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Taurine as a supplement for treating type II diabetes mellitus with metformin and DPP4 inhibitor

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Patients of type 2 diabetes mellitus (T2DM) were grouped into subtypes by a symptom-based diagnostic system of the traditional Chinese medicine for detecting metabolomic information that would otherwise be lost due to the high degree of variations in clinical samples. LC/MS-based metabolomic tools were employed to identify distinct metabolomic features in T2DM patients in one of the subtypes in the symptom-based diagnostic system. Several of the affected metabolic pathways could be ameliorated by the combined treatment of metformin and DPP4 inhibitor, except the taurine/hypotaurine metabolic pathway. It was possible that modulating the taurine/hypotaurine metabolism in the combined treatment with metformin and DPP4 inhibitor would enhance the therapeutic effects. To this end, a mouse diabetic model was treated with taurine in addition to metformin and DPP4 inhibitor. Indeed supplementing taurine during the combined treatment led to improvements in various indicators of the disorder such as those for the fatty acid and glucose metabolisms. It could be concluded that taurine was a useful supplement for treating T2DM with metformin and DPP4 inhibitor.

Keywords Taurine, Metformin, DPP4 inhibitor, Type 2 diabetes mellitus, Syndrome differentiation

Diabetes mellitus is one of the largest public health concerns and an important cause of morbidity and mortality worldwide[1]. Between the two types of diabetes mellitus, type 2 diabetes mellitus (T2DM) accounts for more than 90% of all diabetes patients[2]. T2DM seriously degrades the patients' quality of life and imposes a heavy burden on public health as well as socio-economic development.

Metabolomics is a useful tool to investigate metabolic disorders, such as T2DM. Nevertheless, the high degree of variation among the clinical serum samples makes it difficult to detect subtle metabolic abnormality. It is helpful to improve uniformity in these diverse serum samples by dividing the patients into sub-groups with a set of criteria. A possible venue is to sub-group patients based on nuances in symptoms. It so happens that Traditional Chinese Medicine (TCM) adopts a symptom-based diagnostic system that is time-proven and clinically effective to distinguish T2DM into multiple subtypes for acclimating the prescription of herbal medicine. For example, this so-called syndrome differentiation system in TCM can distinguish T2DM in the early stage into syndromes of consumption of fluid due to excessive-heat, liver-depression and spleen-deficiency, obstruction by turbid sputum, and damp heat accumulation, among others. The intermediate stage of T2DM includes TCM syndromes of lung and kidney Yin-deficiency, spleen-deficiency, as well as spleen and kidney Qi-deficiency. The late stage of T2DM syndromes can be differentiated into liver and kidney Yin-deficiency as well as Yin and Yang-deficiency, among others[3]. Although this is jargonistic for those who are not familiar with the system, this subtyping system enables the prescription of personalized herbal medicine for treatment. Likewise, this subtyping might lead to the collection of samples with better uniformity for metabolomic studies so that subtle differences could be detected.

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In this study, metabolomic tools were employed to characterize one of the T2DM syndromes, damp-heat accumulation (DHA). To probe the molecular characteristics of T2DM of the DHA subtype (T2DM-DHA) with and without drug treatments, plasma samples were analyzed by LC/MS-based (liquid chromatograph-mass spectrometry-based) metabolomics. It was showed that T2DM-DHA was with unique metabolomic patterns, which were altered by drug treatments. A combined treatment with metformin and DPP4 inhibitor seemed to ameliorate some of the abnormality in metabolism. Yet, among the metabolic pathways important to the pathogenesis of T2DM, taurine/hypotaurine pathway was not affected by this combined treatment. As the level of taurine was down-regulated in T2DM-DHA, it was possible that perturbation of this pathway could improve the treatment scheme. Indeed, taurine supplementation in a diabetic mouse model treated with metformin and DPP4 inhibitor ameliorated the fatty acid and glucose metabolisms. This study implicated the basis for using taurine as a supplement for treating T2DM with metformin and DPP4 inhibitor. In addition, it also provides a molecular basis for TCM subtyping and diagnosis.

Results

Selection of patients

Volunteers aged 18–70 were chosen for T2DM subtyping according to Clinical Guide to Chinese Medicine, 2002[4], as well as Guideline for TCM diabetes treatment and prevention, 2020[5]. The focus of this investigation was on the T2DM-DHA subtype which was one of the most prevailing subtypes. The primary symptoms of T2DM-DHA were dyspeptic abdominal distention with heavy head and body. The diagnosis of T2DM-DHA would also be corroborated by symptoms of obesity, xerostomia yet infrequent fluid intake, irritability, depression, lassitude in the limbs, dark urine, and constipation. The tongues of DHA patients were reddish with heavy and yellowish coating as well as indentation made by teeth on the sides. The pulses taken in the radial artery of the wrist were like fast moving beads.

T2DM-DHA is a gradual process. Symptoms for those in the early and those in the later stages are distinctly different in TCM. Accordingly, the blood samples for those patients diagnosed as T2DM-DHA for the very first time were separately grouped into ephDHA (early phase T2DM-DHA) from lphDHA (late phase T2DM-DHA) for LC/MS analysis. Even though it was a prevailing subtype, only 34 of the 197 T2DM patients could be unambiguously diagnosed as T2DM-DHA. In this group, 12 of the ephDHA patients without taking any medicine were separately grouped. The rest were considered to be in lphDHA and separated into groups of untreated (6 patients), treated with metformin (6 patients), with insulin (4 patients), and treated with both metformin and DPP4 inhibitor (6 patients).

Metabolomic patterns in the early phase of T2DM-DHA

Unsurprisingly, plasma samples of ephDHA were metabolomically different from the healthy controls (Fig. 1a) as many metabolites were highlighted in the volcano plot, indicating they were significantly altered statistically (Supplementary Table 1 and Supplementary Fig. 1). The metabolites in the volcano plot could also be identified in the enrichment analysis to implicate multiple metabolic pathways (Fig. 1b).

Purine metabolism was among the most prominently influenced by the disorder with the highest statistical significance. Purine metabolism is involved in the synthesis and degradation of purine nucleotides and regulates the level of adenylate and guanylate in the body. Purines contribute to modulate energy metabolism and signal transduction. A significant increase in purine metabolites such as AMP and inosine was observed in diabetic models[6]. Our data indicated that purine metabolism was up-regulated in ephDHA (Supplementary Table 1).

Taurine/hypotaurine metabolism, as well as biotin metabolism, was also highlighted in the enrichment analysis, albeit in less significant *P* values but with much higher enrichment ratio, indicating more metabolites were identified for the pathway than those in the purine metabolism. The data demonstrated that taurine/hypotaurine metabolism was upregulated in ephDHA patients. Taurine and hypotaurine were sulfur-containing α-amino acids abundantly produced in brain, retina, muscles, and organs throughout the body. Taurine is involved in a wide range of biological processes through its antioxidant and anti-inflammatory effects, among other mechanisms[7]. Taurine is a beneficial agent in many diseases including diabetes[8].

Fatty acid metabolism was also affected. Carnitines, including butyrylcarnitine, isobutyrylcarnitine, and octanoylcarnitine (Supplementary Table 1), are acyl carnitines which contain a fatty acid with the carboxylic acid attached to carnitine through an ester bond. Carnitines participate in the β-oxidation of long-chain fatty acids and play other roles in health and disease[9,10].

Ether lipids are glycerolipids and their metabolism are characteristic for diabetic patients[11]. The metabolism of ether lipids was altered in ephDHA patients (Fig. 1b). The volcano plot indicated that the levels of fatty acids were down, yet the level of acylcarnitines were up, demonstrating the active metabolism of breaking down fatty acids and accumulating their metabolic intermediates (Supplementary Table 1).

Besides compounds relating to energy and food consumption, other compounds characteristic to ephDHA patients could also be detected, which included upregulated bilirubin and taurocholic acid, as well as downregulated 3-indolepropionic acid and glycerophosphocholine (Supplementary Table 1).

Unique metabolomic features in ephDHA

Comparing ephDHA samples with the rest of T2DM samples, the upregulation of taurocholic acid, the downregulation of 3-Indolepropionic acid (IPA), and the significant change in the taurine/hypotaurine metabolism were highlighted (Fig. 1c, d and Supplementary Table 2). It was a demonstration that better metabolomic signals could be detected by the symptom-based grouping of patients into the T2DM-DHA subtype in TCM. It also suggested that abnormality in the taurine/hypotaurine metabolism could be a salient metabolomic feature for the T2DM-DHA subtype (Fig. 1d).

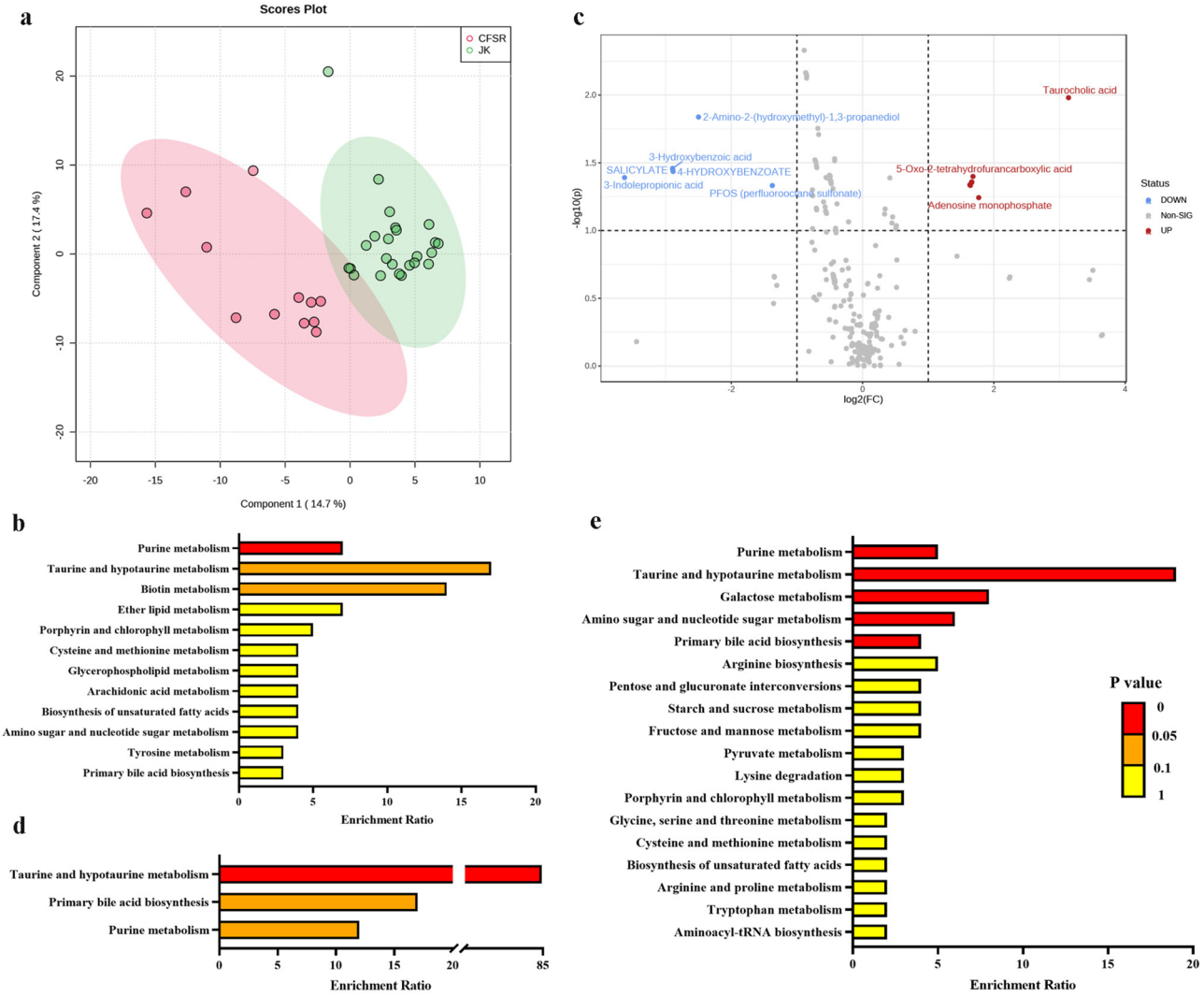

Fig. 1. Metabolomic changes of a T2DM subtype. (**a**) Partial least squares Discriminant Analysis (PLS-DA) analysis of plasma samples from the ephDHA patients (red) and healthy controls (green) indicated that the metabolomic feature of ephDHA deviated from that of the healthy controls. (**b**) Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of differential metabolites in ephDHA patients as compared to that in healthy controls. Purine metabolism was statistically significant. Taurine/hypotaurine metabolism and biotin metabolism were of high enrichment ratios albeit with slightly less significant *P* values. (**c**) The volcano plot of metabolites in ephDHA over that in other T2DM patients. (**d**) KEGG pathway enrichment analysis of plasma samples from ephDHA patients over that of the other T2DM patients. The taurine/hypotaurine metabolism is with significant *P* value and high enrichment ratio. (**e**) As the disorder progressed to lphDHA, changes in purine, taurine/hypotaurine, galactose, amino acid and nucleotide sugar metabolisms, as well as primary bile acid biosynthesis were all become prominent.

The metabolomic changes in the development of DHA

Progressing from ephDHA to lphDHA, the metabolomic difference was appreciable (Supplementary Fig. 2a). Changes in arginine and glutathione metabolisms, as well as fatty acid and amino acid/nucleotide sugar metabolisms, were prominent in enrichment and pathway analyses (Supplementary Fig. 2b). Glutathione is a compound synthesized from cysteine. It is a coenzyme in redox reactions for detoxification and countering the oxidative stress, which is important in T2DM patients[12].

Metabolomic difference between lphDHA and healthy controls

Metabolomically, lphDHA and healthy controls were of significant difference (Fig. 1e and Supplementary Fig. 3). Unsurprisingly, there were also significant differences in the metabolite profile (Supplementary Table 3). Compared to ephDHA, besides purine metabolism, changes in taurine/hypotaurine metabolism, galactose metabolism, amino acid/nucleotide sugar metabolism, and primary bile metabolism became very prominent in lphDHA. In particular, taurine/hypotaurine metabolism was not only of high enrichment ratio, but also

of statistical significance as compared to that in ephDHA (Fig. 1). It corroborated the notion that taurine/hypotaurine metabolism was a characteristic feature for T2DM-DHA.

Drug treatment of T2DM-DHA patients

Metformin is a drug for treating T2DM and gestational diabetes. There were not as many metabolites identified in the volcano plot for T2DM-DHA patients treated with metformin as compared to the untreated (Supplementary Fig. 4 and Supplementary Table 4). The drug seemed to upregulate pyruvate metabolism as well as glycine, serine and threonine metabolism to a slightly less significant *P* value. But they were also of high enrichment ratios (Fig. 2a).

Insulin treatment of T2DM-DHA patients also impacted the metabolisms of fatty acids and unbranched amino acids (Fig. 2b and Supplementary Fig. 5). The level of betaine was up-regulated seemingly in response to the lower level of carnitines (Supplementary Fig. 5 and Supplementary Table 5). Betaine plays a role in the production of carnitine[13]. Deficiency in betaine is known to be associated with T2DM, and insulin seemed to play a positive role for T2DM-DHA patients in this respect.

Dipeptidyl peptidase 4 (DPP4) inhibitors are antihyperglycemic medications for managing T2DM. We also obtained data from T2DM-DHA patients treated with both metformin and DPP4 inhibitor. The combined treatment with these two drugs obviously led to bigger changes in the metabolome and altered more metabolic pathways, as compared to using metformin alone (Fig. 2c and Supplementary Fig. 6). The down-regulation in metabolisms of galactose, amino acid/nucleotide sugar, and purine indicated that the combined treatment of

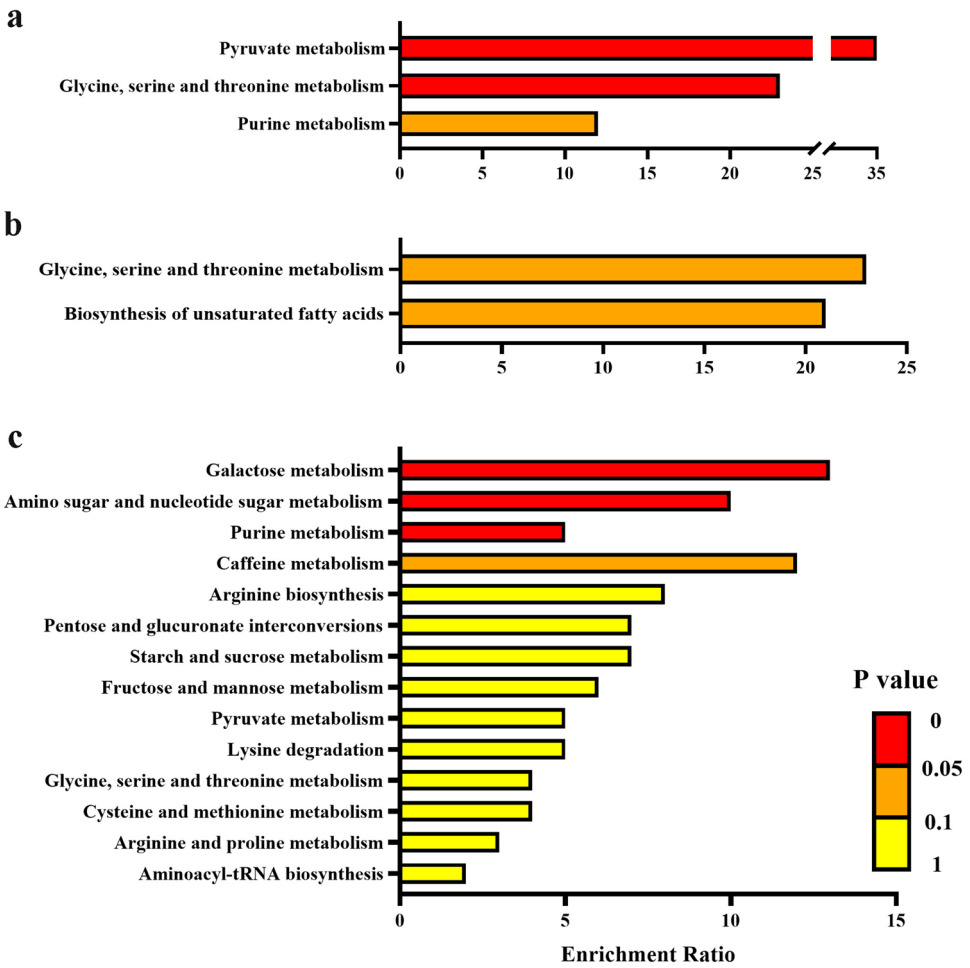

Fig. 2. Metabolomic changes associated with drug treatments. (**a**) KEGG pathway enrichment analysis of plasma in T2DM-DHA patients treated with metformin as compared to that without the treatment. Metformin treatment resulted in upregulating pyruvate metabolism as well as glycine, serine and threonine metabolism. (**b**) KEGG pathway enrichment analysis of plasma in T2DM-DHA patients under insulin treatment as compared to that without the treatment. The treatment perturbed the metabolisms of fatty acids and unbranched amino acids. (**c**) KEGG pathway enrichment analysis of plasma in T2DM-DHA patients treated with both metformin and DPP4 inhibitor as compared to that without the treatment. The combined treatment altered more metabolic pathways, as compared to use metformin alone and down-regulated several metabolic pathways that were up-regulated in T2DM-DHA.

metformin and DPP4 inhibitor was a preferable scheme for treating T2DM-DHA, as it down-regulated several metabolic pathways that were up-regulated in T2DM-DHA (Fig. 1, Fig. 2c, Table 1 and Supplementary Table 6).

Taurine as the supplement in treating the mouse model with metformin and DPP4 inhibitor

Taurine is an important amino acid for treating diabetes[14,15]. However, its mechanism in influencing the diabetic metabolism has not been investigated in depth. In this study, metabolomic parameters for taurine/hypotaurine metabolism were not influenced by the various treatments. Despite the fact that taurine/hypotaurine metabolism was up-regulated, the level of taurine decreased (Table 1). Since the combined treatment with metformin and DPP4 inhibitor was particularly efficacious for the T2DM-DHA patients judging by the metabolomic parameters, it was of great interest to investigate the outcome of the combined treatment with a taurine supplementation.

Unfortunately, there is no generally accepted animal model for T2DM-DHA. However, a typical T2DM model with taurine supplementation during the combined treatment with metformin and DPP4 inhibitor could

Pathway	A	B	C	D	E	F
Purine	C00020 C00212 C00294	C00020 C00212 C00294 C01762				C00020 C00294
Taurine/hypotaurine	C05122	C00245 C00025 C05122				
Glutathione			C00077 C00025	C00025		
Arginine		C00025 C00062	C00025 C00077	C00025		C00025 C00062
Bile acid		C00695 C05122				
Porphyrin		C00025 C00486				
Amino/nucleotide- sugar		C00140 C00181 C00984				C00140 C00181 C00984
Gly/Ser/Thr					C00719	
Fatty acids					C00712	
Pyruvate				C00546		
Ala/Asp/Glu				C00025		

Table 1. A summary of metabolites changed in T2DM-DHA ($P < 0.05$).
A: ephDHA compared to healthy controls; B: lphDHA as compared to healthy controls; C: ephDHA as compared to lphDHA, difference between ephDHA and lphDHA; D: lphDHA patients treated with metformin; E: lphDHA patients treated with Insulin; F: lphDHA patients treated with both metformin and DPP4 inhibitor. Up-regulated metabolites are in red, while the down-regulated metabolites are in blue. KEGG codes for compounds in this table: C00020 = AMP, C00212 = Adenosine, C00294 = Inosine, C01762 = Xanthosine, C05122 = Taurocholate, C00245 = Taurine, C00025 = Glutamate, C00077 = L-Ornithine, C00062 = L-Arginine, C00695 = Cholate, C00486 = Bilirubin, C00140 = N-Acetylchitosamine, C00181 = D-Xylose, C00984 = α-D-Galactose, C00719 = Betaine, C00712 = Oleate, C00546 = Methylglyoxal.

still provide valuable information. To this end, the db/db mice were used. It was shown that the supplementation of taurine in the combined treatment with metformin and DPP4 inhibitor led to a significant decrease in fasting blood glucose (FBG) over the treatment without (Fig. 3a).

Improvements by taurine supplementation in the combined treatment of metformin and DPP4 inhibitor could also be supported by histopathology in liver, white adipose, and colon samples (Fig. 3b-e). For liver samples with HE staining, the significant hepatic steatosis and injuries in db/db mice with increased hepatocyte volume, cellular nuclear consolidation and cytoplasm filled with white vacuolar lesions of varying sizes were reduced after the treatment with either taurine alone or the combined treatment with metformin and DPP4 inhibitor. With the taurine supplementation in the combined treatment, the hepatic structures were orderly arranged and damages were significantly alleviated (Fig. 3b). It could be shown that the number of lipid-loaded hepatocytes was elevated in db/db mice with Oil Red O staining. Treatment with either taurine alone or the combination of metformin and DPP4 inhibitor was with sporadic distribution and decreased number of lipid droplets in hepatocytes. The taurine supplementation with the combined treatment was with more significant less steatosis and lipid deposition in liver cells (Fig. 3c).

The HE staining of adipose tissues showed that the number of adipocytes was significantly reduced yet the area with adipocytes was enlarged in db/db mice, indicating severe accumulation of fat. Treatment with

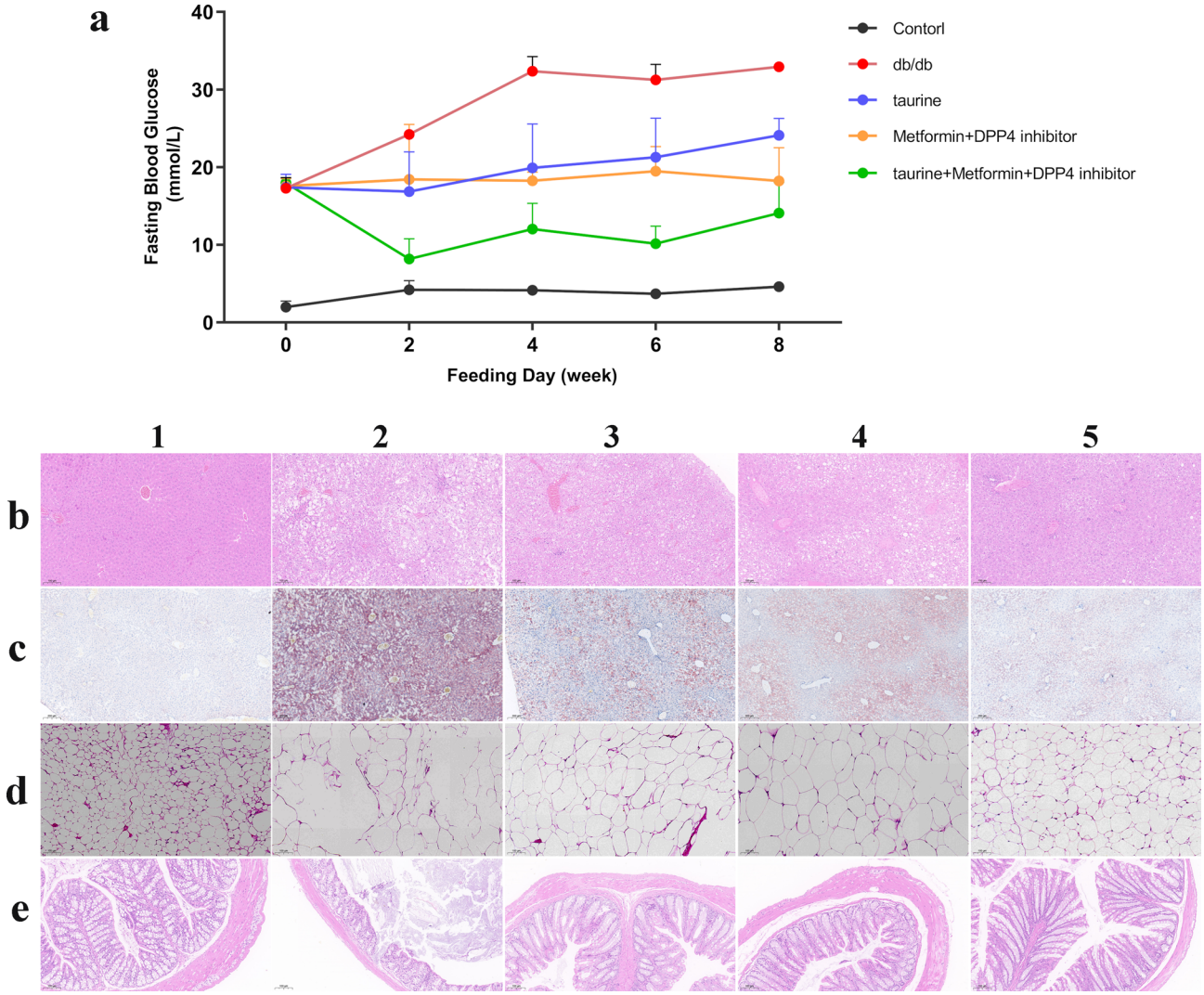

Fig. 3. FBG and Ultra structures of tissues and organs in db/db mice. (**a**) Effect of different treatments on FBG mice (for all groups, n = 8). At week 0, FBG levels were significantly higher in db/db mice than in the normal mice. While the group treated with taurine alone was with slight increase in the FBG level, the FBG level in the group treated with metformin and DPP4 inhibitor was more or less stabilized over time. The supplementation with taurine in the treatment with metformin and DPP4 inhibitor showed a significant reduction in FBG level. (**b**) with HE staining (n = 3) and (**c**) with oil red O staining (n = 3) were hepatic samples, (**d**) with HE staining was adipose tissues (n = 3), and (**e**) with HE staining was colonic samples (n = 3). 1: healthy control; 2: db/db model; 3: db/db treated with taurine; 4: db/db treated with metformin and DPP4 inhibitor; 5: db/db mice treated with metformin and DPP4 inhibitor in conjunction with taurine supplementation.

either taurine alone or a combination of metformin and DPP4 inhibitor led to an increase in the number of adipocytes and a decrease in the area containing the adipocytes. Further improvements were observed with taurine supplementation in those db/db mice treated with both metformin and DPP4 inhibitor with a significant increase in the number of adipocytes and a decrease in the area with these cells (Fig. 3d).

The distal colon samples by HE staining were with notable changes, marked by substantial neutrophil infiltration, depletion of cup cells, and submucosal edema within the intestine. Better morphologies of the colonic crypts and neutrophil infiltration were observed for those treated with either taurine alone or with the combination of metformin and DPP4 inhibitor. With the taurine supplementation in the treatment with metformin and DPP4 inhibitor, significant improvements were with the samples of distal colon (Fig. 3e).

Taurine improves fatty acid and glucose metabolisms in the combined treatment

Metabolomic data showed that combined treatment with metformin and DPP4 inhibitor altered multiple metabolic pathways in db/db mice. However, it seemed also aggravating fatty acid metabolism in enrichment analysis ($P=0.01$, Figs. 4a and b). With the supplementation of taurine though, the fatty acid metabolic pathways were ameliorated ($P=0.006$, Fig. 4c). It was consistent with the histological observation that the combined treatment with metformin and DPP4 inhibitor could lead to increase in the number and area of adipocytes and fat accumulation. With the taurine supplementation, the number of adipocytes significantly increased, and fat accumulation significantly decreased (Fig. 3d).

In addition, taurine also enhanced the glucose metabolism in the combined treatment with metformin and DPP4 inhibitor, as it led to up-regulation of the pentose phosphate pathway ($P=0.03$) and the pyruvate pathway ($P=0.04$, Fig. 4d). The pentose phosphate pathway is an important part of the glucose metabolism[16], and pyruvate is the end-product of glycolysis and an important factor in the TCA (tricarboxylic acid cycle) cycle[17].

The metabolomic data were consistent with the biochemical and histological indicators. All these data together demonstrated that taurine was an effective supplement to the combined treatment of metformin and DPP4 inhibitor to reduce serum glucose levels and alleviate liver fat and other damage in db/db mice.

Discussion

The metabolomic patterns were significantly different for T2DM-DHA patients in different phases and treated with different therapeutic agents. A summary of the metabolites that were changed significantly ($P<0.05$) are listed in Table 1.

Eleven metabolic pathways were affected in T2DM-DHA. In ephDHA, purine and taurine metabolic pathways were first altered (Table 1, column A). As the disorder further progressed, glutathione, arginine, bile acid, porphyrin, amino acid/nucleotide sugar metabolic pathways were also added to the list for T2DM-DHA (Table 1, column B). These changes were already occurred in ephDHA, but only became statistically more significant when it reached the later stage in T2DM-DHA. The differences in glutathione and arginine metabolisms could also be detected between ephDHA and lphDHA, implicating oxidative stress and augment of amino acid metabolism in lphDHA (Table 1, column C).

The treatment with metformin seemed to reduce the oxidative stress and alter the patterns of amino acid metabolism (Table 1, column D), and insulin seemed to mostly attenuate the amino acid and fatty acid metabolisms (Table 1, column E). The data indicated that a combined treatment of metformin and DPP4 inhibitor for T2DM-DHA could not only adjust the metabolism of amino acids but could also adjust the metabolism of purines (Table 1, column F), implicating that this combination was a preferred treatment for T2DM-DHA as it led to the down-regulation of many pathways that were up-regulated in T2DM-DHA. However, this combined treatment did not affect the taurine/hypotaurine metabolism, which seemed to be important in lphDHA. It would be helpful if this pathway could also be attenuated. Since there was no animal model for T2DM-DHA yet taurine/hypotaurine metabolism is a relevant metabolic pathway in diabetes, we used db/db mice as the model for diabetes to corroborate the notion that taurine would be particularly helpful in the combined treatment with metformin and DPP4 inhibitor. Indeed, taurine supplementation greatly improved the outcome in the combined treatment with metformin and DPP4 inhibitor in the db/db mouse model.

The metabolic parameters revealed in this study were consistent with the underlying mechanisms of taurine's function in T2DM. Increased oxidative stress and inflammation are among the most important factors in the pathogenesis of T2DM. Taurine is an antioxidant that could alleviate the oxidative stress in T2DM[18]. Taurine could also suppress inflammatory responses through forming taurine chloramine to inhibit activity of NF-κβ and to suppress gene expression of nitric oxide, IL-6, IL-8, TNF-α, and prostaglandin E2[19,20].

Taurine modulates the AMPK pathway, and its induction of PGC1α activates FOXO1 transcription factor and HNF4α to uplift glycogenesis[21–24]. Taurine can also improve glucose homeostasis by regulating α,β,δ-cell morphophysiology[25], and increase the expression of genes implicated in energy expenditure like PPARα and PPARγ[26,27]. Taurine's regulation of transcription factors, such as SREBPs, PPARα, LXR, PGC1-α, FoxO-1, leads to the inhibition of lipogenesis, promotion of fatty acid β-oxidation and adaptive thermogenesis, as well as the inhibition of cholesterol production and promotion of cholesterol breakdown.

T2DM is divided into different syndromes in TCM for treatment based on its unique diagnostic system. While this subtyping leads to the personalized prescription of herbal medicine, the molecular basis of this system has not been adequately delineated. We employed LC/MS-based metabolomic techniques to investigate the metabolite patterns in the plasma of T2DM-DHA patients. The data indicated that the patterns were distinguishable for the T2DM-DHA in different phases, which suggested that metabolomics could be an objective tool to differentiate TCM subtypes of T2DM and for better prescription of Chinese herbal medicine to improve the treatment of T2DM.

In this study, over 100 patient samples were collected, yet only comparatively few could be selected for the study as human samples were diverse and many information were mingled in the background, which limited the

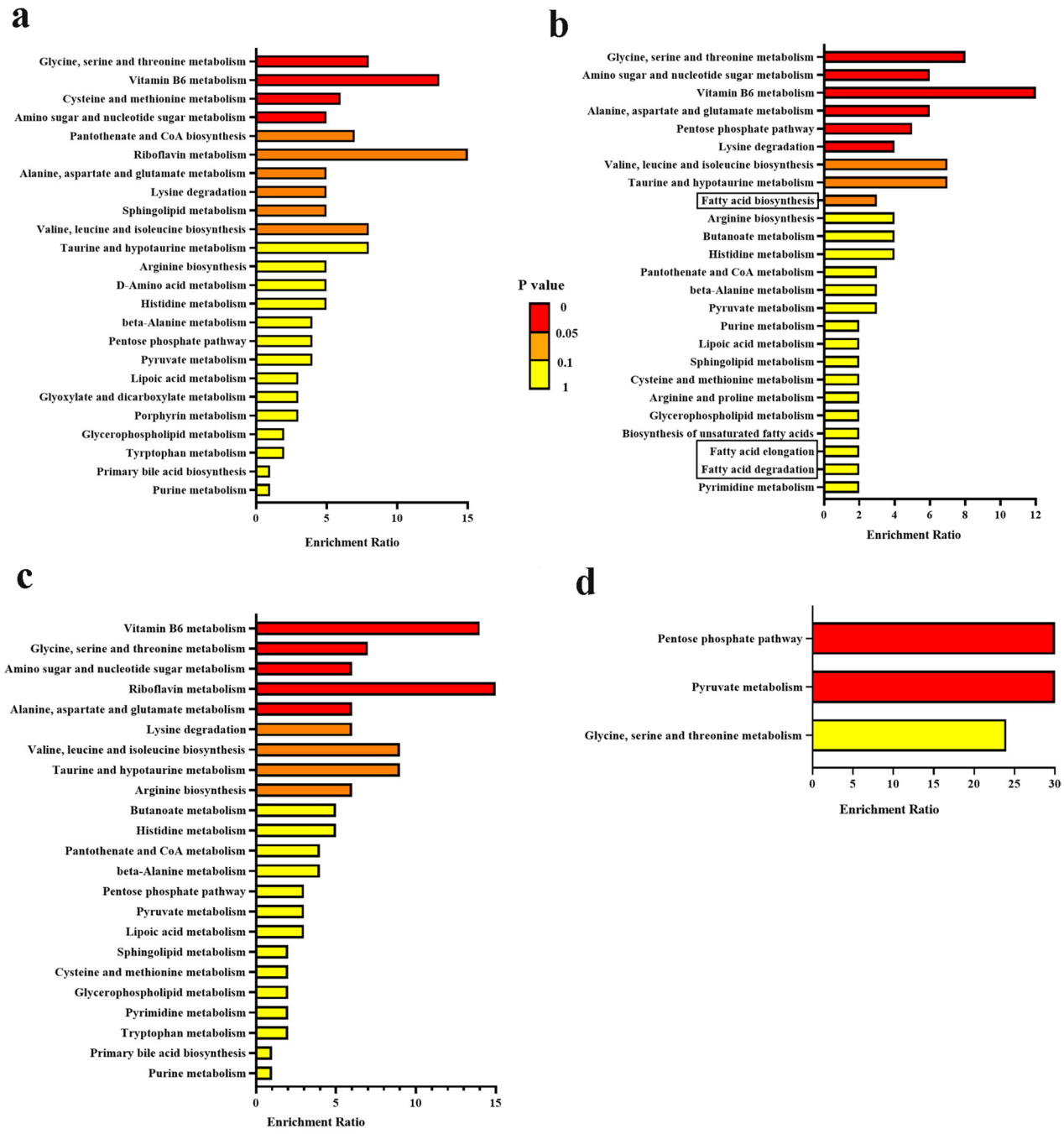

Fig. 4. Taurine ameliorates fatty acid and glucose metabolisms. (**a**) Many metabolic pathways in metformin and DPP4 inhibitor treated group differed from those in db/db controls in enrichment analysis. (**b**) Treatment with metformin and DPP4 inhibitor resulted in the deviation of fatty acid metabolisms over that of the healthy control. (**c**) Taurine supplementation during the combination treatment with metformin and DPP4 inhibitor attenuated the fatty acid metabolisms so it was no longer appeared in the list of metabolomic pathways in the enrichment analysis. (**d**) KEGG pathway enrichment analysis of the sera of the db/db mice treated with taurine, metformin and DPP4 inhibitor over that of the db/db mice treated metformin and DPP4 inhibitor. The pentose phosphate pathway, a part of the glucose metabolism, and pyruvate pathway in TCA cycle were altered in db/db mice treated with taurine in addition to metformin and DPP4 inhibitor over those db/db mice treated with metformin and DPP4 inhibitor but without taurine.

sample size that could be used in this study. It would be certainly better if more samples could be grouped for analysis. Despite the impediment, the currently available samples still allowed useful information to be extracted for analysis.

Taurine has been shown to be a beneficial amino acid for diabetic treatment. In this study, we demonstrated that taurine could attenuate fatty acid and glucose metabolisms in the positive direction, especially to complement

the deteriorating fatty acid metabolisms in the treatment with metformin and DPP4 inhibitor. As metformin and DPP4 inhibitor are widely applicable therapeutics for T2DM, this study supported the notion that taurine enhanced the therapeutic effect of the combined treatment, especially for the T2DM-DHA subtype, and justified further clinical studies with taurine as the supplement.

Methods

Selection of patients

The subtyping of T2DM was carried out according to Clinical Guide to Chinese medicine, 2002[4], as well as Guideline for TCM diabetes treatment and prevention, 2020[5]. Only those who could be unambiguously diagnosed as T2DM-DHA were included in this study. Blood samples from 197 T2DM patients, aged 18 to 70, were taken in Xiamen Hospital of Traditional Chinese Medicine and The First Affiliated Hospital of Xiamen University. Among these, 34 were unambiguously diagnosed as T2DM-DHA. From this group of patients, those in ephDHA without taking any medicine were separately grouped (12). The rest of T2DM-DHA group were considered to be in lphDHA and separated into groups of untreated (6), treated with metformin (6), treated with insulin (4), and treated with both metformin and DPP4 inhibitor (6). The baseline characteristics of the participants in this study were also analyzed (Supplementary Table 7).

Exclusion criteria

The patients with the following conditions were excluded from the study, which include (1) those with either diabetic ketoacidosis, or hyperosmolar hyperglycemia, or acute complications of hypoglycemia within 30 days prior to the collection of blood samples; (2) those with either type I diabetes mellitus, or gestational diabetes, or special types of diabetes, or diabetic foot, or with secondary obesity; (3) those with either complication of severe cardiovascular and cerebrovascular diseases or dysfunction in liver (the activity of transaminases exceeded the upper normal limit by more than 3 times) and kidney (the glomerular filtration rate was less than 60 ml/min/1.73m2); (4) those with either malignant cancer, or lung infection, or any kind of acute/chronic infection; (5) those with mental illness.

Healthy controls

Healthy controls were chosen from those passing the routine physical exams with typical blood indexes, and with neither abnormality in liver and lung, nor cardiovascular and cerebrovascular diseases, nor malignant cancer, nor acute infection, and nor history of chronic infection. Twenty-four healthy controls were included in this study.

Ethics statement

This research was approved by the Ethics Committees of Xiamen Hospital of Traditional Chinese Medicine and The First Affiliated Hospital of Xiamen University. It was carried out in accordance with the Declaration of Helsinki (1996 edition) and relevant clinical research and regulations in China. The investigators had explained the purpose, content, possible benefits and risks of the study in detail to the research subjects. The research was carried out with written informed consent and signature from all the patients involved. (Ethics Acceptance Number: 2022-X021-01).

Serum sample preparation

An aliquot of 100 µL serum was mixed with 400 µl methanol/acetonitrile (1/1, v/v) by vortex for 30 s. The mixture was sonicated for 10 min at 4 °C. After 1 h of incubation at − 20 °C, samples were centrifuged at 13,000 rpm for 15 min at 4 °C. The supernatant was transferred to a new tube and dried at 4 °C by a vacuum concentrator. The samples were then reconstituted with 100 µL of acetonitrile / H_2O solution (1/1, v/v) for LC/MS analysis.

LC/MS analysis

The injection volume was 3 µL. The analysis of plasma metabolites was carried out in a Shimadzu Prominence UPLC system (Nexera UHPLC LC-30A) interfaced with a Triple TOF 5600 + system (SCIEX) equipped with an ESI source. The metabolites were separated through an Acquity UPLC BEH Amide column (100 × 2.1 mm with 1.7 µm particle size, cat. 186,002,878, Waters) with column temperature maintained at 40 °C. Mobile phases consisted of 25 mM ammonium acetate in 25 mM ammonia water (mobile phase A) and acetonitrile (90/10, v/v) (mobile phase B) and was run at a flow rate of 0.3 ml min−1. The gradient was as follows: 95% B for 1 min, 65% B for 14 min, 40% B within 16 min and held for 18 min, then increased to 95% B for 18.1 min, and back to 9% B and held for another 23 min. The flow rate for mobile phases was set at 0.3 ml/ min. The mass spectrometer was run in positive, information-dependent acquisition (IDA) mode, with the source temperature of 550 °C, the ion source gas 1 and 2 at 55 psi, the curtain gas at 35 psi, the collision energy at + 30 eV or − 30 eV, the ion spray voltage floating at 5.5 kV or − 4.5 kV, and the mass range at 60–1250 m/z. The accumulation time for a full scan was set at 150 ms, and the accumulation time for each IDA scan was 45 ms. Peaks of metabolites with intensities larger than 100 c.p.s. after adding up the signal from 10 rounds of IDA scans were chosen for further analysis.

Animal studies

All the animal protocols were approved by the Animal Care Committee of Xiamen University (No. XMULAC20230217). The ARRIVE guidelines were complied. BKS-wt and db/db male mice (7 weeks old) were purchased from Jiangsu GemPharmatech Co., Ltd. The db/db mice were generated by gene editing and embryo injection techniques to construct mutations in the leptin receptor (Lepr) gene, which had close association with obesity, hypertension, diabetes and lipid metabolism disorders, among others[28,29]. The course of the related diseases was greatly affected by the genetic background. The FBG level and body weight of the db/db mice were

significantly higher than those of the control at around 8 weeks and reached stabilization. The mice were raised in the Animal Facility in Xiamen University with a controlled environment of 21∼23 °C of room temperature, 45%–65% of relative humidity and a 12 h-light/dark cycle. During the experiment, mice were allowed free access to diet and water.

After 1 week of accommodation, db/db male mice were randomly selected into a control group and 4 groups treated with water, taurine, metformin and DPP4 inhibitor (sitagliptin), metformin and DPP4 inhibitor plus taurine. All agents are dissolved in water and the intragastric administered dose were: 400 mg/kg/d for taurine, 250 mg/kg/d for metformin, and 20 mg/kg/d for DPP4 inhibitor. All groups were fed with a normal diet for 8 consecutive weeks during the treatment. The FBG level was measured every 2 weeks. In the 8th week, the mice were euthanized and sacrificed for the analyses of the sera and organ tissues.

Sample preparations for light microscopy

Liver, fat, and colon samples were fixed in 4% paraformaldehyde, and the tissues were embedded in paraffin. Tissue Sects. 5 μm thick were prepared using a sectioning machine and subsequently deparaffinized with xylene, hydrated with gradient ethanol, stained with hematoxylin and eosin, and observed under a microscope. Frozen sections of liver tissue were taken, dewaxed, and hydrated. After staining with oil red staining solution and avoiding light, the tissue was re-stained with 60% isopropyl alcohol, distilled water, and hematoxylin, and the images were observed under a microscope.

For the Oil Red O staining, liver tissue needs to be frozen and cut into approximately 20 μm thick frozen sections using a microtome. The sections are then stained with Oil Red O Reagent according to the instructions. Observations are made using a microscope.

Analysis of the fasting blood glucose

The FBG levels in the mice were assessed biweekly using a blood glucose meter following an 8 h fasting period. At the conclusion of the 8-week treatment, the glycosylated hemoglobin content was measured with a hemoglobin A1c meter. All procedures were executed meticulously in adherence to the guidelines.

Data analysis

Data were collected with Analyst TF 1.6 software (SCIEX) and analyzed by MetaboAnalyst 5.0 with the deconvolution and streamline criteria for metabolomics. For volcano plots, the *P* value was 0.1, and the fold-change threshold was 2.0. Demographic and clinical characteristics of the subjects were evaluated with the Student t-test (for normally distributed continuous variables) and the Mann Whitney test (for nonnormally distributed continuous variables). Categorical variables were displayed as numbers (percentage) of subjects within each group and were compared using the X^2 test. For the purpose of comparing between groups in animal studies, a one-way ANOVA was applied. For significant disparities in ANOVA, further pairwise comparisons were conducted using Tukey's post hoc test to delineate the differences. The above analyses were carried out with software SPSS 23.0.

Data availability

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

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Author contributions

TX, CW, YH, WY, WH, SW, YL, and SW carried out the investigations; FZ, NL, CL, and QD collected the samples from the patients and carried out the grouping; LZ and CW analyzed the data; CL, TL, and XC conceptualized the study and supervised the research; TX, CW, YH,WH made the figures and tables; TL and XC wrote and revised the manuscript; XC obtained the funding.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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