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Reduced GDCA 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. BAs change in GDM are still unclear, it 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. BAs metabolism profiles were analyzed both in 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 BMI, elevated serum glucose concentration, raised insulin (both fasting and OGTT), and increased HbA1c levels. Higher insulin resistance index (HOMA-IR) and decreased β-cell compensation (DIo) were also prevalent in this group. Total bile acids (TBA) 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 DIo. No obvious differences in clinical outcome between the GDM and NGT groups were observed. However, GDM patients with high HOMA-IR and low DIo tended to have a higher cesarean delivery rate and younger delivery gestational age. GDCA provides a valuable biomarker to evaluate insulin resistance and DIo, and decreased GDCA levels predict poorer clinical outcomes for GDM.

Key words: Gestational diabetes mellitus; Bile acids; Insulin resistance; β-cell compensation

1 Introduction

Gestational diabetes mellitus (GDM) is a common complication in pregnancy with short- and long-term health risks both for the mother and the developing fetus (Johns et al., 2018). 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) (Group, 2009; O'sullivan et al., 2016; 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 (Yogev et al., 2010; Mortier et al., 2017; Moll et al., 2019).

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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 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). Studies on rodents showed that the induction of postprandial BAs 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 repressed the enzymes involved in hepatic gluconeogenesis, such as phosphoenolpyruvate carboxykinase (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) > cholic acid (CA), 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 bile acid receptor FXR is also differentially activated by CDCA > DCA > LCA > CA (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 have demonstrated that insulin controls BAs 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 was associated with increased 12α-hydroxylated BAs, and the ratios of 12α-hydroxylated / non-12α-hydroxylated BAs were associated with key features of IR. In T2DM patients, levels of total bile acids (TBAs) were nearly twofold compared with healthy subjects, although no disproportionate increases in 12α-hydroxylated BAs were spotted (Haeusler et al., 2013).

Recent results by authors of the present study showed that BAs 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 BAs profile is changed in GDM patients, and determine the clinical significance of any such changes.

In this paper, it is hypothesized that the BAs metabolism profile changes in GDM, and a correlation between insulin metabolism and specific BAs components exists. In order to verify this hypothesis, we analyzed the fasting BAs metabolism profile in GDM and normal pregnant women during the OGTT screening period, and identified the key BAs that changed in GDM, thereby laying the foundations for the assessment of insulin resistance 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 to October 2019 in Women's Hospital, Zhejiang University School of Medicine (Hangzhou, Zheijang, China). Cases of preconceptional diabetes, infections, abnormal liver or kidney function, and those positive for HIV and hepatitis C antibodies were excluded from the study. Blood samples were obtained from all participants after 8-14 hours of fasting.

Subsequently, all women underwent a 75-g oral glucose tolerance test (OGTT). Based on the OGTT data, we defined GDM according to the criteria of International Association of the Diabetes and Pregnancy Study Groups (IADPSG) (Weinert, 2010). Insulin and glucose levels during the OGTT were used to determine the HOMA-IR, 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; Stumvoll et al., 2001). The insulin secretion index and Matsuda index were multiplied to calculate the oral disposition index (DIo), which assesses β-cell compensation for insulin resistance (Elbein et al., 2000). The study was carried out according to the Declaration of Helsinki guidelines. All participants provided informed consent to take part in the follow-up study, and 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 BAs analysis was performed according to the previously published methods with minor modifications (Zhu et al., 2019). Briefly, commercially available reference standards were obtained from TRC lnc. (Toronto, Canada) and Sigma-Aldrich (St. Louis, USA). 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 LC-MS/MS analysis after centrifugation for 10 min at 13,000 × g. Bile acids were separated using a UPLC BEH C18 column (2.1 mm × 100 mm, 1.7 μm; Waters) with 40℃ column temperature. The mass spectrometry detection of BAs were performed using individually optimized cone voltage and collision energy in multiple reaction monitoring (MRM) mode (Table S1).

2.3 Statistical methods

Mass spectrometry data were analyzed using Analyst software v1.6.0 (SCIEX, Framingham, USA). The Student's t test or the Mann-Whitney U test were 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 square discriminant analysis (OPLS-DA) using SIMCA-P (version 13.0, Umetrics, Sweden). All statistical analyses were performed using the GraphPad Prism 7.0 software. Values of $P < 0.05$ were considered statistically significant.

3 Results

3.1 Baseline characteristics of the study population

Compared with NGT, GDM patients had higher body mass index (BMI) ($P < 0.001$). The biochemical indicators included triglyceride (TG), cholesterol (TCH), high-density lipoprotein (HDL), low-density lipoprotein (LDL), glycated albumin (GAL) and hemoglobin A1c (HbA1c). Both HbA1c and TG elevated significantly in the GDM group ($P = 0.013$, $P = 0.047$). Albeit there was no statistical significance in insulin secretion, the levels of fasting insulin and insulin 1 hour and 2 hours post-OGTT were raised in the GDM group compared with NGT ($P < 0.001$, $P = 0.005$, $P < 0.001$). In addition, GDM patients had a deficiency in insulin sensitivity ($P < 0.001$), and poorer β-cell compensation ($P < 0.001$) than that of the NGT group (Table 1). All of the significantly changed parameters in Table 1 were used for Orthogonal Partial Least Squares Discrimination Analysis (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 DIo index,

Matsuda index, HOMA-IR, Stumvoll Phase I and insulin levels in OGTT contributed significantly as a principal component to separate the two groups (Fig. 1B).

3.2 Bile acid metabolism profiles in GDM and NGT

Since glucose and insulin metabolism discriminations exist between 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 BAs profiles. The two cohorts, however, could not be separated by OPLS-DA (data not shown), even though TBA decreased in the GDM group (Fig. 2A). This finding suggested that certain types of BAs may be more intimately involved in glycemia regulation. Indeed, 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 BAs levels remained stable in GDM (Fig. 2B). Furthermore, among individual 12α-hydroxylated BAs, TDCA and 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).

3.3 Correlation between individual BAs and insulin indexes

In order to further describe the relationship between BAs 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, and this correlation coefficiently decreased in GDM. Levels of GDCA also positively correlated with DIo in GDM, but negatively with HOMA-IR and fasting insulin (Potthoff, et al.). No correlation between TDCA and insulin-related indicators was shown in NGT and GDM, although TDCA was markedly reduced in GDM. Among the non-12α-hydroxylated individual BAs, CDCA correlated positively with HOMA-IR and FIN, whilst TUDCA correlated negatively with DIo (Fig. 3).

3.4 Clinical outcome for GDM patients with higher HOMA-IR and lower DIo

Results showed that GDCA declined in GDM, and this decline correlated with insulin sensitivity, IR, DIo and FIN. In order to assess the relationship between GDCA and clinical outcome including delivery gestational age, fetus birthweight 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, DIo, and FIN, respectively. No apparent change in GDCA occurred among the Q1 and Q4 groups divided by Matsuda index and FIN (Figs. 4A and 4B), but it decreased in the HOMA-IR Q4 group and increased in the DIo Q4 groups (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 DIo, 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 DIo 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 BAs 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 fasting insulin and insulin at 1 and 2 hours post the 75-g oral glucose intake increased significantly in GDM. In terms of BAs metabolism profile, TDCA

and GDCA were the most significantly changed BAs in GDM patients. Moreover, GDCA negatively correlated with the insulin resistance index (HOMA-IR) and positively correlated with the β-cell compensation index (DIo). 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 DIo Q1 group showed younger delivery gestational ages. Therefore, our findings suggest that fasting GDCA levels during OGTT may be a useful indicator to evaluate insulin resistance and β-cell compensation, further assisting with the prediction of adverse clinical outcomes of GDM.

Gestational diabetes mellitus shares a similar pathogenesis with T2DM, where β-cell function is insufficient to provide glycemic homeostasis required in pregnancy, and this combined with reduced insulin sensitivity finally results in increased serum glucose (Johns, et al., 2018). In addition to glucose and insulin metabolic disorders, lipids metabolism is also altered in GDM, since levels of total triglycerides, total cholesterol and low-density lipoprotein cholesterol gradually increase throughout such pregnancies (Wang et al., 2019). Our results are consistent with previous reports on clinical characteristics of GDM, including advanced age, higher BMI, TG, and HbA1c.

Variations in BAs metabolism have been partially proved in T2DM, 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 ($\geq$ 4.0 μmol/L) in early pregnancy had a 6.72-fold increased risk of GDM compared with the group presenting the lowest level. Even after adjusting for potential confounders, levels of TBA $\geq$ 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 GDM compared with normal glucose tolerance, 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 mass spectrometry. This prompts us to pay more attention to individual BAs components related to glucose metabolism.

Individual BAs have been evidenced to correlate with GDM. Fasting serum levels of GUDCA $\leq$ 0.07 nmol/mL and DCA $\leq$ 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 (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 BAs metabolism profiles change periodically with gestational age (Zhu, et al., 2019), which difference may 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 fasting insulin and fasting glucose levels resulted in a significant negative correlation between GDCA and HOMA-IR. A study have demonstrated that GDCA is indeed associated with insulin secretion and resistance (Van Nierop et al., 2019). More importantly, insulin resistance can be alleviated by GDCA administration. For example, 40-hour fasting induced insulin resistance, which had no effect on the BAs metabolism profile. However, postprandial GDCA and insulin concentration 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 insulin resistance, and the HOMA-IR of PCOS patients significantly negatively correlates with GDCA(Qi et al., 2019; Moghetti and Tosi, 2020). 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 3 innate lymphoid cell to facilitate interleukin-22 secretion, which in turn improves the PCOS phenotype, including reduced insulin resistance 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 insulin resistance associated with the condition.

In this study, hyperglycemia in GDM lead 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 small sample size, significant data were obtained on the relationship between BAs 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 GLP-1 baseline may influence fasting insulin levels. Changes in levels of GLP-1 and BAs 1 hour and 2 hours after oral glucose intake were not detected either, which limited the possibility to elucidate the dynamic mechanism of BAs 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

Levels 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.

Abbreviations

GDM: gestational diabetes mellitus; BAs: bile acids; OGTT: oral glucose tolerance test; NGT: normal glucose tolerance; HOMA-IR: Homeostatic Model Assessment of Insulin Resistance (index); DIo: disposition index (β-cell compensation); TBA: total bile acids; GDCA: glycodeoxycholic acid; TDCA: taurodeoxycholic acid; T2DM: maternal type 2 diabetes; IR: insulin resistance; FXR: Farnesoid X receptor; TGR5: Takeda G protein receptor 5; PEP-CK: phosphoenolpyruvate carboxy kinase, LCA: lithocholic acid; DCA: deoxycholic acid; CDCA: chenodeoxycholic acid; CA: cholic acid; GLP-1: glucagon like peptide-1; MRM: multiple reaction monitoring; BMI: Body Mass Index, VIP: Variable Importance in Projection; FIN: fasting insulin, OPLS-DA: Orthogonal Partial Least Squares Discrimination Analysis; TG: triglyceride; TCH: cholesterol; HDL: high-density lipoprotein; LDL: low-density lipoprotein; GAL: glycated albumin; HbA1c: HemoglobinA1c.

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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. The bile acid measurements were conducted by Hongwei KONG. Yuning ZHU and Dajing XIA contributed to drafting the manuscript. All authors reviewed and edited the manuscript to produce the final version.

Availability of data and materials

All the data are available to interested researchers upon reasonable request. Requests for access to data should be made to the first author via e-mail: 5202054@zju.edu.cn.

Ethics approval and consent to participate

None of the authors declare any conflict in relation to the study.

This study was carried out according to the declaration of Helsinki guidelines. The research protocol was approved by the ethics committee of the Women's Hospital, Zhejiang University School of Medicine (IRB-20200015-R). All the participants provided their informed consent to enroll in the trial and the follow-up study.

Consent for publication

Not applicable.

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Figures

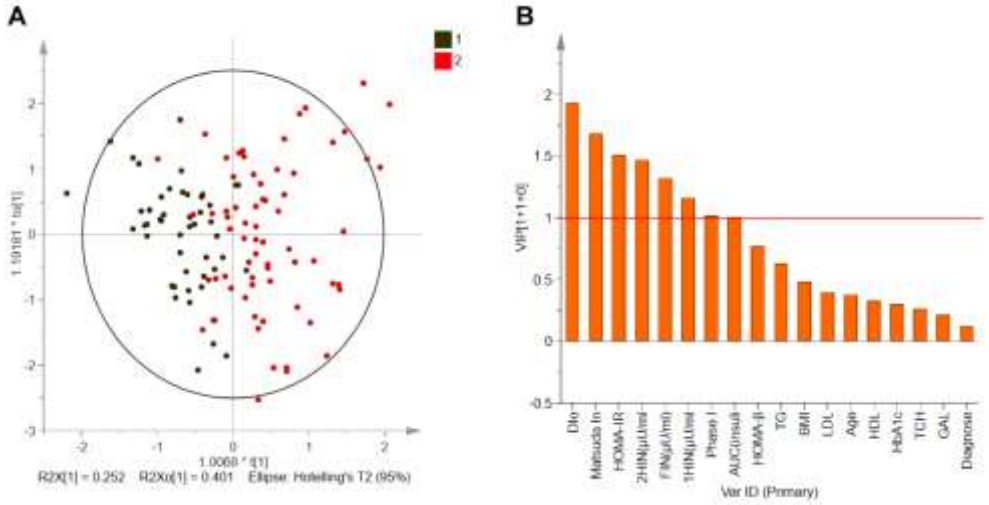

Figure 1. OPLS-DA score plot of NGT and GDM according to the significantly changed parameters, as seen in Table 1. The black and the red square represent the NGT and GDM patients, respectively. The black circle indicates the 95% confidence interval (A). A taxon with a VIP score of > 1 was considered important in the group discrimination (B).

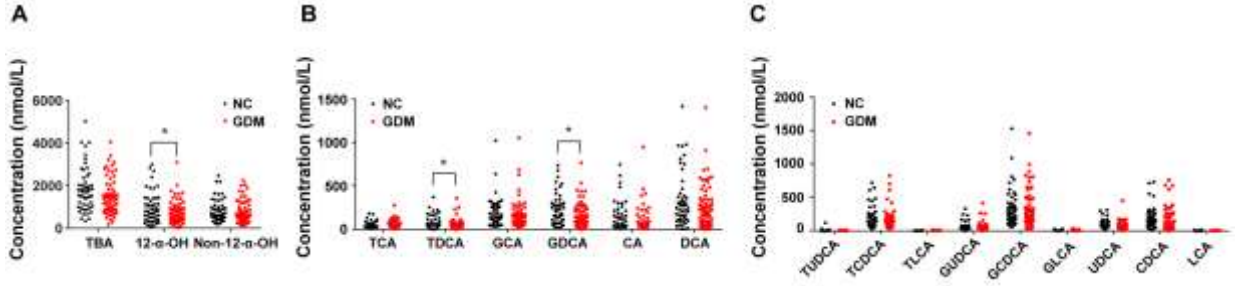

Figure 2. TBA levels remained stable in the GDM group. 12α-hydroxylated bile acids decreased in GDM (B), while non-12α-hydroxylated bile acids showed no obvious changes in the GDM group (A). The 12α-hydroxylated bile acids GDCA and TDCA declined significantly in GDM (B), while non-12α-hydroxylated bile acids presented no apparent variation in individuals where they were detected (C); *: $P < 0.05$.

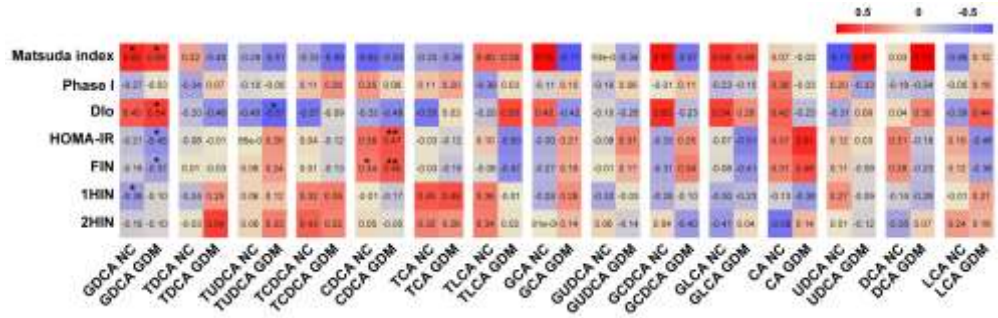

Figure 3. Heat map showing the Spearman correlation between individual bile acids and Matsuda index, Phase I, DIo, HOMA-IR, FIN, 1HIN, 2HIN. The color scheme corresponds to correlation strength as shown by the color bar. Red represents a positive association, blue represents a negative association and white represents no association; *: $P < 0.05$, **: $P < 0.01$.

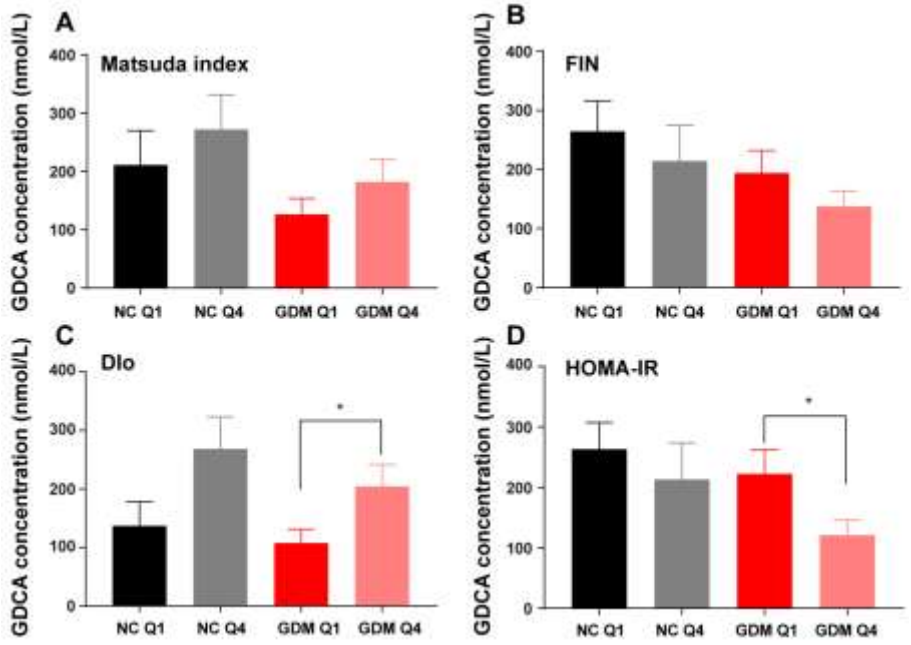

Figure 4. Change of GDCA concentration in the Q1 and Q4 subgroups of NGT and GDM divided by Matsuda index (A), FIN (B), HOMA-IR (C), and DIo (D); *: $P < 0.05$.

Table 1. Characteristics of women with NGT and GDM

	NGT	GDM	
	Median (IQR)/n (%)	Median (IQR)/n (%)	*P*
N	48	67	
Age	30 (28-32.8)	33 (29-36)	0.003
Gestational age	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
1-h glucose OGTT (mg/dL)	137.5 (110.5-150.9)	185.2(165.1-197.5)	< 0.001
2-h glucose OGTT (mg/dL)	116.2 (104.3-130.5)	159.9 (142-172.3)	< 0.001
Fasting insulin (μU/mL)	6.2 (4.9-8.5)	8.9 (6.5-11.9)	< 0.001
1-h insulin OGTT (μU/mL)	40.5 (30.3-63.3)	58.6 (39.7-91.5)	0.005
2-h insulin OGTT (μU/mL)	45.7 (31.1-57.7)	67.9 (46.5-109.0)	< 0.001
Insulin sensitivity (Matsuda and Defronzo)	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
DIo	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

In the table, IQR denotes interquartile range. Differences between the NGT and GDM group 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, triglyceride (TG), cholesterol (TCH), high-density lipoprotein (HDL), low-density lipoprotein (LDL), glycated albumin (GAL) and HemoglobinA1c (HbA1c), were also compared between GDM and NGT. 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 oral disposition index (DIo), which assesses β-cell compensation for insulin resistance. The insulin resistance index and β-cell function were calculated using fasting glucose (mM) × fasting insulin (μIU/mL)/22.5 and 20 × fasting insulin (μIU/mL)/[fasting glucose (mM) -3.5] formulation, respectively. $P < 0.05$ was considered to be statistically significant. Data were expressed as median and IQR.

Table 2. Clinical outcomes for women with NGT and GDM. The GDM group was divided into subgroups by the level HOMA-IR and DIo

	NGT	All-GDM	GDM Q1 (IR)	GDM Q4 (IR)		GDM Q1 (DIo)	GDM Q4 (DIo)	
	Median (IQR)/n (%)	Median (IQR)/n (%)	Median (IQR)/n (%)	Median (IQR)/n (%)	$P^{\#}$	Median (IQR)/n (%)	Median (IQR)/n (%)	$P^{\#}$
N	44/48	59/67	17/17	12/17		15/17	16/17	
Missing data	4	8	0	5		2	1	
Delivery								
Cesarean delivery	36.4% (16/44)	54.2% (32/59) §	41.2% (7/17) §	91.7% (11/12) ***	0.008	66.7% (10/15) §	43.8% (7/16) §	0.285
Gestational age	39.3 (39-41.1)	39 (38-39.6) §	39 (38.4-39.3) §	38.6 (37.4-39.4) §	0.718	38.4 (37-39.3) *	39 (38.3-39.4) §	0.073
Infant birth weight (g)	3300 (2970-3635)	3400 (3090-3670) §	3200 (2950-3430) §	3430 (3098-3980) §	0.134	3400 (2870-3810) §	3235 (3065-3428) §	0.445

In the table, IQR denotes interquartile range. Clinical outcomes, including cesarean delivery rate, delivery gestational age and infant birth weight were compared between women in the NGT, GDM and GDM subgroups. Patients with GDM were subdivided into four quartiles (Q1, Q2, Q3, Q4) according to the level of HOMA-IR and DIo. §indicates no statistically significant difference when compared to the NGT group, and $P^{\#}$ represents the difference across Q1 and Q4 groups. $P < 0.05$ was considered to be statistically significant. Data were expressed as median and IQR.