Fasting serum total bile acid levels are associated with insulin sensitivity, islet β-cell function and glucagon levels in response to glucose challenge in patients with type 2 diabetes

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Abstract. Type 2 diabetes (T2D) is characterized by islet β-cell dysfunction and impaired suppression of glucagon secretion of α-cells in response to oral hyperglycaemia. Bile acid (BA) metabolism plays a dominant role in maintaining glucose homeostasis. So we evaluated the association of fasting serum total bile acids (S-TBAs) with insulin sensitivity, islet β-cell function and glucagon levels in T2D. Total 2,952 T2D patients with fasting S-TBAs in the normal range were recruited and received oral glucose tolerance tests for determination of fasting and postchallenge glucose, C-peptide and glucagon. Fasting and systemic insulin sensitivity were assessed by homeostasis model assessment (HOMA) and Matsuda index using C-peptide, i.e., ISHOMA-cp and ISI⁹⁹-cp, respectively. Islet β-cell function was assessed by the insulin-secretion-sensitivity-index-2 using C-peptide (ISSI²-cp). The area under the glucagon curve (AUCgla) was used to assess postchallenge glucagon. The results showed ISHOMA-cp, ISI⁹⁹-cp and ISSI²-cp decreased, while AUCgla notably increased, across ascending quartiles of S-TBAs but not fasting glucagon. Moreover, S-TBAs were inversely correlated with ISHOMA-cp, ISI⁹⁹-cp and ISSI²-cp (r = –0.21, –0.15 and –0.25, respectively, p < 0.001) and positively correlated with AUCgla (r = 0.32, p < 0.001) but not with fasting glucagon (r = 0.033, p = 0.070). Furthermore, after adjusting for other clinical covariates by multiple linear regression analyses, the S-TBAs were independently associated with ISHOMA-cp (β = −0.04, t = −2.82, p = 0.005), ISI⁹⁹-cp (β = −0.11, t = −7.05, p < 0.001), ISSI²-cp (β = −0.15, t = −10.26, p < 0.001) and AUCgla (β = 0.29, t = 19.08, p < 0.001). Increased fasting S-TBAs are associated with blunted fasting and systemic insulin sensitivity, impaired islet β-cell function and increased glucagon levels in response to glucose challenge in T2D.

Key words: Total bile acids, Insulin sensitivity, β-cell function, Glucagon, Type 2 diabetes

Received online in J-STAGE as advance publication Jul. 17, 2020
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intestinal digestion and absorption of lipophilic nutrients [1]. In recent studies, BAs were also shown to act as important signalling molecules in the regulation and maintenance of glucose, lipid, and energy homeostasis [2]. The BA pool is maintained mainly via enterohepatic circulation (approximately 95%) and to a small extent (approximately 5%) by hepatic synthesis of BAs [3]. BAs emerge in the peripheral circulation when BA levels increase in hepatocytes, and the BAs spill over into sinusoidal blood. Elevation in the serum total BA (S-TBA) in the fasting state have been reported in several studies conducted in obese individuals or individuals with diabetes with a background of insulin resistance [4, 5]. Haeusler et al. [6] revealed that S-TBA levels in patients with type 2 diabetes (T2D) were nearly two-fold higher than those in healthy subjects. In addition, increased S-TBA levels were also found to be positively associated with fasting insulin resistance in both nondiabetic subjects and patients with type 2 diabetes (T2D) [7] and could increase the risk of gestational diabetes [8]. However, the roles of S-TBAs in pathogenesis of T2D is not well clear. We needed a study pooling a large sample set with T2D to explore the association of fasting S-TBA levels with multiple metabolic features and to contribute evidence regarding the roles of S-TBAs in T2D.

The pathophysiological changes of T2D are characterized by decreased insulin sensitivity, impaired islet β-cell function [9] and impaired suppression of glucagon secretion from α-cells in response to oral hyperglycaemia [10-12]. The core pathogenesis of T2D involves disorders in multiple organs and tissues, including the pancreas, liver, muscles and intestine [13]. Meanwhile, BAs affect host glucose and energy metabolism via interactions with their receptors, mainly nuclear farnesoid X receptor (FXR) and transmembrane Takeda G protein-coupled receptor 5 (TGR5) [4, 14], in multiple organs and tissues that are also involved in T2D. Therefore, we hypothesized that fasting S-TBAs may be connected to decreased insulin sensitivity, impaired islet β-cell function and dysregulation of glucagon secretion of α-cells in patients with T2D.

Therefore, the present cross-sectional study was conducted in a large population with T2D to explore the association of S-TBAs with indices of core mechanisms of T2D derived from an oral glucose tolerance test (OGTT). We addressed the following questions: (1) Are fasting S-TBAs independently associated with fasting insulin sensitivity (primarily the liver), systemic insulin sensitivity (primarily the liver and muscles) and islet β-cell function? (2) Are fasting S-TBAs independently associated with fasting and postchallenge glucagon levels in these patients?

**Methods**

**Patient recruitment**

Patients with T2D who visited the outpatient department or were hospitalized as inpatients of the Department of Endocrinology, Affiliated Hospital 2 of Nantong University, between 2015 and 2018 were consecutively recruited for this study. During the recruitment stage, the major inclusion criteria were as follows: (1) T2D met the diagnostic criteria of the American Diabetes Association’s position statement issued in 2015 [15]; (2) fasting S-TBAs were within the normal reference range (≤10.0 μmol/L); (3) patients were aged 20–75 years; and (4) fasting C-peptide levels were ≥0.5 ng/mL. We also excluded patients with any of the following conditions: (1) type 1 diabetes; (2) history of cancer, especially in the digestive system; (3) severe cardiovascular and cerebrovascular complications; (4) excessive alcohol consumption, with alcohol intake >20 g daily for women and >30 g daily for men; (5) hepatotoxic drug consumption, which may adversely affect liver function; (6) recent use of cholesterol absorption inhibitors (ezetimibe) or BA sequestrants; (7) chronic cholestasis; (8) chronic viral hepatitis and hepatic cirrhosis; (9) inflammation of the gallbladder and bile ducts; (10) cholelithiasis, which refers to stones in the gallbladder or bile ducts; (11) surgeries associated with the liver, bile ducts, gastrointestinal tract and pancreas; (12) chronic renal dysfunction; (13) abnormal thyroid function; (14) recent administration of steroid hormones. At the final stage of the study, 2,952 patients with T2D with complete data were aggregated for analysis. The procedures of our study conformed with the Helsinki Declaration. Our study received ethics approval from the Medical Ethics Committee of the Academic Committee at the Affiliated Hospital 2 of Nantong University, with written informed consent collected from all patients.

**Data collection**

Because the patients were consecutively recruited from among outpatients and inpatients with a focus on endocrinology, we had the facility to obtain medical histories, perform physical examinations, and access biochemical and imaging data from the information platform for all patients. The complete medical history contained recorded demographic parameters, present history of illness, past medical history and personal history. The demographic parameters documented were age, sex, body mass index (BMI), systolic/diastolic blood pressure (SBP/DBP), etc. The present history of illness focused on duration of diabetes, self-reported other types of diabetes, glucose-lowering therapies such as intervention by lifestyle alone, insulin treatments, insulin secretagogues,
metformin, pioglitazone, α-glucosidase inhibitors (AGIs), glucagon-like peptide-1 (GLP-1) receptor agonists (GLP-1RAs), dipeptidyl peptidase-4 inhibitors (DPP-4Is), and other recent medications such as statins, steroid hormones (i.e., glucocorticoids), antihypertensive agents, cholesterol absorption inhibitors (i.e., ezetimibe), BA sequestrants (i.e., cholestyramine), etc. Past medical history included chronic hepatitis (i.e., hepatitis B or C virus infection), hepatic cirrhosis (due to hepatitis virus, alcoholism, etc.), inflammation in the bile system (i.e., cholecystitis and cholangitis), cholestasis (stones in the gallbladder or bile ducts), surgeries in the digestive system (liver, bile ducts, gastrointestinal tract and pancreas), history of cancer (especially in the digestive system), chronic cholestasis, cardiovascular diseases (i.e., stroke, myocardial infarction, heart failure, revascularization, peripheral arterial stenosis or occlusion, etc.), hypertension history, chronic renal dysfunction, thyroid dysfunction (i.e., hyperthyroidism and hypothyroidism), and other specific types of diabetes. Drinking behaviour was categorized into three levels: no alcohol consumption, alcohol consumption (mild-to-moderate alcohol intake) and excessive alcohol consumption (excessive alcohol intake, >20 g daily for women and >30 g daily for men). Patients in our study with an SBP ≥140 mmHg, a DBP ≥90 mmHg or a history of hypertension or recent use of antihypertensive medication were identified as having hypertension.

**OGTT and evaluation of insulin sensitivity, islet β-cell function and glucagon levels**

All patients were required to consume their usual stable diet and avoid greasy foods for 3 days before the OGTT and pause all glucose-lowering therapies 1 day before the OGTT. On the test day, a 75-g OGTT was carried out in all patients in the early morning who had been in a fasting state for at least 8 hours. Venous blood samples were collected at fasting (0) and at 0.5, 1, 2, and 3 hours after glucose loading for the synchronous determination of serum glucose, C-peptide and glucagon levels. We used C-peptide instead of insulin in the evaluation parameters to eliminate cross-contamination of exogenous insulin with detection reagents. Fasting insulin sensitivity was converted from the inverse of homeostasis model assessment of insulin resistance (HOMA-IR) using C-peptide (IS\textsubscript{HOMA-cp}), which was defined as 22.5/(glucose \times C-peptide in fasting status) [16, 17]. Systemic insulin sensitivity was assessed by the Matsuda index using C-peptide (IS\textsubscript{M-cp}) [16, 17]. Islet β-cell function was assessed by the insulin secretion sensitivity index 2 using C-peptide (ISSI\textsubscript{2-cp}) [18, 19], which was defined as IS\textsubscript{M-cp} multiplied by the ratio of the area under the C-peptide curve (AUC\textsubscript{cp}) to the area under the glucose curve (AUC\textsubscript{glu}). Postchallenge glucagon levels were assessed by the area under the glucagon curve (AUC\textsubscript{gla}).

**Laboratory tests**

As biomarkers from the OGTT, glucose levels were detected with the oxidase method in an automated biochemical instrument (Model 7600, Hitachi), serum C-peptide levels were detected with the chemiluminescence method in an immunoassay system (Dxi 800, Beckman Coulter), and glucagon levels were detected with the radioimmunoassay method in an automated γ-counter (GC-1200, USTC Zonkia). On the test day, fasting venous blood samples were also collected from all patients for the detection of clinical biochemical indices. The fasting serum total bile acids (S-TBAs, using the enzymatic cycling assay), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (γ-GT), creatinine (Cr), cystatin C (Cys C), uric acid (UA), triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDLC) and high-density lipoprotein cholesterol (HDLC) were detected with an automated biochemical analyser (Model 7600, Hitachi). The HbA1c levels were detected with an ion exchange-based HPLC method in a haemoglobin analysis system (D-10, Bio-Rad). The CKD-EPI creatinine–cystatin C equation (2012) was used to estimate the glomerular filtration rate (GFR\textsubscript{cp}) [20].

**Statistical analyses**

The frequency distribution of fasting S-TBAs in all patients was evaluated (Fig. 1), and the data displayed a positively skewed distribution. To analyse the associations of S-TBAs with metabolic features and other clinical variables, all patients were categorized by quartiles of the fasting S-TBA levels (Table 1). Clinical characteristics are displayed for the total and the four subgroups based on fasting S-TBA quartiles and are expressed as the means ± SDs for continuous variables and as frequencies (percentages) for categorical variables. If the distributions of the data were skewed, a natural logarithm transformation (ln) was applied to achieve a normal distribution for further analysis, such as lnS-TBAs.

One-way analysis of variance (ANOVA) followed by a post-test for linear trend was applied to detect the differential trends in continuous variables among the four subgroups based on fasting S-TBA quartiles, and a linear-by-linear association chi-squared test was used to compare rate trends in categorical variables among the four subgroups of fasting S-TBA quartiles. Corresponding test statistics (F and x² values) and p values for the trend are also provided. Bivariate correlations of fasting S-TBAs with major metabolic parameters, including IS\textsubscript{HOMA-cp}, IS\textsubscript{M-cp}, ISSI\textsubscript{2-cp}, fasting glucagon, and AUC\textsubscript{gla},
were analysed by means of Pearson’s correlation tests, and scatter plots for these correlations are graphically displayed in Fig. 2 and Fig. 3. Only the correlation between fasting S-TBAs and fasting glucagon was not significant. Considering that alcohol consumption may have an effect on the levels of S-TBAs, the correlations of fasting S-TBAs with major metabolic parameters were adjusted for alcohol consumption by partial correlation analyses.

Furthermore, we used multivariable linear regression analyses to explore independent effects of fasting S-TBAs on fasting insulin sensitivity (IS\textsubscript{HOMA-cp}), systemic insulin sensitivity (IS\textsubscript{M-cp}), islet β-cell function (ISSI\textsubscript{2cp}) and glucagon levels (AUC\textsubscript{gla}) by adjusting for the other clinical covariates in each regression model. In each metabolic outcome of regression analysis (Table 2), the initial model 0 was unadjusted; model 1 was adjusted for age, sex, BMI, SBP, DBP, diabetes duration, and alcohol consumption; model 2 was further adjusted for hypertension, statin medication and glucose-lowering therapies; and model 3 was further adjusted for hepatic enzymes, lipid profiles, GFR\textsubscript{epi}, UA, HbA1c and other metabolic features. SPSS for Windows, standard version 19.0, was used to input and analyse the data, and statistical significance was identified when the threshold of the \( p \) value was less than 0.05.

Results

Clinical characteristics of the patients

Table 1 summarizes the clinical characteristics of the 2,952 recruited patients for the total and according to quartiles of the fasting S-TBA levels. The recruited patients had normal S-TBA levels of 3.29 ± 1.38 μmol/L, ages of 52.7 ± 8.2 years, and diabetes durations of 5.27 ± 3.90 years. The ranges of the fasting S-TBA quartiles were 1.3–2.3 μmol/L (first quartile, Q1), 2.4–2.8 μmol/L (second quartile, Q2), 2.9–3.8 μmol/L (third quartile, Q3) and 3.9–10.0 μmol/L (fourth quartile, Q4). From Q1 to Q4 of fasting S-TBA levels, the major metabolic parameters IS\textsubscript{HOMA-cp}, IS\textsubscript{M-cp} and ISSI\textsubscript{2cp} progressively decreased, while AUC\textsubscript{gla} notably increased progressively (all \( p \) values for trend <0.001), but this was not observed for fasting glucagon (\( p \) for trend = 0.099). Moreover, with increasing fasting S-TBA quartiles, age, diabetes duration, prevalence of hypertension, TG, CysC, ALT, γ-GT, AUC\textsubscript{gla} and AUC\textsubscript{cp} significantly increased, whereas GFR\textsubscript{epi} decreased (all \( p \) values for trend <0.05). However, the female ratio, BMI, SBP, DBP, frequency of statin use, TC, HDLC, LDLC, UA or HbA1c did not exhibit any differences among the fasting S-TBA quartiles (all \( p \) values for trend >0.05). Additionally, comparisons of glucose-lowering therapies showed that the frequency of metformin and pioglitazone use decreased, whereas the AGIs increased, as the S-TBA quartiles increased (all \( p \) values for trend <0.05), but intervention by lifestyle alone, insulin treatments, insulin secretagogues, DPP-4Is and GLP-1RAs were comparable among the S-TBA quartiles (all \( p \) values for trend >0.05). Regarding alcohol consumption, we excluded patients with excessive alcohol intake, and we still found increased trends in the ratio of alcohol consumption (mild to moderate) with increasing fasting S-TBA quartiles (\( p \) for trend <0.001), which implies that alcohol consumption may have an effect on the levels of S-TBAs.

Relationships between fasting S-TBAs and major metabolic parameters

Pearson’s correlation tests showed that fasting S-TBAs were inversely correlated with IS\textsubscript{HOMA-cp}, IS\textsubscript{M-cp} and ISSI\textsubscript{2cp} (\( r = -0.21 \), -0.15 and -0.25, respectively, \( p < 0.001 \)) and positively correlated with AUC\textsubscript{gla} (\( r = 0.32 \), \( p < 0.001 \)) but not with fasting glucagon levels (\( r = 0.033 \), \( p = 0.070 \)). Scatter plots for these correlations are graphically displayed in Fig. 2 and Fig. 3. After adjusting for alcohol consumption by partial correlation analyses, we observed that fasting S-TBAs were still inversely correlated with IS\textsubscript{HOMA-cp}, IS\textsubscript{M-cp} and ISSI\textsubscript{2cp} (\( r = -0.20 \), -0.14 and -0.24, respectively, \( p < 0.001 \)) and positively correlated with AUC\textsubscript{gla} (\( r = 0.32 \), \( p < 0.001 \)).

Multiple linear regression models displaying independent associations of fasting S-TBAs with major metabolic outcomes

Table 2 shows the associations of fasting S-TBAs with
| Variables | Total | Q1 | Q2 | Q3 | Q4 | $F / \chi^2$ value | $p$ for trend |
|-----------|-------|----|----|----|----|-----------------|-------------|
| $n$       | 2,952 | 725 | 710 | 753 | 764 | 2.308           | 0.129       |
| S-TBAs (μmol/L) | 3.29 ± 1.38 | 2.03 ± 0.24 | 2.58 ± 0.15 | 3.29 ± 0.26 | 5.14 ± 1.33 | —             | —           |
| S-TBAs range (μmol/L) | 1.3–10.0 | 1.3–2.3 | 2.4–2.8 | 2.9–3.8 | 3.9–10.0 | —             | —           |
| lnAUC | 0.75 ± 0.45 | 0.89 ± 0.48 | 0.75 ± 0.45 | 0.71 ± 0.42 | 0.65 ± 0.40 | 168.3          | <0.001      |
| lnIS | 6.04 ± 0.38 | 6.14 ± 0.40 | 6.05 ± 0.38 | 6.02 ± 0.37 | 5.98 ± 0.36 | 79.99          | <0.001      |
| lnM-cp | 4.84 ± 0.23 | 4.92 ± 0.22 | 4.85 ± 0.23 | 4.82 ± 0.21 | 4.78 ± 0.22 | 155.0          | <0.001      |
| lnGFP values for trends are also provided. | | | | | | | |

Note: ANOVA followed by a post-test for linear trend and linear-by-linear association chi-squared test were applied to detect trends in continuous data and categorical data among S-TBA quartiles, respectively. Corresponding test statistics and $p$ values for trends are also provided.
major metabolic outcomes by multiple linear regression analyses. In the basal unadjusted model 0, fasting S-TBAs were significantly associated with IS_HOMA-cp ($\beta = -0.21$, $t = -11.8$, $p < 0.001$, adjusted $R^2 = 0.045$), ISI_M-cp ($\beta = -0.25$, $t = -8.44$, $p < 0.001$, adjusted $R^2 = 0.023$), ISSI2 cp ($\beta = -0.25$, $t = -13.7$, $p < 0.001$, adjusted $R^2 = 0.060$) and AUC_gla ($\beta = 0.32$, $t = 18.5$, $p < 0.001$, adjusted $R^2 = 0.104$). After gradually adding the other clinical covariates in each model, we observed a gradual increase in the adjusted $R^2$. In the fully adjusted model 3, fasting S-TBAs were still independently associated with IS_HOMA-cp ($\beta = -0.04$, $t = -2.82$, $p = 0.005$, adjusted $R^2 = 0.060$), ISI_M-cp ($\beta = -0.11$, $t = -7.05$, $p < 0.001$, adjusted $R^2 = 0.501$), ISSI2 cp ($\beta = -0.15$, $t = -10.26$, $p < 0.001$, adjusted $R^2 = 0.528$) and AUC_gla ($\beta = 0.29$, $t = 19.08$, $p < 0.001$, adjusted $R^2 = 0.473$).

Considering that metformin, pioglitazone or AGIs treatment may have an effect on the levels of S-TBAs, the independent effects of fasting S-TBAs on major metabolic features were explored in patients not receiving metformin, pioglitazone or AGIs treatment. We found increased fasting S-TBAs within the normal range are still significantly associated with major metabolic features in patients with T2D (Supplementary Tables S1, S2 and S3).

**Discussion**

Measurement of S-TBA levels using the enzymatic method is a routine clinical application in the detection of hepatic function and has potential clinical utility in the assessment of metabolic diseases. In the present study, we analysed the associations of differential fasting S-TBA levels split into quartiles with the main metabolic characteristics in patients with T2D ($n = 2,952$). The main findings of our study are as follows: first, increased fasting S-TBAs within the normal reference range were independent in relation to both blunted fasting and systemic insulin sensitivity indices derived from OGTT; second, increased fasting S-TBA levels were inversely
associated with integrated islet β-cell function assessed by ISSI2; and third, increased fasting S-TBA levels contributed to increased glucagon levels in response to glucose challenge in patients with T2D but not to fasting glucagon levels. Ongoing studies worldwide are attempting to investigate how pathological factors and conditions have effects on islet β-cells and α-cells to interfere with the progression of T2D. Our present study revealed that fasting serum BAs may be potential endocrine molecules mediating numerous metabolic pathways involved in the pathogenesis of T2D, which implies that the regulation of fasting S-TBAs may be a therapeutic target for T2D.

Table 2  Multiple linear regression models displaying adjusted estimates for fasting S-TBAs for outcomes of IS\textsubscript{HOMA-\text{cp}}, IS\textsubscript{M-\text{cp}}, ISSI2\textsubscript{cp} and AUC\textsubscript{glu} adjusted for the other clinical covariates in each model

| Models | B (95% CI) | \(\beta\) | \(t\) | \(p\) | Adjusted \(R^2\) |
|--------|------------|------------|------|------|----------------|
| lnIS\textsubscript{HOMA-\text{cp}} | | | | | |
| Model 0: unadjusted | –0.26 | –0.21 | –11.81 | <0.001 | 0.045 |
| Model 1: age, sex, BMI, SBP, DBP, diabetes duration, and alcohol consumption | –0.24 | –0.20 | –11.75 | <0.001 | 0.194 |
| Model 2: model 1 + hypertension, statin medication and glucose-lowering therapies | –0.24 | –0.19 | –12.04 | <0.001 | 0.285 |
| Model 3: model 2 + ALT, γ-GT, GFR\textsubscript{ep}, S-UA, lipid profiles, HbA1c, fasting glucagon, ISSI2\textsubscript{cp}, and AUC\textsubscript{glu} | –0.05 | –0.04 | –2.822 | 0.005 | 0.606 |
| lnIS\textsubscript{M-\text{cp}} | | | | | |
| Model 0: unadjusted | –0.16 | –0.15 | –8.444 | <0.001 | 0.023 |
| Model 1: age, sex, BMI, SBP, DBP, diabetes duration, and alcohol consumption | –0.14 | –0.14 | –7.958 | <0.001 | 0.189 |
| Model 2: model 1 + hypertension, statin medication and glucose-lowering therapies | –0.13 | –0.13 | –8.096 | <0.001 | 0.302 |
| Model 3: model 2 + ALT, γ-GT, GFR\textsubscript{ep}, UA, lipid profiles, HbA1c, fasting glucagon and AUC\textsubscript{glu} | –0.11 | –0.11 | –7.051 | <0.001 | 0.501 |
| lnISSI2\textsubscript{cp} | | | | | |
| Model 0: unadjusted | –0.15 | –0.25 | –13.70 | <0.001 | 0.060 |
| Model 1: age, sex, BMI, SBP, DBP, diabetes duration, and alcohol consumption | –0.15 | –0.24 | –13.59 | <0.001 | 0.108 |
| Model 2: model 1 + hypertension, statin medication and glucose-lowering therapies | –0.16 | –0.25 | –14.73 | <0.001 | 0.194 |
| Model 3: model 2 + ALT, γ-GT, GFR\textsubscript{ep}, UA, lipid profiles, HbA1c, fasting glucagon, IS\textsubscript{HOMA-\text{cp}} and AUC\textsubscript{glu} | –0.10 | –0.15 | –10.26 | <0.001 | 0.528 |
| lnAUC\textsubscript{glu} | | | | | |
| Model 0: unadjusted | 0.40 | 0.32 | 18.51 | <0.001 | 0.104 |
| Model 1: age, sex, BMI, SBP, DBP, diabetes duration, and alcohol consumption | 0.39 | 0.32 | 17.86 | <0.001 | 0.111 |
| Model 2: model 1 + hypertension, statin medication and glucose-lowering therapies | 0.39 | 0.32 | 12.63 | <0.001 | 0.119 |
| Model 3: model 2 + ALT, γ-GT, GFR\textsubscript{ep}, UA, lipid profiles, HbA1c, fasting glucagon, IS\textsubscript{HOMA-\text{cp}} and ISSI2\textsubscript{cp} | 0.35 | 0.29 | 19.08 | <0.001 | 0.473 |
S-TBAs in turn lead to tissue inflammatory responses [21], hepatic fibrosis [22], blunted cardiac contractility [23], metabolic impairments in neurons and neurological decline [24, 25]. Pregnant subjects with elevated fasting S-TBA levels fulfilling criteria for intrahepatic cholestasis present with increased glycaemic concentrations, aggravated glycaemic fluctuations, dyslipidaemia, high risks of pre-eclampsia, foetal overgrowth and gestational diabetes [26, 27]. More intriguingly, alterations in bile flow caused by acute extrahepatic cholestasis may not only impair glucose tolerance and insulin secretion but also enhance the secretion of glucagon and GLP-1 in both fasting and postprandial states [28]. These phenomena suggested that altered bile flow and subsequent elevated S-TBAs may decrease insulin sensitivity, dysregulate incretin hormone and lead to islet β-cell dysfunction. In our present study, the recruited patients with T2D exhibited normal bile flow in contrast to the above mentioned previous studies, but these patients with fasting S-TBA levels in the high-normal range still presented with blunted insulin sensitivity, inferior islet β-cell function and abnormally increased glucagon levels.

In addition to aberrantly elevated S-TBAs under the condition of cholestasis, increased fasting S-TBAs under the normal bile flow condition may contribute to multiple adverse metabolic features. An observational study by Prinz et al. [29] conducted in 74 patients with a broad range of BMIs showed that fasting S-TBA levels were higher in obese individuals than in underweight controls and positively correlated with BMI. In a study of non-alcoholic fatty liver disease (NAFLD), Ferslew et al. [30] revealed that both fasting and postprandial S-TBAs were approximately two-fold higher in patients with non-alcoholic steatohepatitis (NASH) than in healthy controls, which indicated that increased S-TBA levels may account for hepatic injury and pathogenesis in NASH. A community-based study by Sun et al. [7] demonstrated that a slight but significant positive correlation existed between increased fasting S-TBA levels and fasting insulin resistance in both non-diabetic subjects (n = 8,463) and T2D patients free of glucose-lowering drugs (n = 1,140). Another study in 241 individuals showed that fasting S-TBA levels were significantly increased based on the increased presence of insulin resistance irrespective of glycaemic status [31]. Moreover, pregnant women with higher first-trimester S-TBA levels, even those within the normal reference range, were more likely to have a high risk of gestational diabetes [8]. Our present study found that subjects with fasting S-TBAs at the highest quartile levels (3.9–10 μmol/L) presented with blunted fasting insulin sensitivity (IS_{HOMA-cp}), even after adjusting for demographic data and glucose-lowering therapies. Based on the fasting glucose and C-peptide levels, IS_{HOMA-cp} predominantly represents hepatic insulin sensitivity [16, 32], so increased fasting S-TBA levels were closely associated with blunted hepatic insulin sensitivity. Syring et al. [33] documented that direct elevation of S-TBAs by BA infusion could reduce hepatic insulin sensitivity and disturb insulin levels to suppress hepatic glucose production, which supported our results. In addition, our study also showed that systemic insulin sensitivity assessed by ISI_{M-cp} was inversely associated with fasting S-TBAs. ISI_{M-cp} is a whole-body insulin sensitivity index that effectively reflects a composite of both hepatic and peripheral tissue (primarily the muscles) sensitivity to insulin [16, 17], so increased fasting S-TBA levels were inversely associated with integrated hepatic and muscular insulin sensitivity. Furthermore, we found that increased fasting S-TBAs were negatively correlated with integrated islet β-cell function assessed by ISSI_{2cp} and positively correlated with postchallenge glucagon levels, which implies that increased fasting S-TBAs may exert adverse effects on islet β-cells and dysregulate α-cell secretions in response to oral hyperglycaemia. However, no significant relationship between fasting S-TBAs and fasting glucagon levels was found in our study.

Additionally, an increase in fasting S-TBA levels in our study was revealed to be weakly associated with other clinical features of insulin resistance, such as increased serum liver enzymes, TG, raised glucose levels (AUC_{glu}) after glucose loading and an increased prevalence of hypertension. Our study also demonstrated that increased fasting S-TBA levels may account for some chronic diabetic complications. The trend of decreased GFR_{epi} and increased serum CycC, which indicated the propensity for kidney injury, was consistent with the trend of increased fasting S-TBA quartiles. Meanwhile, multiple clinical factors may influence the levels of fasting S-TBAs. In our study, older T2D patients were prone to have higher S-TBA levels, and T2D patients who received metformin or pioglitazone treatment presented with lower S-TBA levels. A study by Li et al. [34] showed that metformin could inhibit CYP8B1 expression and in turn decrease BA synthesis, which may explain the relationship of lower TBA levels with metformin administration. However, effects of metformin are generally known to be intestinal actions, the mechanism of lower TBA levels with metformin administration is still within the speculation.

There are some possible mechanisms of increased S-TBAs with metabolic diseases. BAs exert important effects on the regulation of the glycaemic response in healthy subjects and in patients with T2D. In healthy conditions with normal insulin sensitivity, insulin infusion was found to strongly reduce S-TBA levels by inhibiting BA synthesis and promoting BA uptake from
sinusoidal blood into hepatocytes [5, 35]. However, multiple BA metabolic defects arise under conditions of insulin resistance, such as obesity and T2D [4-6, 36]. Those defects in BA metabolism were associated with high levels of markers of total BA synthesis and defective hepatic BA transport [5], which may lead to increased S-TBA levels in peripheral circulation. Increased systemic BAs, regardless of the status of BAs (conjugation or unconjugated species), can directly induce hepatic insulin resistance and impair insulin action to inhibit hepatic glucose production [33]. However, in the portal vein, increased BAs cannot impair hepatic insulin sensitivity. Defects in BA metabolism may also interfere with the metabolic pathways mediated by FXR and TGR5. In addition, impairment in FXR and TGR5 signalling, in turn, may result in an inflammatory cascade in immune cells, increases in hepatic gluconeogenesis, a decline in insulin sensitivity and energy expenditure in muscle and brown adipose tissue, dysregulation in islet β-cell and α-cell secretions, and gut dysbiosis, all of which have been convincingly demonstrated to be responsible for T2D and other metabolic diseases associated with obesity [37]. Actually, mutual enhancement between increased serum BAs and insulin resistance promoted the incidence of metabolic diseases. In addition, glucagon secretion from pancreatic α-cells was increased after absorption of mixed BAs from the intestine, which was secondary to the activation of the basolateral TGR5 receptor of enterocytes and release of glucagonotropic hormones [38]. Increased glucagon secretion is dependent on the absorption of BAs into blood circulation, which may account for the association of serum BAs with postchallenge glucagon (AUC_{gl}) but not with fasting glucagon. Increased fasting S-TBAs are associated with metabolic abnormality in T2D, which implies that reducing fasting S-TBAs may be a therapeutic target for T2D. In the clinical practice, ursodeoxycholic acid (UDCA) therapy can decrease S-TBAs in intrahepatic cholestasis of pregnancy [39], and UDCA was lower in T2D compared with health subjects [40], therefore UDCA therapy could be applied in T2D to reduce S-TBAs. Additionally, mutual enhancement between increased BAs and insulin resistance promoted the incidence and progression of metabolic diseases, such as T2D, and the therapy target to insulin resistance may reduce serum S-TBAs and improve metabolic diseases.

Our present study has several limitations. First, we analysed only the relationships between S-TBAs in fasting status and metabolic features and did not determine S-TBA levels after glucose challenge or analyse their roles in metabolic outcomes. Second, different BA species, such as primary/secondary BAs, amino acid-conjugated BAs and 12α-hydroxylated BAs, may play different roles in T2D and other metabolic diseases. Although diverse serum BA species contribute to insulin resistance [31, 33], we did not analyse the associations of BA species with major metabolic features in T2D. Third, serum BAs play vital roles in regulating intestinal hormones, such as GLP-1 and 2, which in turn regulate β-cell and α-cell secretions. We did not determine the intestinal hormone levels for an in-depth study. Fourth, the gold standard for determining insulin sensitivity and islet β-cell function is glucose clamp tests, and indices derived from the OGTT may not fully evaluate insulin sensitivity and islet β-cell function. However, glucose clamp tests are too laborious and cumbersome to be applied in large-sample studies.

**Conclusions**

Increased fasting S-TBA levels within the normal range are significantly associated with blunted fasting and systemic insulin sensitivity, impaired islet β-cell function and increased glucagon levels in response to glucose challenge in patients with T2D, which implies that the regulation of fasting S-TBAs may be a therapeutic target for T2D.

**Acknowledgements**

The study was funded by the Social Development Projects of Nantong (MS22015065, MS12019019), the Medical Research Project of Nantong Health Commission (MB2019011) and the Medical Research Project of Jiangsu Health Commission (QNRC2016408).

**Disclosure**

The authors have stated no conflicts of interest.

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