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Research Article

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Reduced glycodeoxycholic acid levels are associated with negative clinical outcomes of gestational diabetes mellitus

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Abstract: Gestational diabetes mellitus (GDM) is characterized by glycemia and insulin disorders. Bile acids (BAs) have emerged as vital signaling molecules in glucose metabolic regulation. BA change in GDM is still unclear, which exerts great significance to illustrate the change of BAs in GDM. GDM patients and normal pregnant women were enrolled during the oral glucose tolerance test (OGTT) screening period. Fasting serums were sampled for the measurement of BAs. BA metabolism profiles were analyzed in both pregnant women with GDM and those with normal glucose tolerance (NGT). Delivery characteristics, delivery gestational age, and infant birthweight were extracted from medical records. GDM patients presented distinctive features compared with NGT patients, including higher body mass index (BMI), elevated serum glucose concentration, raised insulin (both fasting and OGTT), and increased hemoglobin A1c (HbA1c) levels. Higher homeostasis model assessment of insulin resistance (HOMA-IR) and decreased β-cell compensation (i.e., oral disposition index (DI_o)) were also prevalent in this group. Total BAs (TBAs) remained stable, but glycodeoxycholic acid (GDCA) and taurodeoxycholic acid (TDCA) levels declined significantly in GDM. GDCA was inversely correlated with HOMA-IR and positively correlated with DI_o. No obvious differences in clinical outcome between the GDM and NGT groups were observed. However, GDM patients with high HOMA-IR and low DI_o tended to have a higher cesarean delivery rate and younger delivery gestational age. In conclusion, GDCA provides a valuable biomarker to evaluate HOMA-IR and DI_o, and decreased GDCA levels predict poorer clinical outcomes for GDM.

Key words: Gestational diabetes mellitus (GDM); Bile acid; Insulin resistance; β-Cell compensation

1 Introduction

Gestational diabetes mellitus (GDM) is a common complication in pregnancy with short- and long-term health risks for both the mother and the developing fetus (Johns et al., 2018; Zhou et al., 2020). Women with GDM show an increased incidence of hypertensive disorders during pregnancy, including gestational hypertension, preeclampsia, and eclampsia, and thus may need preterm caesarean section, or even develop subsequent maternal type 2 diabetes (T2DM) (The HAPO Study Cooperative Research Group, 2009; O'Sullivan et al., 2011; Wei et al., 2017). Offspring born to mothers with GDM are at increased risk of multiple immediate complications, including macrosomia, preterm birth, injury, shoulder dystocia, respiratory distress, and other conditions (Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study Cooperative Research Group, 2010; Mortier et al., 2017; Moll et al., 2020).

Insulin resistance (IR) progresses with advancing gestation to supply adequate energy for the growing fetus. Increased IR promotes endogenous glucose production and the breakdown of fat stores, resulting in

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raised glycemic index and free fatty acid concentration (di Cianni et al., 2003). The abnormal metabolic adaptations of IR in pregnancy always consequently lead to GDM. Bile acids (BAs) have also emerged as crucial signaling molecules in the glucose metabolism via the nuclear hormone farnesoid X receptor (FXR) and the Takeda G protein receptor 5 (TGR5) signaling pathways (Shapiro et al., 2018; Jia et al., 2019; Jin et al., 2019). Studies on rodents showed that the induction of postprandial BA secretion leads to the stimulation of glycogen storage and the inhibition of hepatic glycolytic and lipogenic gene expression in an FXR-dependent manner (Watanabe et al., 2004; Duran-Sandoval et al., 2005). The activation of FXR represses the enzymes involved in hepatic gluconeogenesis, such as phosphoenolpyruvate carboxy kinase (PEP-CK) and glucose 6-phosphatase (Potthoff et al., 2011; Zhang et al., 2012). In addition to direct regulation, BAs are also involved in glucose metabolism through insulin secretion.

The TGR5 receptor, which is differentially activated by lithocholic acid (LCA), deoxycholic acid (DCA), chenodeoxycholic acid (CDCA), and cholic acid (CA) (in the order of activity), promotes the secretion of glucagon-like peptide-1 (GLP-1) by intestinal L cells acting on pancreatic β-cells to stimulate insulin secretion (Katsuma et al., 2005). The BA receptor FXR is also differentially activated by CDCA, DCA, LCA, and CA (in the order of activity) (Cariou et al., 2006). Although GLP-1 secretion is negatively regulated by FXR, TGR5 activation in L cells rapidly occurs postprandially, whereas FXR activation induces a more delayed response that requires transcriptional activation (Trabelsi et al., 2015; Kim and Fang, 2018). Research on interactions between BAs and insulin has demonstrated that insulin controls BA composition by regulating 12α-hydroxylase called cyp8b1 through forkhead box-O1 (FoxO1), and the serum insulin level is positively correlated with the concentration of 12α-hydroxylated BAs (CA, DCA, and their conjugated forms) (Haeusler et al., 2012). In healthy subjects, IR is associated with increased 12α-hydroxylated BAs, and the ratios of 12α-hydroxylated/non-12α-hydroxylated BAs are associated with key features of IR. In T2DM patients, levels of total BAs (TBAs) are nearly two-fold compared with healthy subjects, although no disproportionate increases in 12α-hydroxylated BAs are spotted (Haeusler et al., 2013).

Our recent results showed that BA metabolic profile changes with advancing gestation. Specifically, unconjugated BAs dominate during the second trimester, whilst conjugated BAs are more prevalent in the third trimester (Zhu et al., 2019). Since individual BAs exert distinctive affinity to FXR and TGR5 and they also play different roles in glucose metabolism, it is necessary to clarify whether the BA profile is changed in GDM patients and determine the clinical significance of any such changes.

In this paper, it is hypothesized that the BA metabolism profile changes in GDM and a correlation between insulin metabolism and specific BA components exists. In order to verify this hypothesis, we analyzed the fasting BA metabolism profile in GDM and normal pregnant women during the oral glucose tolerance test (OGTT) screening period and identified the key BAs that changed in GDM, thereby laying the foundations for the assessment of IR and β-cell compensation using BAs.

2 Materials and methods

2.1 Study design and population

A total of 67 GDM patients and 48 patients with normal pregnancy, with a maternal age of 23–44 years and a gestational age of 21–29 weeks, were enrolled between May 2019 and October 2019 in Women's Hospital, Zhejiang University School of Medicine (Hangzhou, China). Cases of pre-conceptional diabetes, infections, and abnormal liver or kidney function, and those positive for human immunodeficiency virus (HIV) and hepatitis C antibodies were excluded from the study. Blood samples were obtained from all participants after 8–14 h of fasting. Subsequently, all women underwent a 75-g OGTT. Based on the OGTT data, we defined GDM according to the criteria of the International Association of the Diabetes and Pregnancy Study Groups (IADPSG) (Weinert, 2010). Insulin and glucose levels during the OGTT were used to determine the homeostasis model assessment of insulin resistance (HOMA-IR), homeostasis model assessment β cell function (HOMA-β), insulin secretion (using the Stumvoll first-phase estimate), and insulin sensitivity (using the Matsuda index) values (Matsuda and DeFronzo, 1999; Stumvoll et al., 2000, 2001). The insulin secretion index and Matsuda index were multiplied to calculate the oral disposition index (DI_o),

which assesses β-cell compensation for IR (Elbein et al., 2000). The research was approved by the Ethics Committee of the Women's Hospital, Zhejiang University School of Medicine (IRB-20200015-R).

2.2 Measurement of BAs

The procedure for BA analysis was performed according to the previously published methods with minor modifications (Zhu et al., 2019). Briefly, commercially available reference standards were obtained from Toronto Research Chemicals (TRC) Inc. (Toronto, Canada) and Sigma-Aldrich (St. Louis, USA). Total volumes of 100 μL serum specimen, standard solutions, and quality control were vortexed with 300 μL of the stable isotope-labeled internal standard (IS) stock solutions. A total of 150 μL supernatant was aspirated by the autosampler for liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis after centrifugation for 10 min at 13000*g*. BAs were separated using an ultra-high performance liquid chromatography (UHPLC) BEH C18 column (2.1 mm×100 mm, 1.7 μm; Waters, Massachusetts, USA) with 40 ℃ column temperature. The MS detection of BAs was performed using individually optimized cone voltage and collision energy in multiple reaction monitoring (MRM) mode (Table S1).

2.3 Statistical methods

MS data were analyzed using Analyst software v1.6.0 (SCIEX, Framingham, USA). The Student's *t*-test or Mann-Whitney *U*-test was used to evaluate differences among groups for continuous variables. One-way analysis of variance (ANOVA) or Kruskal-Wallis test was conducted to compare the differences for categorical variables. Data of BAs were log-transformed for orthogonal partial least squares discrimination analysis (OPLS-DA) using SIMCA-P (Version 13.0, Umetrics, Sweden). All statistical analyses were performed using the GraphPad Prism 7.0 software. *P* values of <0.05 were considered statistically significant.

3 Results

3.1 Baseline characteristics of the study population

Compared with normal glucose tolerance (NGT), GDM patients had a higher body mass index (BMI) ($P<0.001$). The biochemical indicators included triglyceride (TG), total cholesterol (TCH), high-density lipoprotein (HDL), low-density lipoprotein (LDL), glycated albumin (GAL), and hemoglobin A1c (HbA1c). Both HbA1c and TG were elevated significantly in the GDM group ($P=0.013$ and $P=0.047$, respectively). Albeit there was no statistical significance in insulin secretion, the levels of fasting insulin (FIN) and insulin 1 and 2 h post-OGTT were raised in the GDM group compared with the NGT group ($P<0.001$, $P=0.005$, and $P<0.001$, respectively). In addition, GDM patients had a deficiency in insulin sensitivity (3.33−6.30 for GDM vs. 5.84−10.33 for NGT, $P<0.001$), and poorer β-cell compensation (3628−5186 for GDM vs. 6584−9121 for NGT, $P<0.001$) than the NGT group (Table 1). All of the significantly changed parameters in Table 1 were used for OPLS-DA calculations to evaluate differences between GDM and NGT patients. A distinct clustering pattern was observed between samples from GDM and NGT individuals (Fig. 1a). The variable importance in projection (VIP) scores for the mentioned indicators in Table 1 showed that the DI_o index, Matsuda index, HOMA-IR, Stumvoll Phase I, and insulin levels in OGTT contributed significantly as a principal component to separating the two groups (Fig. 1b).

3.2 BA metabolism profiles in GDM and NGT

Since glucose and insulin metabolism discriminations exist between the GDM and NGT groups and BAs are known to participate in the glycemic homeostasis via direct and indirect pathways, we postulated that GDM and NGT could be distinguished by BA profiles. The two cohorts, however, could not be separated by OPLS-DA (data not shown), even though TBAs decreased in the GDM group (Fig. 2a). This finding suggested that certain types of BAs may be more intimately involved in glycemic regulation. Indeed, the levels of 12α-hydroxylated BAs decreased obviously ((759.57±64.38) nmol/L vs. (994.74±106.30) nmol/L, $P=0.048$), whereas non-12α-hydroxylated BA levels remained stable in the GDM group (Fig. 2b). Furthermore, among individual 12α-hydroxylated BAs, taurodeoxycholic acid (TDCA) and glycodeoxycholic acid (GDCA) in fasting serum decreased significantly in the GDM group (TDCA: (38.96±7.80) nmol/L vs. (68.98±11.98) nmol/L, $P=0.030$; GDCA: (156.68±17.95) nmol/L vs. (226.50±26.69) nmol/L, $P=0.026$), while there was no obvious change in concentrations of non-12α-hydroxylated BAs (Fig. 2c).

Table 1 Characteristics of women with NGT and GDM

Parameter	NGT (*n*=48)	GDM (*n*=67)	*P*
Age (year)	30 (28–33)	33 (29–36)	0.003
Gestational age (year)	24 (24–25)	25 (24–26)	0.423
BMI (kg/m^2)	22.26 (21.17–23.07)	24.44 (22.19–26.58)	<0.001
Fasting glucose (mg/dL)	78.0 (74.9–82.1)	85.0 (78.1–94.0)	<0.001
One-hour glucose OGTT (mg/dL)	137.5 (110.5–150.9)	185.2 (165.1–197.5)	<0.001
Two-hour glucose OGTT (mg/dL)	116.2 (104.3–130.5)	159.9 (142.0–172.3)	<0.001
FIN (μU/mL)	6.2 (4.9–8.5)	8.9 (6.5–11.9)	<0.001
One-hour insulin OGTT (μU/mL)	40.5 (30.3–63.3)	58.6 (39.7–91.5)	0.005
Two-hour insulin OGTT (μU/mL)	45.7 (31.1–57.7)	67.9 (46.5–109.0)	<0.001
Insulin sensitivity (Matsuda index)	7.78 (5.84–10.33)	4.58 (3.33–6.30)	<0.001
Insulin secretion (Stumvoll)	981.5 (865.1–1264.0)	1054.0 (636.4–1321.0)	0.683
DI_o	7572 (6584–9121)	4409 (3628–5186)	<0.001
HOMA-IR	1.20 (0.92–1.71)	1.87 (1.28–2.64)	<0.001
HOMA-β	157.7 (130.6–234.5)	150.3 (111.8–194.0)	0.694
AUC (insulin/glucose)	5.15 (4.31–7.22)	5.83 (4.11–9.31)	0.136
TG (mmol/L)	2.00 (1.76–2.52)	2.32 (1.89–2.88)	0.046
TCH (mmol/L)	5.96 (5.25–6.63)	5.68 (5.06–6.55)	0.216
HDL (mmol/L)	1.96 (1.63–2.24)	1.79 (1.56–2.00)	0.094
LDL (mmol/L)	2.97 (2.54–3.61)	2.74 (2.34–3.13)	0.073
GAL (%)	12.2 (11.6–13.0)	12.3 (11.7–12.9)	0.311
HbA1c (%)	4.9 (4.8–5.1)	5.1 (4.9–5.3)	0.013

The data are described as median (IQR). The differences between the NGT and GDM groups were compared using the Kruskal-Wallis test for continuous variables and the Fisher's exact test for categorical variables. Biochemical indicators, including fasting glucose and insulin, 1/2-h-glucose and insulin, TG, TCH, HDL, LDL, GAL, and HbA1c, were also compared between GDM and NGT groups. Insulin and glucose levels during the OGTT were used to estimate insulin secretion (using the Stumvoll first-phase estimate) and insulin sensitivity (using the Matsuda index). These indices were multiplied to calculate the DI_o, which assesses β-cell compensation for insulin resistance. The insulin resistance index and β-cell function were calculated using fasting glucose concentration (mmol/L)×FIN concentration (μU/mL)/22.5 and 20×FIN concentration (μU/mL)/(fasting glucose concentration (mmol/L)−3.5), respectively. $P<0.05$ was considered to be statistically significant. NGT: normal glucose tolerance; GDM: gestational diabetes mellitus; BMI: body mass index; OGTT: oral glucose tolerance test; FIN: fasting insulin; DI_o: oral disposition index; HOMA-IR: homeostasis model assessment of insulin resistance; HOMA-β: homeostasis model assessment β cell function; AUC: area under the curve; TG: triglyceride; TCH: total cholesterol; HDL: high-density lipoprotein; LDL: low-density lipoprotein; GAL: glycated albumin; HbA1c: hemoglobin A1c; IQR: interquartile range.

3.3 Correlation between individual BAs and insulin indexes

In order to further describe the relationship between BA components and the apparently changed insulin-related indicators, their correlation was analyzed. Fasting GDCA was found to positively correlate with insulin sensitivity in both NGT and GDM groups, and this coefficient correlation decreased in the GDM group. The level of GDCA also positively correlated with DI_o in the GDM group, but negatively with HOMA-IR and FIN. No correlation between TDCA and insulin-related indicators was shown in the NGT or GDM group, although TDCA was markedly reduced in the GDM group. Among the non-12α-hydroxylated individual BAs, CDCA correlated positively with HOMA-IR and FIN, whilst tauroursodeoxycholic acid (TUDCA) correlated negatively with DI_o (Fig. 3).

3.4 Clinical outcomes for GDM patients with higher HOMA-IR and lower DI_o

Results showed that GDCA declined in the GDM group, and this decline correlated with insulin sensitivity, IR, DI_o, and FIN. In order to assess the relationship between GDCA and clinical outcomes including delivery gestational age, fetus birth weight, and cesarean delivery rate, GDM patients were subdivided into four quartiles (Q1: <25%, Q2: 25%−50%, Q3: >50%−75%, and Q4: >75%) according to the Matsuda index, HOMA-IR, DI_o, and FIN, respectively. No apparent change in GDCA occurred among the Q1 and Q4 groups divided by Matsuda index and FIN (Figs. 4a

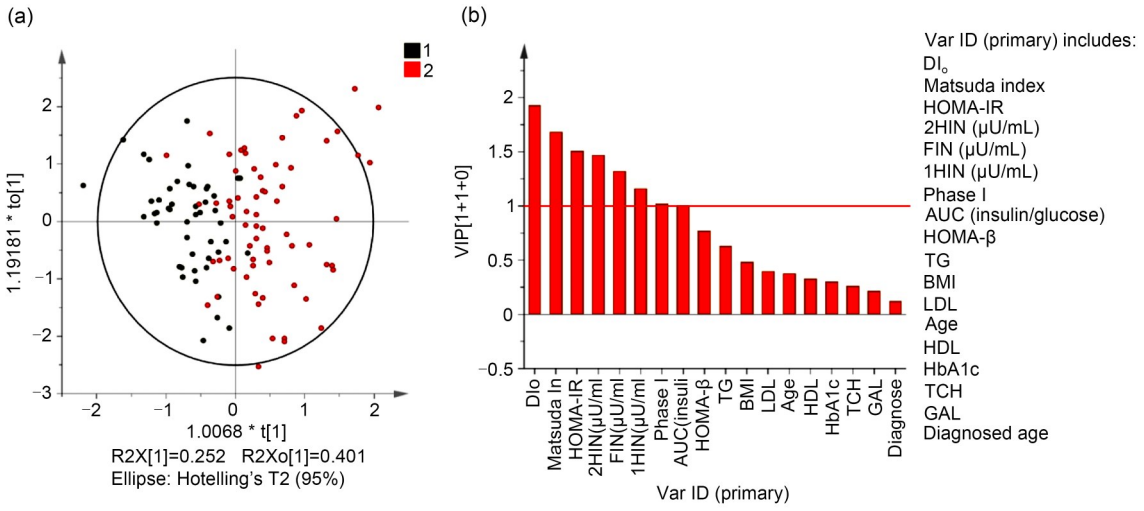

Fig. 1 OPLS-DA score plots of NGT and GDM according to the significantly changed parameters, as seen in Table 1. (a) The black and the red dots represent the NGT (1) and GDM (2) patients, respectively. The black circle indicates the 95% confidence interval. (b) A taxon with a VIP score of >1 (red line) was considered important in the group discrimination. OPLS-DA: orthogonal partial least squares discrimination analysis; NGT: normal glucose tolerance; GDM: gestational diabetes mellitus; VIP: variable importance in projection; Var: variable; DI_o: oral disposition index; HOMA-IR: homeostasis model assessment of insulin resistance; 1/2HIN: 1/2-h postprandial serum insulin; FIN: fasting insulin; AUC: area under the curve; HOMA-β: homeostasis model assessment β cell function; TG: triglyceride; BMI: body mass index; LDL: low-density lipoprotein; HDL: high-density lipoprotein; HbA1c: hemoglobin A1c; TCH: total cholesterol; GAL: glycated albumin.

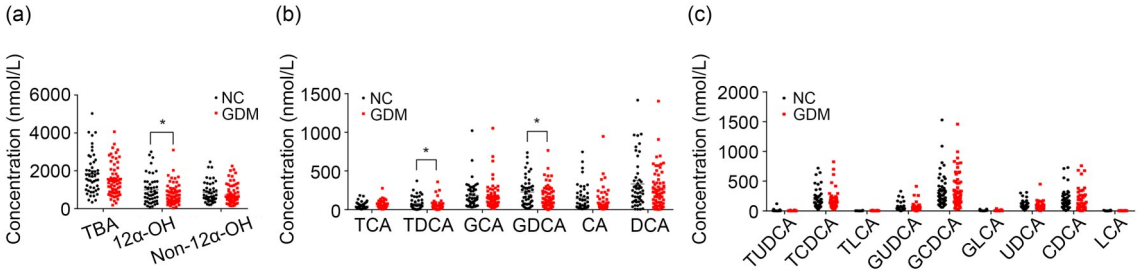

Fig. 2 TBA levels remained stable in the GDM group. 12α-Hydroxylated BAs decreased in the GDM group (b), while non-12α-hydroxylated BAs showed no obvious changes in the GDM group (a). The 12α-hydroxylated BAs GDCA and TDCA declined significantly in the GDM group (b), while non-12α-hydroxylated BAs presented no apparent variation in individuals where they were detected (c). *P<0.05. BA: bile acid; NC: negative control; GDM: gestational diabetes mellitus; TBA: total bile acid; 12α-OH: 12α-hydroxylated BAs; Non-12α-OH: non-12α-hydroxylated BAs; TCA: taurocholic acid; TDCA: taurodeoxycholic acid; GCA: glycocholic acid; GDCA: glycodeoxycholic acid; CA: cholic acid; DCA: deoxycholic acid; TUDCA: tauroursodeoxycholic acid; TCDCA: taurochenodeoxycholic acid; TLCA: taurolithocholic acid; GUDCA: glycoursodeoxycholic acid; GCDCA: glycochenodeoxycholic acid; GLCA: glycolithocholic acid; UDCA: ursodeoxycholic acid; CDCA: chenodeoxycholic acid; LCA: lithocholic acid.

and 4b), but it increased in the DI_o Q4 group and decreased in the HOMA-IR Q4 group (Figs. 4c and 4d). With regard to clinical outcomes, no variations were shown for GDM patients as a whole compared with the NGT group. However, when the GDM subgroup was divided by HOMA-IR and DI_o, it was found that GDM patients with elevated HOMA-IR and decreased GDCA (HOMA-IR Q4 group) had increased cesarean delivery rates. Patients with GDM and low DI_o presented a younger gestational age before delivery compared with patients with NGT (Table 2).

4 Discussion

In the present work, it was established that the abnormalities of insulin-related indicators were the main factors to distinguish GDM and NGT. Combining BA

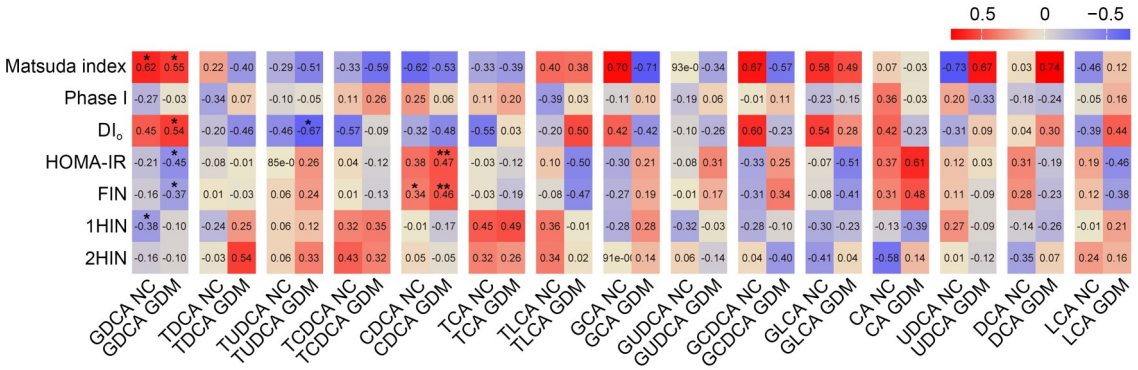

Fig. 3 Heat map showing the Spearman correlation between individual BAs and Matsuda index, Phase I, DI_0, HOMA-IR, FIN, 1HIN, and 2HIN. The color scheme corresponds to correlation strength as shown by the color bar. Red represents a positive association, and blue represents a negative association. * *P*<0.05, ** *P*<0.01. BA: bile acid; DI_0: oral disposition index; HOMA-IR: homeostasis model assessment of insulin resistance; FIN: fasting insulin; 1/2HIN: 1/2-h postprandial serum insulin; GDCA: glycodeoxycholic acid; TDCA: taurodeoxycholic acid; TUDCA: tauroursodeoxycholic acid; TCDCA: taurochenodeoxycholic acid; CDCA: chenodeoxycholic acid; TCA: taurocholic acid; TLCA: taurolithocholic acid; GCA: glycocholic acid; GUDCA: glycoursodeoxycholic acid; GCDCA: glycochenodeoxycholic acid; GLCA: glycolithocholic acid; CA: cholic acid; UDCA: ursodeoxycholic acid; DCA: deoxycholic acid; LCA: lithocholic acid; NC: negative control; GDM: gestational diabetes mellitus.

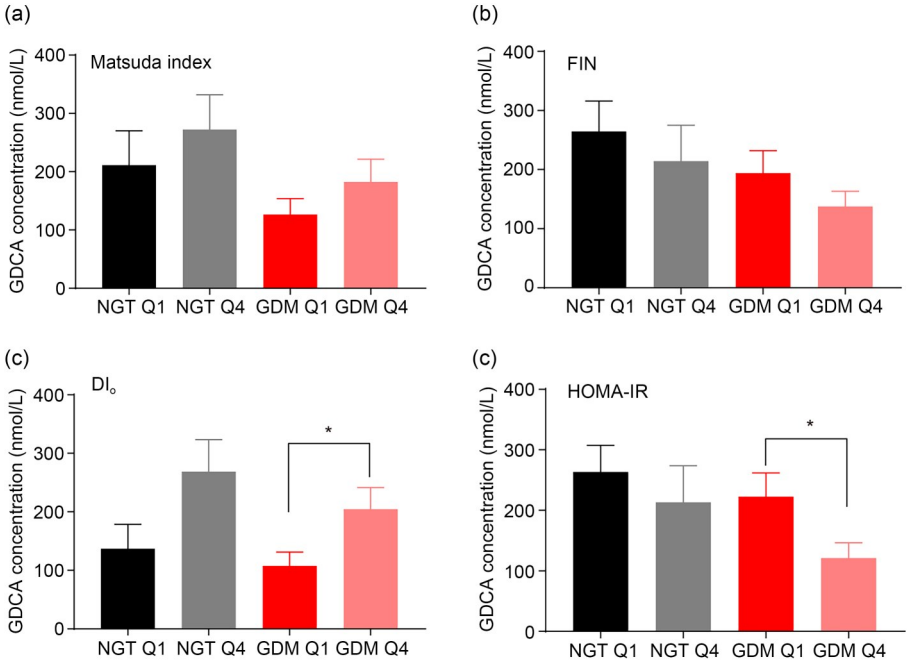

Fig. 4 Changes of GDCA concentration in the Q1 and Q4 subgroups of NGT and GDM divided by Matsuda index (a), FIN (b), DI_0 (c), and HOMA-IR (d). Data are expressed as mean±standard error of the mean (SEM) (NGT Q1/Q4: *n*=12; GDM Q1/Q4: *n*=17). * *P*<0.05. GDCA: glycodeoxycholic acid; NGT: normal glucose tolerance; GDM: gestational diabetes mellitus; FIN: fasting insulin; DI_0: oral disposition index; HOMA-IR: homeostatic model assessment of insulin resistance.

metabolism profiles, the correlation between specific BAs and insulin-related indicators was clarified. Overall, GDM patients showed higher levels of BMI and HbAc1 during the OGTT screening period. Furthermore, levels of FIN and insulin at 1 and 2 h post the 75-g oral glucose intake increased significantly in GDM patients. In terms of BA metabolism profile, TDCA and GDCA were the most significantly changed BAs in GDM patients. Moreover, GDCA negatively correlated with the HOMA-IR and positively correlated

Table 2 Clinical outcomes for women with NGT and GDM

Group	*n*	Missing data	Cesarean delivery rate (%)	Gestational age (year)	Infant birth weight (g)
NGT	44/48	4	36.4 (16/44)	39.3 (39.0–41.1)	3300 (2970–3635)
All-GDM	59/67	8	54.2 (32/59)^{NS}	39.0 (38.0–39.6)^{NS}	3400 (3090–3670)^{NS}
GDM Q1 (HOMA-IR)	17/17	0	41.2 (7/17)^{NS}	39.0 (38.4–39.3)^{NS}	3200 (2950–3430)^{NS}
GDM Q4 (HOMA-IR)	12/17	5	91.7 (11/12)***	38.6 (37.4–39.4)^{NS}	3430 (3098–3980)^{NS}
$P^{\#}$			0.008	0.718	0.134
GDM Q1 (DI_o)	15/17	2	66.7 (10/15)^{NS}	38.4 (37.0–39.3)*	3400 (2870–3810)^{NS}
GDM Q4 (DI_o)	16/17	1	43.8 (7/16)^{NS}	39.0 (38.3–39.4)^{NS}	3235 (3065–3428)^{NS}
$P^{\#}$			0.285	0.073	0.445

The data are described as median (IQR) for gestational age and infant birth weight. Patients with GDM were subdivided into four quartiles (Q1, Q2, Q3, and Q4) according to the levels of HOMA-IR and DI_o. Clinical outcomes, including cesarean delivery rate, delivery gestational age, and infant birth weight, were compared between women in the NGT group, GDM group, and GDM subgroups (Q1 and Q4). ^{NS}: no statistically significant difference when compared to the NGT group; * $P<0.05$; *** $P<0.001$, compared to the NGT group; $P^{\#}$ represents the difference between Q1 and Q4 groups, and $P<0.05$ was considered to be statistically significant. NGT: normal glucose tolerance; GDM: gestational diabetes mellitus; HOMA-IR: homeostasis model assessment of insulin resistance; DI_o: oral disposition index; IQR: interquartile range.

with the β-cell compensation index. In the clinical outcomes assay, no significant differences between GDM and NGT were observed, while GDM patients in the HOMA-IR Q4 group had elevated rates of cesarean section delivery, and patients in the DI_o Q1 group showed younger delivery gestational ages. Therefore, our findings suggest that fasting GDCA levels during OGTT may be a useful indicator to evaluate IR and β-cell compensation, further assisting with the prediction of adverse clinical outcomes of GDM.

GDM shares a similar pathogenesis with T2DM, where β-cell function is insufficient to maintain the glycemic homeostasis during pregnancy. Insufficience of β-cell function combined with reduced insulin sensitivity finally results in increased serum glucose (Johns et al., 2018). In addition to glucose and insulin metabolic disorders, lipid metabolism is also altered in GDM, since levels of total TGs, TCH, and LDL gradually increase throughout such pregnancies (Wang et al., 2019). Our results were consistent with previous reports on clinical characteristics of GDM, including advanced age, higher BMI, TG, and HbA1c.

Variations in BA metabolism have been partially proved in T2DM patients, as TBA almost doubled in T2DM patients compared with healthy subjects, suggesting its role in the development of T2DM (Haeusler et al., 2013; Zhu et al., 2020). Intrahepatic cholestasis of pregnancy (ICP) is characterized by increased TBA, and patients with ICP are much more vulnerable to suffering from GDM (Martineau et al., 2015). These studies suggest that TBA is likely related to the occurrence of T2DM and GDM. Indeed, in Chinese prospective cohort studies, the incidence of GDM in the group with the highest TBA level (≥4.0 μmol/L) in early pregnancy had a 6.72-fold increased risk of GDM compared with the group presenting the lowest level, and even after adjusting for potential confounders, levels of TBA of ≥2.0 μmol/L still presented an increased risk for developing GDM (Hou et al., 2016; Kong et al., 2020). In our research, TBA remained stable in the GDM group compared with the NGT group, which may be partially caused by the difference between the TBA detected by enzymatic cycling assay and the TBA consisting of 15 individual BAs detected by MS. This prompts us to pay more attention to individual BA components related to glucose metabolism.

Individual BAs have been evidenced to correlate with GDM. Fasting serum levels of glycoursodeoxycholic acid (GUDCA) of ≤0.07 nmol/mL and DCA of ≤0.28 nmol/mL in early pregnancy were independently associated with an increased risk of GDM development (Li et al., 2018). An untargeted metabolomics analysis of pregnant women's fasting serum during the OGTT screening period spotted that CA, ios-DCA, and dehydio-LCA were decreased in GDM patients (Hou et al., 2018). In a rodent model, increased serum CA concentration combined with diminished BAs receptors, including FXR and TGR5, was correlated with GDM (Bellafante et al., 2020). In the present study, GDCA and TDCA were both clearly decreased in GDM. In contrast to other studies, our previous research indicated that BA metabolism profiles changed periodically with gestational age (Zhu et al., 2019), which difference might be caused by dissimilar detection platforms and trimesters when the samples were collected.

In women with normal pregnancy, serum GDCA concentration cumulated in a time-dependent manner after 75-g oral glucose intake (Haslam et al., 2020). In GDM patients, reduced GDCA combined with increased FIN and fasting glucose levels resulted in a significant negative correlation between GDCA and HOMA-IR. A study has demonstrated that GDCA is indeed associated with insulin secretion and resistance (van Nierop et al., 2019). More importantly, IR can be alleviated by GDCA administration. For example, 40-h fasting induced IR, which had no effect on the BA metabolism profile. However, postprandial GDCA and insulin concentrations changed in a statistically significant positive correlation pattern. Increased GDCA triggered the secretion of insulin in a GLP-1-dependent manner (van Nierop et al., 2019). This is helpful to explain that even if GDCA elevates after glucose intake in GDM patients, the declined GDCA baseline still makes this increase insufficient to promote insulin secretion via GLP-1, finally leading to the failure of glycemic regulation. Polycystic ovary syndrome (PCOS) has been associated with IR, and the HOMA-IR of PCOS patients significantly negatively correlates with GDCA (Qi et al., 2019; Zhang et al., 2020; Moghetti and Tosi, 2021). Mechanistically, the significantly increased *Bacteroides vulgatus* presence in the gut microbiota of PCOS individuals causes the reduction of GDCA. The latter combines with GATA-3 in the intestinal group innate lymphoid cell to facilitate interleukin-22 secretion, which in turn improves the PCOS phenotype, including reduced IR and ovarian function recovery (Qi et al., 2019). It is yet to be established, however, whether variations in the gut microbiome occur in GDM, subsequently decreasing GDCA levels and thereby resulting in the IR associated with the condition.

In this study, hyperglycemia in GDM led to severe maternal and fetal outcomes, while the overall clinical outcome for GDM patients was not significantly different from that of NGT patients, which may be attributed to early lifestyle changes and even pharmacotherapy. Moreover, it is worth noting that GDM patients with serious insulin compensation deficiency, as well as high HOMA-IR subgroups, showed worse clinical outcomes.

Despite that our study was single-center with a small sample size, significant data were obtained on the relationship between BA metabolism and GDM. It is considered that abnormalities in specific BAs may predict the adverse clinical outcomes of GDM. The greatest limitation of this study was its cross-sectional nature, which conversely provoked the determination of the metabolic profile of BAs in early gestational age to spot potentially valuable biomarkers for earlier GDM screening. In addition, the GLP-1 baseline was not measured in the fasting serum, as variations in the GLP-1 baseline may influence FIN levels. Changes in the levels of GLP-1 and BAs 1 and 2 h after oral glucose intake were not detected either, which limited the possibility to elucidate the dynamic mechanism of BA metabolism regulating insulin secretion through GLP-1. Moreover, the sample size was fairly small, resulting in a failure to establish a cut-off point for GDCA.

5 Conclusions

The level of GDCA declined significantly in GDM patients, which was inversely correlated with insulin sensitivity and positively correlated with β-cell compensation. Therefore, GDCA could be a valuable biomarker candidate for the assessment of insulin sensitivity and β-cell compensation. Reduced GDCA levels increased the risk of adverse pregnancy outcomes in GDM patients.

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Author contributions

Bo ZHU and Zhixin MA designed the study. Lei FANG and Hong ZHANG collected the data. Zhixin MA performed the statistical data analyses. Hongwei KONG conducted the BA measurements. Yuning ZHU and Zhixin MA contributed to drafting the manuscript. Dajing XIA designed, organized, and supervised the project, and revised the manuscript. All authors have reviewed and approved the manuscript to produce the final version.

Compliance with ethics guidelines

Bo ZHU, Zhixin MA, Yuning ZHU, Lei FANG, Hong ZHANG, Hongwei KONG, and Dajing XIA declare that they have no conflict of interest in relation to the study.

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008 (5). All the participants provided their informed consent to enroll in the trial and the follow-up study.

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Supplementary information

Table S1