl Med*. 2014;2(1):7. PMID: 25332983; PMCID: PMC4200650. doi:10.3978/j.issn.2305-5839.2013.03.02
58. Chiang JYL, Ferrell JM. Up to date on cholesterol 7 alpha-hydroxylase (CYP7A1) in bile acid synthesis. *Liver Res*. 2020;4(2):47–63. PMID: 34290896; PMCID: PMC8291349. doi:10.1016/j.livres.2020.05.001
59. Yan Y, Niu Z, Sun C, et al. Hepatic thyroid hormone signalling modulates glucose homeostasis through the regulation of GLP-1 production via bile acid-mediated FXR antagonism. *Nat Commun*. 2022;13(1):6408. PMID: 36302774; PMCID: PMC9613917. doi:10.1038/s41467-022-34258-w
60. Modica S, Gadaleta RM, Moschetta A. Deciphering the nuclear bile acid receptor FXR paradigm. *Nucl Recept Signal*. 2010;8:e005. PMID: 21383957; PMCID: PMC3049226. doi:10.1621/nrs.08005

61. Müllenbach R, Weber SN, Lammert F. Nuclear receptor variants in liver disease. *J Lipids*. 2012;2012:934707. PMID: 22523693; PMCID: PMC3317184. doi:10.1155/2012/934707
62. Chiang JY. Bile acid metabolism and signaling. *Compr Physiol*. 2013;3(3):1191–1212. PMID: 23897684; PMCID: PMC4422175. doi:10.1002/cphy.c120023
63. He L. Metformin and Systemic Metabolism. *Trends Pharmacol Sci*. 2020;41(11):868–881. doi:10.1016/j.tips.2020.09.001
64. Zhu H, Jia Z, Li YR, Danelisen I. Molecular mechanisms of action of metformin: latest advances and therapeutic implications. *Clin Exp Med*. 2023;23(7):2941–2951. doi:10.1007/s10238-023-01051-y
65. Agius L, Ford BE, Chachra SS. The metformin mechanism on gluconeogenesis and AMPK activation: the metabolite perspective. *Int J Mol Sci*. 2020;21(9):3240. doi:10.3390/ijms21093240
66. Galicia-Garcia U, Benito-Vicente A, Jebari S, et al. Pathophysiology of type 2 diabetes mellitus. *Int J Mol Sci*. 2020;21(17):6275. doi:10.3390/ijms21176275
67. Ojo OA, Ibrahim HS, Rotimi DE, Ogunlakin AD, Ojo AB. Diabetes mellitus: from molecular mechanism to pathophysiology and pharmacology. *Med Novel Technol Dev*. 2023;19:100247. doi:10.1016/j.medntd.2023.100247
68. Banday MZ, Sameer AS, Nissar S. Pathophysiology of diabetes: an overview. *Avicenna J Med*. 2020;10(4):174–188. doi:10.4103/ajm.ajm_53_20