Diabetes, Metabolic Syndrome and Obesity

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ORIGINAL RESEARCH

Profile of Serum Bile Acids in Elderly Type 2 Diabetic Patients with Various Obesity Types: A Cross-Sectional Study

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Objective: The distribution of body fat plays a critical role in the pathogenesis of type 2 diabetes mellitus (T2DM). However, the specific metabolic profiles and biomarkers that distinguish the different obesity phenotypes in T2DM remain to be fully elucidated. Bile acids (BAs), which are recognized as pivotal signaling molecules in the regulation of glucose and lipid metabolism, warrant further investigation to characterize their profiles across different obesity phenotypes. Understanding the clinical significance of these BAs in the management of T2DM is essential and merits thorough exploration.

Design: In this cross-sectional study conducted at the Zhangjiang Community Health Service Center in Shanghai, ninety-nine elderly participants were recruited and categorized into four groups: non-diabetic controls (NC), T2DM with lean phenotype (TN), T2DM with overweight phenotype (TO), and T2DM with abdominal obesity phenotype (TA). Biochemical indices, visceral adiposity indices, and bile acid (BA) profiles were analyzed and compared across the groups.

Results: Healthy individuals exhibited lower triglyceride levels, waist-to-hip ratio (WHR), visceral adiposity index (VAI), and Chinese visceral adiposity index (CVAI), as well as higher HDL-c level and total BA levels compared to T2DM patients. T2DM patients with different obesity phenotypes displayed distinct BA profiles. Specifically, the TN group showed higher levels of conjugated DCA BA species, GDCA, and TDCA, compared to the TO group. These BA species are essential for regulating lipid and glucose metabolism. In contrast, the TA group exhibited higher ratios of 12α-hydroxylated BAs to non 12α-hydroxylated BAs, taurine-conjugated BAs to glycine-conjugated BAs, and higher levels of LCA compared to the TO group. Additionally, CVAI was positively associated with unconjugated SBAs, CA-7S, and DLCA.

Conclusion: These results revealed that T2DM patients with different obesity phenotypes exhibit distinct BA profiles. Specific BAs, particularly GDCA, TDCA, and LCA, are closely associated with adiposity indices and may serve as crucial signaling molecules in modulating visceral adiposity, serum lipid profiles, and glucose homeostasis in obese T2DM patients. These BA species play a pivotal role in the pathogenetic process underlying diabetes and various forms of obesity. Furthermore, their significance highlights their potential contributors to drug development and as therapeutic targets for T2DM patients with specific obesity subtypes.

Keywords: bile acids and salts, diabetes mellitus, obesity

Introduction

The prevalence of type 2 diabetes mellitus (T2DM) has emerged as a significant global health concern.[1] The adverse effects of diabetes extend beyond hyperglycemia, leading to the development of severe complications such as macroangiopathy (eg,

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cardiovascular disease) and microangiopathy (eg, diabetic kidney disease, diabetic retinopathy, and neuropathy).[2,3] However, T2DM is a heterogeneous disease characterized by diverse metabolic phenotypes. Among these, obesity-prone and obesity-resistant phenotypes are particularly notable in T2DM patients.[4] For obesity-prone T2DM, various therapeutic strategies are available, including antidiabetic medications, incretin-based therapies such as glucagon-like peptide-1 (GLP-1) receptor agonists and dipeptidyl peptidase inhibitors, lifestyle modifications, anti-obesity agents, and bariatric surgery. However, these approaches are not entirely suitable for obesity-resistant T2DM.[5,6] Therefore, identifying personalized treatment strategies tailored to specific metabolic phenotypes is of great clinical significance.

Obesity is a major risk factor for the pathogenesis of T2DM, with approximately 60% of individuals with diabetes being obese.[1] Body fat distribution has a unique impact on developing metabolic diseases, including T2DM.[7] Notably, visceral fat is more strongly associated with metabolic complications than subcutaneous fat.[8] A recent population-based study revealed that overweight individuals with a high waist circumference (WC) have a significantly higher cumulative incidence of T2DM compared to those with a normal WC.[9] In recent years, researchers have expanded the assessment of visceral obesity beyond traditional measures as WC and waist-to-hip ratio (WHR) to include more comprehensive indicators like the visceral adiposity index (VAI) and the Chinese visceral adiposity index (CVAI).[10,11] These indices have been extensively validated in the literature as reliable predictors of diabetes.[11–13] However, whether different obesity phenotypes in T2DM are associated with specific metabolic biomarkers and their relationship with visceral adiposity indices remains an area requiring further exploration.

Bile acids (BAs), a key component of bile, play an essential role in the digestion and absorption of nutrients.[14] Beyond their digestive functions, BAs act as critical signaling molecules that regulate glucose and lipid metabolism.[15] Previous studies have shown that T2DM patients have lower circulating BA concentrations compared to healthy individuals.[16] Additionally, the ratio of 12α-hydroxylated BAs to non-12α-hydroxylated BAs has been linked to key features of insulin resistance (IR).[17] The interplay between lipid and BA metabolism has also been well-documented. Research has demonstrated that genetic and microbiological factors influence plasma and fecal BA profiles, plasma lipid levels, and hepatic fat accumulation in obese individuals.[18] Despite these findings, a comprehensive understanding of the distinct characteristics of BAs in diabetic patients and their variations across different obesity phenotypes remains incomplete.

Our study aims to lay the groundwork for future research exploring the relationship between serum BAs, T2DM, and body fat distribution. To achieve this, we analyzed the clinical characteristics and specific BA metabolites in obese T2DM patients. Additionally, we investigated the correlations between clinical markers and serum BAs to further elucidate their interrelationships.

Materials and Methods

Study Population

The study cohort consisted of 99 participants aged 60 to 85, including 33 non-diabetic individuals (18 females and 15 males) and 66 patients with T2DM (37 females and 29 males). Participants were recruited from the Zhangjiang Community Health Service Center in Shanghai. Exclusion criteria included individuals with type 1 diabetes, gestational diabetes, or other specific types of diabetes, as well as those with a history of mental illness, abnormal liver or kidney function, or drug abuse.

Based on the screening results, the study participants were categorized into four groups (Figure 1): non-diabetic controls (NC) (fasting blood glucose (FBG) < 6.1 mmol/L; Body mass index (BMI) < 24 kg/m^2; WC: males < 90 cm, females < 85 cm; n = 33), T2DM with lean phenotype (TN) (FBG ≥ 7.0 mmol/L; BMI < 24 kg/m^2, WC: males < 90 cm, females < 85 cm; n = 21), T2DM with overweight phenotype (TO) (FBG ≥ 7.0 mmol/L; BMI ≥ 24 kg/m^2; WC: males < 90 cm, females < 85 cm; n = 22), and T2DM with abdominal obesity phenotype (TA) (FBG ≥ 7.0 mmol/L; BMI ≥ 24 kg/m^2; WC: males ≥ 90 cm, females ≥ 85 cm; n = 23).

Clinical Data Collection

Data on age, gender, and medical history were collected through structured questionnaires. Height and body weight were measured using electronic measurement instruments (Sheng-yuan, Zhengzhou, China), while participants wore light clothing and removed their hats or shoes. Measurements were recorded to the nearest 0.1 cm for height and 0.1 kg for weight. WC and

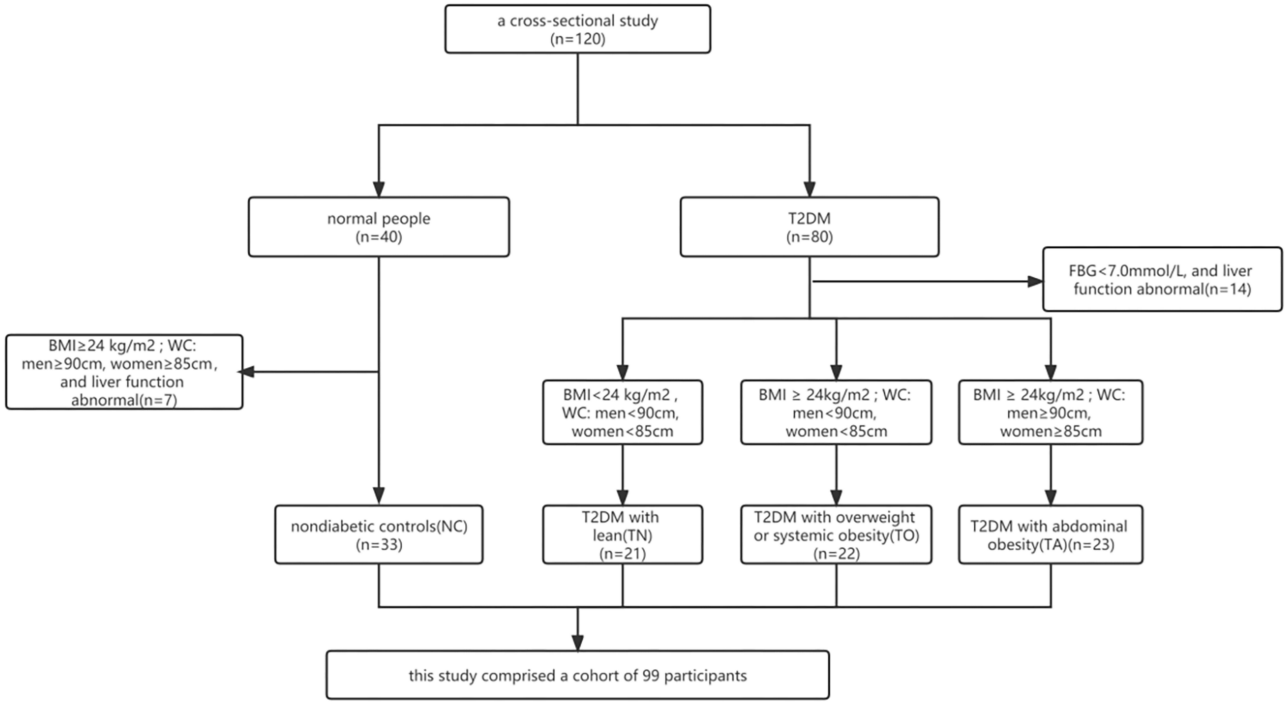

Figure 1 Flowchart of study population inclusion and exclusion.

hip circumference (HC) were measured using a tape measure while participants stood upright. WC was measured at the midpoint between the lowest rib and the iliac crest, while HC was measured at the level of the greater trochanter. Blood pressure (BP) was measured using electronic sphygmomanometers (Bio-space in Cheonan, South Korea) after participants rested in a seated position for 10 minutes. All anthropometric measurements and questionnaires data were collected by trained physicians. BMI was calculated as weight (in kilograms) divided by the square of height (in meters). WHR was determined by dividing WC by HC.

Blood samples were collected from the antecubital vein in the morning after an overnight fast. FBG, total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), alanine transaminase (ALT), aspartate transaminase (AST), uric acid (UA), and creatinine (Cr) were measured using an automatic biochemistry analyzer (Hitachi, Tokyo, Japan).

The VAI and CVAI were calculated using the following formulas (with TG and HDL-C expressed in mmol/L) males: VAI = (WC/39.68 + (1.88 BMI)) × (TG/1.03) × (1.31/HDL-c); females: VAI = (WC/36.58 + (1.89 × BMI)) × (TG/0.81) × (1.52/HDL-c); Males: CVAI = −267.93 + 0.68 × age + 0.03 × BMI + 4.00 × WC (cm) + 22.0/log 10 TG-16.32 × HDL-c; Females: CVAI = −187.32 + 1.71 × age + 4.23 × BMI + 1.12 × WC (cm) + 39.76 × log 10 TG-11.66 × HDL-c. The VAI,[19] a composite measure incorporating WC, BMI, TG, and HDL-c levels, was initially developed to assess visceral adiposity. To account for differences in body fat distribution between Asian and Caucasian populations, Xia et al[20] introduced the CVAI, a novel index derived from a multivariate regression model that includes age as a variable. This index has since been validated as a reliable tool for evaluating visceral fat dysfunction.

Disease Definition

Incident diabetes was defined as meeting any of the following criteria: FBG ≥ 7.0 mmol/L, 2-hour post-load plasma glucose (2hPG) ≥11.1 mmol/L, or self-reported previous diagnosis of diabetes by a physician along with the current use of antidiabetic medications.[21] Overweight was defined as BMI ≥ 24.0 kg/m^2,[22] while abdominal obesity was defined as WC ≥ 90.0 cm for males and ≥85.0 cm for females.[23]

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BA Measurement

BA content was quantified using ultra-high performance liquid chromatography-tandem mass spectrometry (LC-MS) on an ACQUITY UPLC system (ExionLC™ AD, USA) coupled with a triple quadrupole mass spectrometer (QTRAP®6500+, USA). LC-MS is widely recognized as a powerful analytical technique for complex biological samples due to its exceptional separation capabilities and versatility across a broad range of compounds.[24,25] The liquid chromatography conditions were as follows: the chromatographic column was a Waters ACQUITY UPLC HSS T3 C18 column (1.8 μm, 100 nm*2.1 mm i.d.); the mobile phase consisted of phase A containing ultrapure water with 0.01% acetic acid and 5 mmol/L ammonium acetate, and Phase B containing acetonitrile with 0.01% acetic acid, with a flow rate of 0.35 mL/min; the column temperature was maintained at 40°C; and the injection volume was 3 μL. The gradient elution involved a series of A/B ratios, including 0 min A/B 95:5 (V/V), 0.5 min A/B 60:40 (V/V), 4.5 min A/B 50:50 (V/V), 7.5 min A/B 25:75 (V/V), and 10.0 min A/B 5:95 (V/V), with a final ratio of 95:5 (V/V) at 12.0 min A/B. The mass spectrometry conditions included electrospray ionization (ESI) at a temperature of 550°C, a mass spectrometry voltage of −4500 V, and a curtain gas (CUR) of 35 psi. Each ion pair was scanned and detected in the triple quadrupole using optimized declustering potential (DP) and collision energy (CE). To ensure data quality, a quality control (QC) sample, prepared as a mixed solution, was analyzed for every ten test samples. The instrument's high stability provided critical assurance for the consistency and dependability of the data. After acquiring mass spectrometry data from various samples, the chromatographic peaks of all target analytes were integrated and quantified using standard curves.

Statistical Analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS25.0). The Shapiro–Wilk test was used to assess the normality of the data distribution. Normally distributed data were expressed as mean ± standard deviation and compared using one-way analysis of variance (ANOVA). Non-normally distributed data were expressed as median and interquartile range M (P25, P75) and compared using the Mann–Whitney test of two independent samples. Categorical variables were expressed as percentages and compared using the Chi-square test. The Mantel test and Procrustes analysis were employed to evaluate potential relationships between BA profiles and clinical indicators. Spearman correlation analysis was employed to explore associations between biochemical parameters and BA metabolic profiles. For data normalization in the forest model, we used the transformation $\log_{10}$ (x*=$\log_{10}$ (x)/$\log_{10}$ (max)). Area under the curve (AUC) model evaluation of serum BAs was used to estimate each BA species across the four groups. A two-tailed p-value < 0.05 was considered statistically significant throughout the study.

Results

Baseline Characteristics of Participant

The baseline characteristics of the study participants are presented in Table 1. The WHR of the three groups of T2DM patients showed significant differences compared to the NC group. Both the TO and TA groups exhibited higher TG levels and lower HDL-c values than the NC group. According to the findings of this study, the BMI, WC, HC, WHR, VAI, and CVAI values were significantly higher in the TO and TA groups compared to the NC and TN groups. Notably, the WC, WHR, and CVAI values were significantly greater in the TA group than in the TO group. Additionally, the TA group demonstrated the highest levels of the inflammatory marker ALT and the renal function index UA.

Distribution of BAs in the Study Population

Among the four groups, the TA group exhibited the lowest percentage of unconjugated primary bile acids (PBAs) and the highest percentage of conjugated PBAs. In contrast, the TO group showed the lowest percentages of conjugated PBAs and conjugated secondary bile acids (SBAs) (Figure 2A). However, no significant differences were observed in the levels of total BAs, PBAs, SBAs, or the SBAs/PBAs ratio across the groups (Figure 2B–E). Although serum levels of 12α-hydroxylated BAs and non-12α-hydroxylated BAs did not differ significantly, the ratio of 12α-hydroxylated BAs to non-12α-hydroxylated BAs was significantly lower in the TO group compared to the TA group. Specifically, the TO group had a median (interquartile

Table 1 Baseline Characteristics of the Participants

	NC	TN	TO	TA
Clinical characteristics				
No. (F:M)	33 (18:15)	21 (12:9)	22 (9:13)	23 (16:7)
Age (y)	70.00 (66.00–74.00)	74.00 (69.50–77.50)	72.50 (69.00–78.00)	69.00 (67.00–73.00)
BMI (kg/m^2)	21.72 (21.02–22.48)	22.19 (21.66–23.48)*	25.63 (24.68–26.32)*$^{\#}$	26.54 (25.44–27.87)*$^{\#,\dagger}$
WC (cm)	74.00 (72.05–77.20)	79.00 (73.00–82.10)	83.10 (80.15–84.45)*$^{\#}$	91.30 (89.20–93.10)*$^{\#,\dagger}$
HC (cm)	90.00 (88.10–92.55)	89.10 (87.65–90.70)	95.15 (93.53–96.85)*$^{\#}$	98.00 (95.00–101.00)*$^{\#}$
WHR	0.82 (0.80–0.88)	0.89 (0.83–0.92)*	0.87 (0.84–0.89)*	0.94 (0.91–0.96)*$^{\#,\dagger}$
SBP (mmHg)	132.24±17.20	136.71±21.67	149.64±17.81*$^{\#}$	151.00±20.76*$^{\#}$
DBP (mmHg)	74.21±10.20	72.62±8.41	74.59±5.69	75.39±9.06
Blood glucose				
FBG (mmol/L)	5.15 (4.88–5.52)	9.69 (8.28–11.82)*	8.20 (7.45–9.12)*$^{\#}$	8.44 (7.40–9.73)*$^{\#}$
Lipid profile				
TC (mmol/L)	4.67 (4.16–5.08)	4.74 (4.15–5.46)	4.88 (4.16–5.61)	4.96 (3.84–5.79)
TG (mmol/L)	0.95 (0.75–1.35)	1.24 (0.80–2.19)	1.61 (0.99–2.18)*	1.55 (0.85–2.44)*
HDL-c (mmol/L)	1.37 (1.19–1.65)	1.28 (1.03–1.52)	1.10 (1.05–1.33)*	1.09 (0.93–1.26)*
LDL-c (mmol/L)	2.85±0.75	2.97±0.78	3.19±0.69	2.92±1.11
Hepatorenal function				
ALT (U/L)	16.00 (13.00–18.50)	17.00 (14.50–24.00)	18.50 (14.00–22.25)	20.00 (15.00–28.00)*
AST (U/L)	20.00 (18.50–25.00)	18.00 (15.50–21.50)*	18.50 (14.75–22.00)*	20.00 (18.00–24.00)
UA (μmmol/L)	300.40 (272.60–332.50)	285.70 (238.80–366.70)	295.50 (258.03–345.55)	366.20 (278.40–419.40)*$^{\#}$
Cr (μmmol/L)	67.00 (58.00–74.50)	64.00 (50.50–79.50)	61.00 (54.75–70.00)	70.00 (59.00–92.00)
Visceral adiposity index				
VAI	46.65 (31.40–59.21)	63.18 (44.20–142.87)	100.19 (63.78–159.95)*	121.20 (68.83–262.22)*$^{\#}$
CVAI	77.96±18.18	95.65±31.74*	114.62±20.21*$^{\#}$	144.54±21.13*$^{\#,\dagger}$

Notes: Values are n, mean ± SD, or median (interquartile range); *$p < 0.05$ for comparison with NC group; $^{\#}p < 0.05$ for comparison with TN group; $^{\dagger}p < 0.05$ for comparison with TO group.

range) of 0.25 (0.14–0.35), while the TA group had a median (interquartile range) of 0.39 (0.16–0.65) ($p = 0.048$). Similarly, no significant differences were observed in the serum levels of total taurine-conjugated and glycine-conjugated BAs. However, the ratio of taurine-conjugated BAs to glycine-conjugated BAs was significantly higher in the TA group than in the TO group (0.13 (0.12–0.20) vs 0.09 (0.06–0.15); $p = 0.026$) (Figure 2F–K). Furthermore, conjugated deoxycholic acid (DCA) BA species were more abundant in the TN group compared to the TO group, while unconjugated lithocholic acid (LCA) BA species were less abundant in the TN group than in the TA group (both $p < 0.05$; Figure 2L and M).

Conjugated DCA BA species, glycodeoxycholic acid (GDCA), and taurodeoxycholic acid (TDCA), belonging to DCA BA species, were significantly higher in the TN group compared to the TO group (all $p < 0.05$; Figures 2L,3A and B). The TO group exhibited higher levels of glycoursodeoxycholic acid-3-sulfate (GUDCA-3S) and β-muricholic acid (β-MCA) than the NC group (both $p < 0.05$), while levels of TDCA and 3β-hydroxycholenoic acid (3β-HDCA) were lower in the TO group (both $p < 0.05$; Figure 3B–E). In contrast, unconjugated LCA BA species and isoallolithocholic acid (IALCA) were less abundant in the TN group compared to the TA group (both $p < 0.05$; Figures 2M and 3F). Serum levels of taurolithocholic acid (TLCA) and LCA were significantly elevated in TN and TA groups, respectively, compared to the TO group (25.60 (0.00–25.79) vs 25.83 (12.80–26.09) nmol/L, and 8.94 (3.74–13.81) vs 13.23 (6.21–30.31) nmol/L; $p < 0.05$; Figure 3G and H). Furthermore, the TN group showed higher levels of β-glycocholic acid (βGCA) compared to the TA group (Figure 3I).

Random Forest Analysis and AUC Model Evaluation of Serum BAs

AUC was selected as the metric to identify differential BA at the greatest value in the four groups' random forest pairwise comparisons, which were used to create the Venn diagram (Figure 4A). The combined presence of 3-oxo-cholic acid (3-oxo-CA) and 12-oxo-chenodeoxycholic acid (12-oxo-CDCA) produced the highest AUC value (AUC = 0.74;

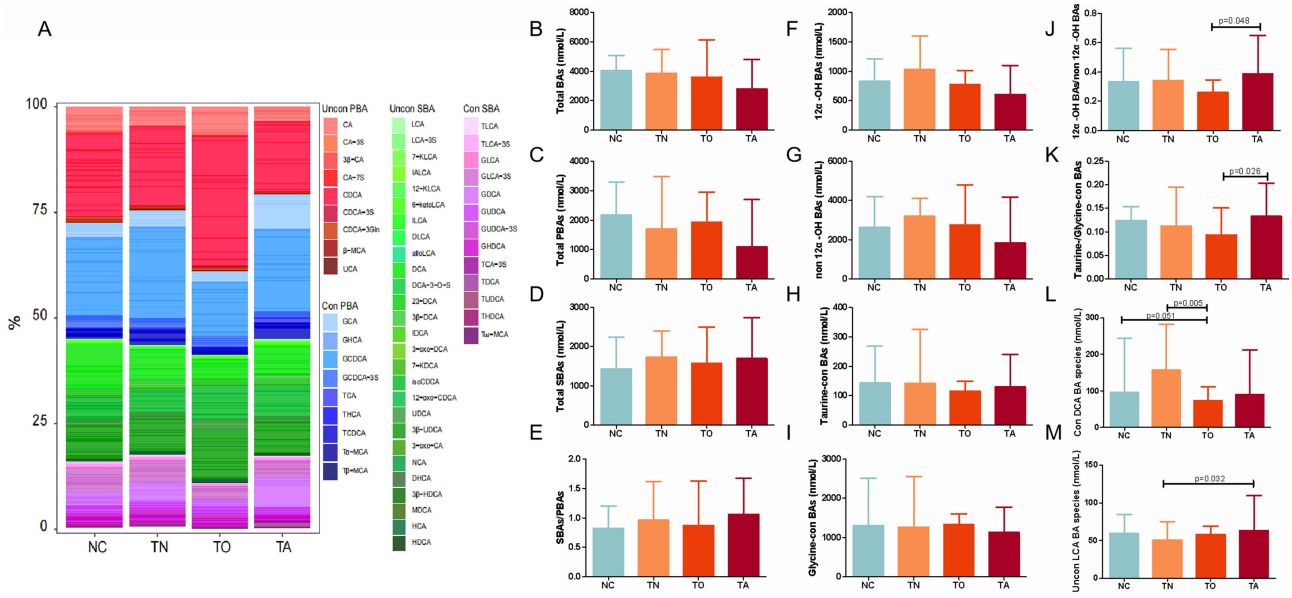

Figure 2 Distribution of BAs in the Study Population. (**A**) The relative percentage content of BAs in different groups. Unconjugated PBA in red, blue for conjugated PBA, Unconjugated SBA in green, and conjugated SBA in purple. (**B**–**M**) Total BAs; Total PBAs; Total SBAs; SBAs/PBAs; 12α-OH BAs; non-12α-OH BAs; Taurine-conjugated BAs; Glycine-conjugated BAs; 12α-OH BAs/non-12α-OH BAs; Taurine-/Glycine-conjugated BAs; Conjugated DCA BA species; Unconjugated LCA BA species. Mann–Whitney *U*-test.

Abbreviations: Con, conjugated; Uncon, unconjugated; PBAs, primary bile acid; SBAs, secondary bile acid.

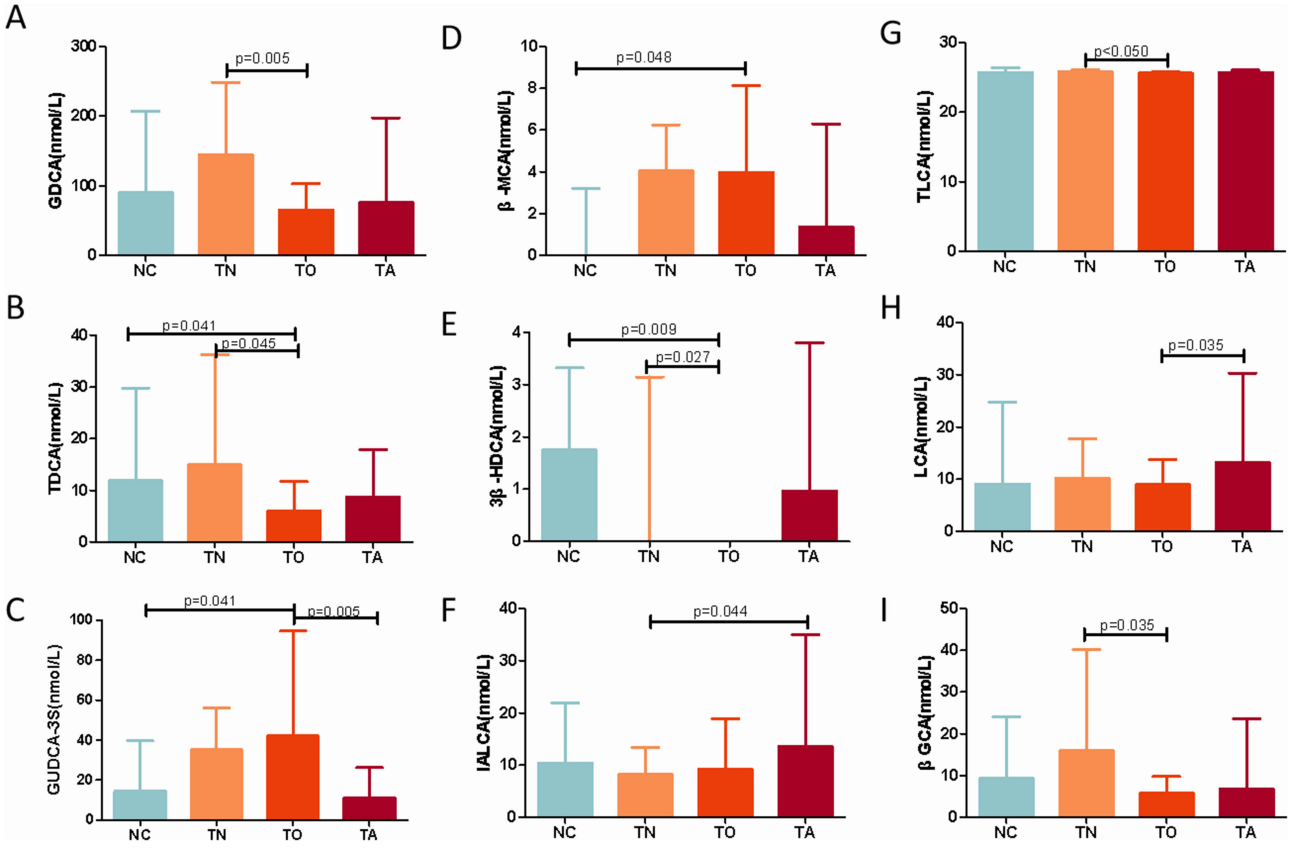

Figure 3 Distribution of differentiation BAs in the Study Population. (**A**–**I**) GDCA; LCA; TDCA; GUDCA-3S; IALCA; β-MCA; βGCA; TLCA; 3β-HDCA. Mann–Whitney *U*-test.

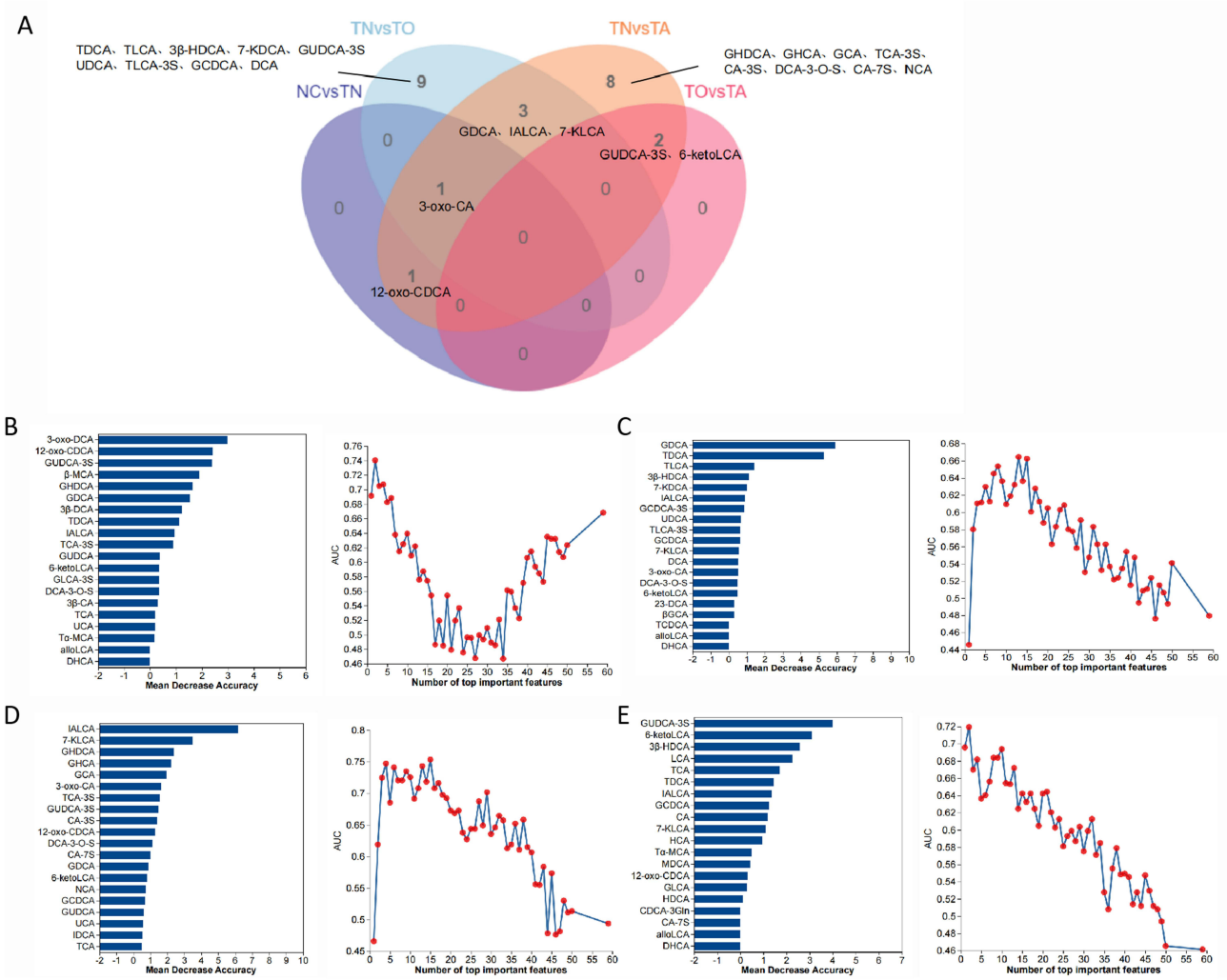

Figure 4 Random forest analysis and AUC model evaluation of serum BAs among four groups. (**A**) The Venn diagram of the random forest analysis and AUC model evaluation of serum BAs in four groups: (**B**–**E**) The figure on the left displays the top 20 differential metabolites, while the figure on the right presents the model evaluation using top important metabolism. If serum BA levels are recorded as 0, it is recommended to assign a value of 0.1 before log10 transformation of the data. (**B**) NC vs TN; (**C**) TN vs TO; (**D**) TN vs TA; (**E**) TO vs TA.

Figure 4B) in the comparison between the NC and TN groups. Similarly, TN and TO groups were compared, and the top 13 differential metabolites, namely GDCA, TDCA, TLCA, 3β-HDCA, 7-ketodeoxycholic acid (7-KDCA), IALCA, glycochenodeoxycholic acid-3-sulfate (GCDCA-3S), ursodeoxycholic acid (UDCA), taurolithocholic acid-3-sulfate (TLCA-3S), glycochenodeoxycholic (GCDCA), 7-ketolithocholic acid (7-KLCA), DCA, and 3-oxo-CA, exhibited the highest AUC values (AUC = 0.66; Figure 4C). When the TN and TA groups were compared, the top 15 differential metabolites had the highest AUC value of 0.75, including IALCA, 7-KLCA, glycohyodeoxycholic acid (GHDCA), glycohyocholic acid (GHCA), glycocholic acid (GCA), 3-oxo-CA, taurocholic acid-3-sulfate (TCA-3S), GUDCA-3S, cholic acid-3-sulfate (CA-3S), 12-oxo-CDCA, deoxycholic acid-3-O-sulfate (DCA-3-O-S), cholic acid-7-sulfate (CA-7S), GDCA, 6-keto LCA, and NCA (Figure 4D). When the TO and TA groups were compared, the GUDCA-3S and 6-keto-lithocholic acid (6-keto LCA) showed the greatest AUC value (AUC = 0.72; Figure 4E).

Association Between Serum BAs and Clinical Parameters

Clinical phenotype and BA composition significantly correlated, according to a Procrustes analysis of physiological measurements based on Bray–Curtis distance and principal coordinates analysis (PCoA) of BA composition (Procrustes analysis, $p < 0.05$; Mantel test, $p < 0.05$, permutations = 999; Figure 5A). Furthermore, Spearman correlation analysis

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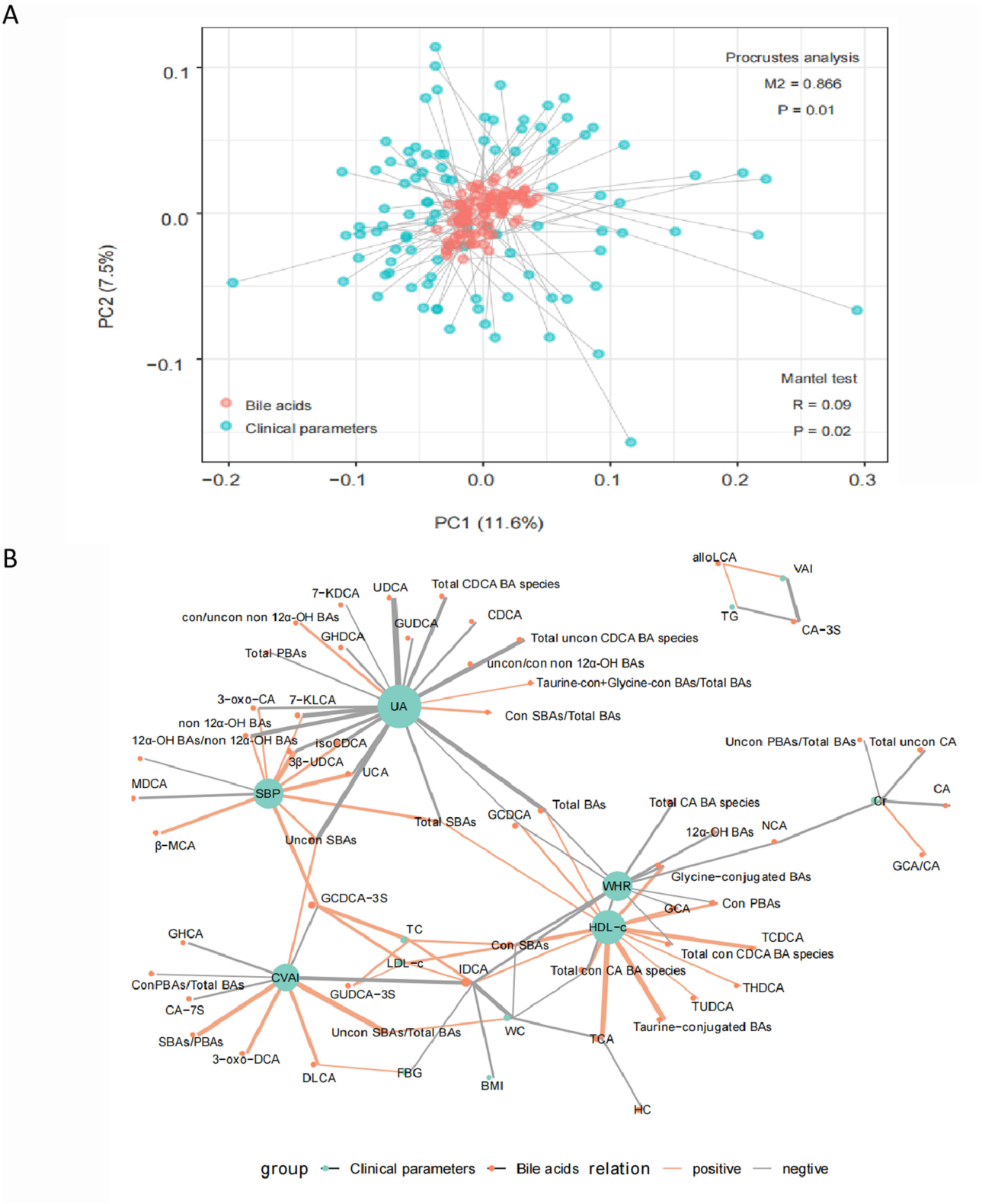

Figure 5 Association between Serum BAs and Clinical Parameters. (**A**) Mantel test and Procrustes analysis to assess the correlation between bile acids and clinical indicators. (**B**) Spearman correlation analysis of the association between serum BAs and clinical parameters.

Abbreviations: PBA, the sum of primary BAs; SBA, the sum of secondary BAs; con, conjugated; uncon, unconjugated; T/G, Taurine-conjugated BAs/Glycine-conjugated BAs.

demonstrated significant relationships between the subgroup of serum BA composition and several biochemical measures and metabolic parameters in the study participants. GCDCA-3S, GHCA, CA-7S, and the ratio of ConPBAs to total BAs showed negative correlations with CAVI. These relationships are illustrated in the correlation network presented in Figure 5B. Additionally, allolithocholic acid (alloLCA) correlated positively with TG and VAI, while CA-3S correlated negatively. HDL-c, LDL-c, and TC correlated positively with a serum of all measured BAs, while WC, HC, WHR, UA, and Cr correlated negatively. All BAs had positive connections with SBP, except for the ratio of 12α-hydroxylated BAs to non-12α-hydroxylated BAs and murideoxycholic acid (MDCA). However, deoxycholic acid (DLCA) was positively associated with CAVI and FBG, while isodeoxycholic acid (IDCA) correlated negatively with CAVI, BMI, and FBG.

Discussion

This study investigated the clinical characteristics and BA profiles in healthy individuals and T2DM patients. Our findings revealed that healthy individuals exhibited lower levels of TG, VAI, and CVAI while displaying higher levels of HDL-c and total BAs compared to T2DM patients. Additionally, distinct BA profiles were observed among different obesity phenotypes of T2DM. Specifically, we found that the TA group had higher ratios of 12-hydroxylated BAs to non-12-hydroxylated BAs and taurine-conjugated BAs to glycine-conjugated BAs compared to the TO group. Furthermore, obese T2DM patients exhibited variations in specific BAs, including GDCA, TDCA, LCA, and TLCA. Moreover, serum BA levels were positively associated with lipid profiles, such as TC and HDL-c. CVAI was also associated with uncon SBAs, CA-7S, and DLCA.

Our results highlighted distinct metabolic phenotype differences in obese patients with T2DM, showing significantly different VAI and CVAI. The WHR and CVAI of the three T2DM groups showed significant differences compared to the NC group, underscoring the close relationship between visceral adiposity and T2DM. TA group had the highest VAI and CVAI levels, while healthy individuals had the lowest. VAI and CVAI have gained considerable attention as novel, easy-to-use indices for assessing IR and are recognized as reliable biomarkers for cardiometabolic risk and incident hypertension.[11,26,27] CVAI has been validated as a valuable predictor for evaluating glucose and lipid metabolism in T2DM patients,[12] particularly within the Chinese population, which aligns with our findings. The potential applications of VAI and CVAI as biomarkers and predictors for various metabolic phenotypes are extensive and warrant further exploration.

BAs can ameliorate dysmetabolic diseases by modulating the BA pool and BA receptor activities.[28] Our study revealed that T2DM patients exhibiting distinct metabolic phenotypes displayed unique BA profiles.[28] Notably, healthy individuals exhibited the highest total BA levels, which decreased with increasing BMI, WC, VAI, and CVAI. This observation aligns with a randomized controlled trial demonstrating an increase in total BA levels following reductions in body weight, BMI, WC, and abdominal fat.[29] Weight loss was associated with improved insulin sensitivity, which in turn led to a proportional increase in BA production and absorption.[28] Our study also found a significant elevation in the ratios of 12α-hydroxylated BAs to non 12α-hydroxylated BAs and taurine-conjugated BAs to glycine-conjugated BAs in the TA group compared to the TO group. Similar alterations in BA species were observed in obese patients who underwent Roux-en-Y gastric bypass surgery.[30] Postoperative increases in these BA species were found to enhance dietary fat and cholesterol absorption through the action of fibroblast growth factor (FGF) 19, a key regulator of BA synthesis.[31]

Our study revealed that specific BA species within different metabolic phenotype groups were significantly associated with clinical characteristics. Obese T2DM patients exhibited variations in GDCA, TDCA, LCA, TLCA, and other BAs. Specifically, the TO group showed lower levels of GDCA and TDCA compared to the TN group. A previous observational study has reported a positive correlation between GDCA and IR in lean young males, while TDCA was associated with IR in obese individuals.[32,33] A cohort study conducted in a Chinese population suggested that GDCA and TDCA, both linked to IR, may play a significant role in modulating insulin clearance during the early stages of hyperinsulinemia.[34] In the study examining bile acids in patients with gestational diabetes, a significant reduction in GDCA and TDCA levels was observed, which were inversely correlated with the homeostasis model assessment of insulin resistance (HOMA-IR).[35] Furthermore, GDCA administration has been shown to increase GLP-1 levels and reduce late postprandial glucose levels.[32] These findings suggest that GDCA and TDCA likely influence T2DM patients and obese individuals through mechanisms involving IR. DCA capsules have been shown to reduce blood glucose levels in animal models of type 2 diabetes, highlighting their potential as a promising therapeutic agent.[36] Moreover, our research identified distinct LCA BA species among the three groups of T2DM patients. Several studies have shown that

LCA enhances metabolism by promoting the synthesis of bile acid-7-sulfate (CA-7S) in human hepatocytes and rat liver, as well as stimulating GLP-1 secretion in enteroendocrine cells.[37,38] Furthermore, CA-7S, an antidiabetic molecule, was negatively correlated with CVAI. These BA species may play a critical role in regulating visceral adiposity, serum lipid profiles, and glucose homeostasis in obese T2DM, thereby contributing to the pathogenetic process underlying diabetes and various forms of obesity.

The novelties of this research include the use of visceral adiposity index and targeted BA analysis in obese T2DM patients. However, this study has several limitations. First, the clinical trial was conducted at a single center with a limited sample size, highlighting the need for future studies to adhere to adhere to rigorous clinical research standards and expand the sample size to enhance generalizability. Second, serum BA levels may be influenced by a variety of complex exogenous and genetic factors, such as lifestyle and dietary habits, which could introduce variability in the results. Lastly, the lack of evaluation of insulin-related indicators prevented a comprehensive analysis of the relationship between BAs and insulin in glucose and lipid metabolism. Consequently, it is essential to acknowledge that these factors cannot be entirely disregarded and should be addressed in future research.

Conclusions

These findings revealed that novel changes in BAs profiles exist in obese T2DM patients. Specific BA species, especially GDCA, TDCA and LCA, may play a critical role in regulating visceral adiposity, serum lipid profiles, and glucose homeostasis in obese T2DM patients, thereby contributing to the pathogenetic process underlying diabetes and various forms of obesity. The potential utility of BAs, along with visceral adiposity indexes such as the VAI and the CVAI, as biomarkers and predictors of diverse metabolic phenotypes is particularly noteworthy. However, further research is necessary to fully elucidate the therapeutic effects of these BA species on diabetes management in individuals with varying metabolic phenotypes.

Abbreviations

ALT, alanine transaminase; alloDCA, allodeoxycholic acid; AST, aspartate transaminase; AUC, area under the curve; BAs, Bile acids; BP, blood pressure; BMI, body mass index; CA, cholic acid; CA-3S, cholic acid-3-sulfate sodium salt; CA-7S, bile acid-7-sulfate; CDCA, chenodeoxycholic acid; CE, Collision energy; Con, conjugated; Cr, creatinine; CVAI, Chinese visceral adiposity index; DBP, diastolic blood pressure; DCA, deoxycholic acid; DCA-3-O-S, deoxycholic acid 3-O-sulfate disodium salt; DP, Declustering potential; FBG, fasting blood glucose; FGF, fibroblast growth factor; GCA, glycocholic acid; GCDCA, glycochenodeoxycholic acid; GCDCA-3S, glycochenodeoxycholic acid-3-sulfate disodium salt; GDCA, glycodeoxycholic acid; GHCA, glycohyocholic acid; GHDCA, glycohyodeoxycholic acid; GLP-1, glucagon-like peptide-1; GUDCA-3S, glycoursodeoxycholic acid-3-sulfate sodium; HC, hip circumference; IALCA, isoallolithocholic acid; IR, insulin resistance; LCA, lithocholic acid; LC-MS, liquid chromatography-tandem mass spectrometry; NCA, norcholic acid; PBAs, primary bile acids; PCoA, principal coordinates analysis; SBAs, secondary bile acids; SBP, systolic blood pressure; T2DM, type 2 diabetes mellitus; TC, total cholesterol; TCA-3S, taurocholic acid-3-sulfate sodium salt; TDCA, taurodeoxycholic acid; TG, triglyceride; TLCA, taurolithocholic acid; TLCA-3S, taurolithocholic acid-3-sulfate; UA, uric acid; UDCA, ursodeoxycholic acid; Uncon, unconjugated; VAI, visceral adiposity index; WC, waist circumference; WHR, waist-to-hip ratio; β-MCA, β-muricholic acid; 3β-HDCA, 3β-hyodeoxycholic acid; 3-oxo-CA, 3-oxocholic acid; 6-keto LCA, 5-β-cholanic acid-3α-ol-6-one; 7-KDCA, 7-ketodeoxycholic acid; 7-KLCA, 7-ketolithocholic acid; 12-oxo-CDCA, 12-oxochenodeoxycholic acid.

Data Sharing Statement

All data are available at: https://osf.io/pnd9s/?view_only=484ecfce53474515905958b8ec692bcc (accessed on 09 October 2023).

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the patients to publish this paper.

Institutional Review Board Statement

The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of Longhua Hospital affiliated with Shanghai University of Traditional Chinese Medicine (Approval Letter No.: 2017LCSY069 and 28 December 2017).

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Author Contributions

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.

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Disclosure

The authors declare no conflicts of interest in this work.

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