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Research paper

Bile acid metabolites in early pregnancy and risk of gestational diabetes in Chinese women: A nested case-control study

Jing Li [a], Xiaoxu Huo [a], Yun-Feng Cao [b,c], Sai-Nan Li [d], Zuo Du [d], Ping Shao [e], Junhong Leng [e], Cuiping Zhang [e], Xiao-Yu Sun [b,c], Ronald C.W. Ma [f], Zhong-Ze Fang [d,*], XilinYang [a,**]

[a] Department of Epidemiology and Biostatistics, School of Public Health, Tianjin Medical University, Tianjin, China
[b] Key Laboratory of Liaoning Tumor Clinical Metabolomics (KLLTCM), Jinzhou, Liaoning, China
[c] RSKT Biopharma Inc, Dalian, Liaoning, China
[d] Department of Toxicology, School of Public Health, Tianjin Medical University, Tianjin, China
[e] Tianjin Women and Children's Health Center, Tianjin, China
[f] Department of Medicine and Therapeutics, Prince of Wales Hospital, The Chinese University of Hong Kong, Hong Kong, China

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ABSTRACT

Background: Bile acid metabolism plays an important role in metabolism but it is uncertain whether bile acid metabolites in early pregnancy are associated with risk of gestational diabetes mellitus (GDM).
Methods: We organized a 1:1 case-control study nested in a prospective cohort of 22,302 pregnant women recruited from 2010 to 2012 in China: 243 women with GDM were matched with 243 non-GDM controls on age (±1 year). Conditional logistic regression and restricted cubic spline were used to examine full-range associations of bile acid metabolites with GDM.
Findings: All the 9 detectable bile acids were inversely associated with the risk of GDM, among them, 8 in nonlinear and one in largely linear manners in multivariable analysis. Glycoursodeoxycholic acid (GUDCA) at ≤0.07 nmol/mL and deoxycholic acid (DCA) at ≤0.28 nmol/mL had threshold effects and their decreasing levels below the cutoff points were associated with rapid rises in the risk of GDM. In traditional risk factor model, the stepwise procedure identified that GUDCA ≤ 0.07 nmol/mL and DCA ≤ 0.280 nmol/mL were still significant (OR: 6.84, 95%CI: 1.10–42.48 & 2.06, 1.26–3.37), while other bile acids were not. Inclusion of the two bile acids in the model increased the area under operating characteristic's curve from 0.69 to 0.76 (95% CI: 0.71–0.80) ($P < .05$).
Interpretation: Serum GUDCA ≤ 0.07 nmol/mL and DCA ≤ 0.28 nmol/mL in early pregnancy were independently associated with increased risk of GDM in Chinese pregnant women.
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1. Introduction

The prevalence of GDM has been increasing steadily worldwide, especially in Asia countries [1]. In Tianjin, China, the prevalence of GDM increased from 2.3% in 1999 to 9.3% in 2010–2012 [2]. It is critically important to prevent occurrence of GDM, especially in high risk pregnant women, with possible intervention commencing before pregnancy or in early pregnancy. However, our recent meta-analysis of 29 randomized controlled trials showed that lifestyle intervention initiated within 15 weeks of gestation was only associated with a 20% reduction in the risk of GDM and lifestyle intervention after the 15th gestational week was ineffective [3]. Given the high residual risk of GDM despite early lifestyle intervention and GDM's harmful long-term effects on increased risks of diabetes [4] and cardiovascular disease in women [5], as well as childhood obesity in their offspring [6], there is a strong need to accurately identify high risk women early in pregnancy and to better understand the etiology of GDM for design of more effective interventions.

Bile acid metabolism plays an important role in the metabolism of glucose, fat, and energy [7, 8]. In this connection, studies both in rodents and humans have shown that bile acid metabolism impacts on obesity [9] and type 2 diabetes mellitus (T2DM) [10, 11]. High-fat diet (HFD)-

* Corresponding author at: Department of Toxicology, School of Public Health, Tianjin Medical University, 22 Qixiangtai Road, Heping District, Tianjin 300070, China.
** Corresponding author at: P.O. Box 154, Department of Epidemiology & Biostatistics, School of Public Health, Tianjin Medical University, 22 Qixiangtai Road, Heping District, Tianjin 300070, China.
E-mail addresses: fangzhongze@tmu.edu.cn (Z.-Z. Fang), yangxilin@tmu.edu.cn (XilinYang).

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Research in context

Evidence before this study

We performed a systematic search of the PubMed database for human studies evaluating differences in individual bile acids between normal pregnant women and women with gestational diabetes mellitus (GDM) published up to July 20, 2018. The searching terms were "(gestational diabetes OR gestational diabetes mellitus OR GDM) and (bile acids OR bile acid)". Only three small studies (n < 300) were identified. One study from Poland observed that serum levels of 4 bile acids and derivatives markedly decreased in GDM compared with non-GDM, and two studies from China found that one primary bile acid and six secondary bile acids as well as unconjugated and conjugated bile acids were significantly up- or down- regulated in women with GDM versus normal pregnant women. These findings suggest that changes in individual bile acids may be associated with risk of GDM. However, because all the three studies did not adjust for traditional confounding factors and other bile acid metabolites, it remains unknown which of these bile acid metabolites are independently associated with risk of GDM.

Added value of this study

We found that deoxycholic acid (DCA) and glycoursodeoxycholic acid (GUDCA), the glycine conjugates of ursodeoxycholic acid (UDCA), were inversely associated with risk of GDM in non-linear manners, and both had clear threshold effects on the risk of GDM. DCA < 0.28 nmol/mL and GUDCA < 0.07 nmol/mL were associated with markedly increased risk of GDM after adjusting for traditional GDM risk factors and the other bile acid. Inclusion of the two bile acids in a model with traditional GDM risk factors greatly increased the area under the receiver's operating characteristic curve from 0.69 to 0.76.

Implications of all the available evidence

Our study is the first reporting that DCA < 0.28 nmol/mL and GUDCA < 0.07 nmol/mL were independently associated with GDM. Inclusion of these two markers has potential to greatly improve the prediction of GDM in early pregnancy. Given the close link of DCA and GUDCA with gut microbiota, it is worthwhile to conduct randomized controlled trials to test the effect of interventions targeting gut microbiota for prevention of GDM in early pregnancy.

fed mice are characterized by increased fasting glucose and a decreased bile acid pool size but improvement of insulin resistance after supplement with cholic acids (CA) [9]. An animal study found that secondary bile acids played a protective role in pancreatic islet beta-cells in diabetic rats [12]. Pilot studies utilizing metabolomics ($n < 50$) observed significant changes in bile acid species in GDM individuals in the 2nd trimester of pregnancy [13, 14]. In another study ($n < 300$), significant alteration of bile acid metabolome was also observed in the 1st trimester of pregnancy between normal pregnant women and women with GDM [15]. However, it remains unknown which patterns of bile acid metabolites in early pregnancy are associated with the risk of GDM.

Using a universal screening system of Chinese pregnant women established from 2010 to 2012 in Tianjin, China, we aimed to organize a nested case-control study from the cohort of pregnant women [2] and used metabolomics approach to explore the associations between bile acid metabolite patterns and subsequent risk of GDM in Chinese pregnant women in Tianjin, China.

2. Materials and methods

2.1. Research design and population

The study cohort and methods were described previously [2]. Briefly, from October 2010 to August 2012, we established a population-based cohort of pregnant women based on a universal screening and management system for GDM in the six central urban districts of Tianjin, China, which was initiated in 1999 [16]. A total of 22,302 pregnant women were recruited in this prospective study at their first antenatal care. They were followed longitudinally from their first antenatal care visit to the time of glucose challenge test (GCT) at 24–28 gestational weeks and through the postpartum period. Ethics approval was obtained from the Ethics Committee for Clinical Research of Tianjin Women and Children's Health Centre (TWCHC), Tianjin, China, and written consent was obtained from all the women.

Among these recruited women, a two-step screening procedure was used to identify GDM cases. At first, all pregnant women underwent a 50-g 1-h GCT in non-fasting status at 24–28 weeks of gestation at a primary care hospital. Women with plasma glucose (PG) ≥ 7.8 mmol/L were referred to the GDM clinic within TWCHC where they underwent a 75-g 2-h oral glucose tolerance test (OGTT) in the morning after at least 8-h of fasting. PGs at fasting, 1-h and 2-h after the glucose load were measured at the Central Laboratory of TWCHC using an automatic analyzer (TBA-120FR, Toshiba, Japan). GDM was diagnosed according to the World Health Organization 2013 criteria [17].

In the large cohort, 2991 pregnant women in early stage of the study provided overnight fasting blood samples. The sera and clotted blood were separated immediately and stored at −80 °C. In this study, 227 pregnant women were excluded due to lack of GCT results or lack of OGTT results if their GCT ≥ 7.8 mmol/L. Of the remaining 2764 women (Comparison of baseline characteristics of the 2764 women with the rest of the entire cohort is listed in the Appendix Table S1), 243 women developed GDM and were used as the cases in the current study and 243 women without GDM matched on age (±1 year of the case) were used as the controls. Finally, we organized 243 pairs of GDM cases and their controls in the 1:1 nested case-control study, using a macro written and conducted in the Statistical Analysis System (SAS). The flow chart of selection of the pregnant women is shown in Fig. 1.

2.2. Data collection procedures

Detailed data collection methods have been described previously [2]. Briefly, at the first antenatal care visit, standardized procedures were used to measure maternal height, weight and blood pressure (BP) by nurses or obstetricians. Weight was measured to an accuracy of 0.1 kg and was re-measured when the GCT was performed. Height was measured to the nearest 0.5 cm. Because weight gain during the first trimester of pregnancy is minimal, we used body weight at first antenatal care visit as the pre-pregnancy body weight to estimate pre-pregnancy body mass index (BMI). BMI was calculated as weight at first antenatal care visit in kilogram divided by squared body height in meter. BMI groups at first antenatal care visit were defined based on the criteria recommended for Chinese adults [18]: underweight (<18.5 kg/m^2), normal weight (18.5–23.9 kg/m^2), overweight (24.0–27.9 kg/m^2) and obesity (≥28.0 kg/m^2). Repeated measurements of body weight were performed and the difference between the first antenatal visit and the GCT was calculated.

Other data were collected by a series of structured questionnaires completed by care providers and/or pregnant women at their first antenatal care visit, at the time of the GCT, and at subsequent antenatal care visits, respectively. Some information such as pregnancy outcomes was retrieved from a centralized computer database within Tianjin Maternal and Child Health Information System. The collected data included maternal age, family history of diabetes in first degree relatives, parity,

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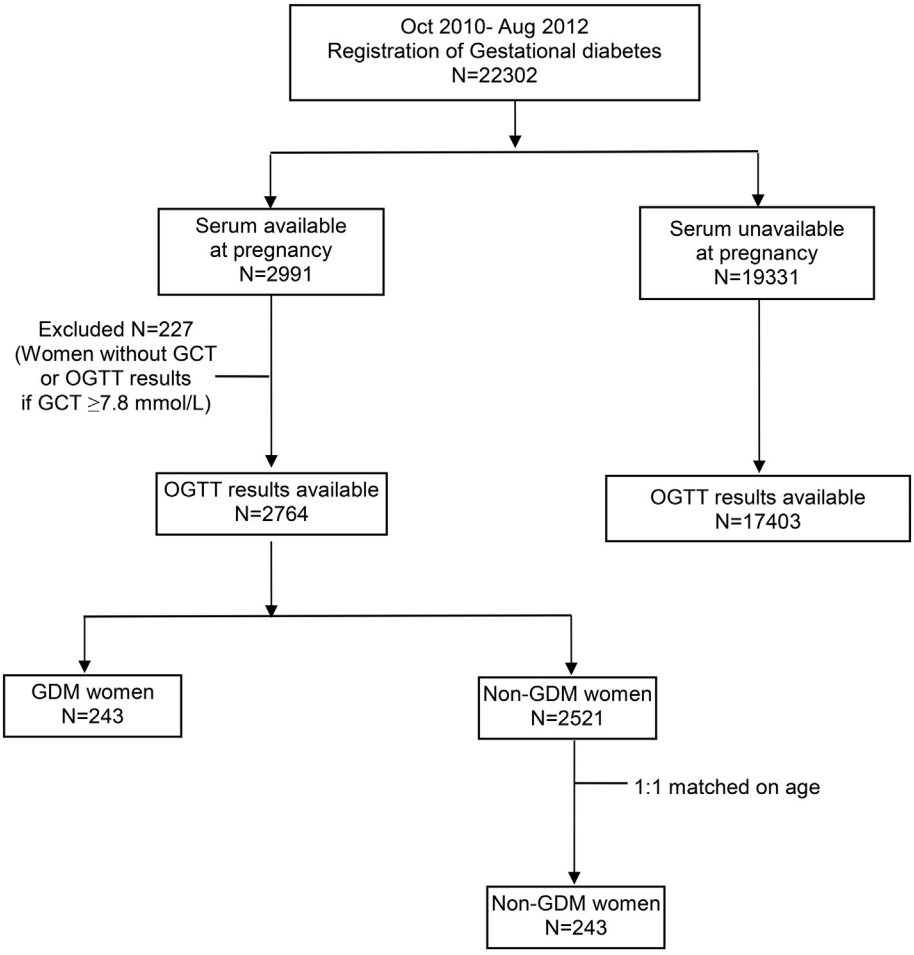

Fig. 1. Flow diagram of selection of the study women in the nested case-control study.

ethnicity, education attainment, habitual smoking before or during pregnancy, and alcohol consumption before or during pregnancy. Education attainment was classified into >12 or ≤12 years of schooling. Habitual smoking before or during pregnancy was defined as continuously smoking one or more cigarettes per day for at least 6 months before pregnancy or smoking one or more cigarettes per day during pregnancy.

2.3. Metabolomics analysis of serum bile acids components

2.3.1. Sample pretreatment

−80 °C low temperature preservation of sample thawed in 4 °C. Quantitative weighing 50 μL sample in 1.5 mL EP, adding 2 μg/mL internal standard solution 10 μL, vortex 10 s, then 300 μL cold protein precipitation liquid (a methanol solution containing 0.1% ammonia) was added to the mixture, vortex 45 s, at 4 °C, the mixture was centrifuged for 10 min at a rotation speed of 16,000 g. After that, 200 μL of supernatant was transferred and concentrated to dry under nitrogen. Finally, the dried supernatant was dissolved with 50 μL methanol and 20 μL sample injection for LC/MS analysis. To ensure data quality, quality control (QC) samples were prepared by mixing all of the samples. During analysis of the sample sequence, one QC sample was run after every 30 injections.

2.3.2. LC-MS/MS analysis

Quantification of bile acids was performed according to previous studies with slight modification [19, 20]. Specifically, an Eksigent ultral liquid chromatography 100 coupled with an AB 5600 Triple TOF system (AB SCIEX) was used to identify and quantify the bile acids components. A 2.1 × 100 mm XBridge Peptide BEH C18 column (waters) with a 4 × 2.0 mm guard column (phenomenex) was equipped to separate the different components. The separation was achieved under a column temperature of 40 °C using a controlled gradient of mobile phase A, which consisted of 0.1% (v/v) formic acid and 10 mM acetic acid amine in water, and mobile phase B, composed of 0.1% formic acid in 80% (v/v) methanol and 20% (v/v) acetonitrile, at a flow rate of 0.4 mL/min. The gradient flow was first set at 35% (v/v) B for 0.5 min, linearly increased to 60% B during the next 2.5 min, linearly increased to 80% B during the next 7 min, linearly increased to 90% B during the next 6 min, linearly decreased to 35% B during the next 4.5 min and maintained at this composition for an additional 2.5 min. The injection volume of the sample was 5 μL. ESI source configurations for Triple TOF were set as follows: ion source gas 1 (GS1) as 50, ion source gas 2 (GS2) as 50, curtain gas (CUR) as 30, source temperature as 550 °C, and ion spray voltage floating as −4500 V in negative mode. The instrument was set to acquire over the *m/z* range 200–800 Da for TOF MS scans and the m/z range 50–800 Da for the production of ion scans in auto MS/MS acquisition. The accumulation time for TOF MS scans and the production ion scans were set at 19.993 min. The collision energy of the production ion scan was set at -45 V ±20 V spread, and the declustering potential was set at −80 V. The detection limits of individual bile acids are listed in Appendix Table S2.

2.3.3. Data processing

The raw data were acquired using the PeakView 1.2 software and MultiQuant 2.1 software based on the *m/z* value and the sample retention time.

2.4. Statistical analysis

Statistical analyses were performed using SAS version 9.3 (SAS Institute Inc., Cary, NC, USA). Quantitative data were expressed as means (standard deviation [SD]) or median (interquartile range [IQR]) and categorical data were presented as n (%) where appropriate. We used paired *t*-test or the Wilcoxon signed-rank test where normal distribution was rejected by checking the Q-Q plot, to compare differences of continuous variables and McNemar test or Fisher's exact test where appropriate to compare differences of proportions between the GDM group and the non-GDM group. Conditional binary logistic regression [21] was performed to estimate the odds ratios (ORs) and 95% confidence intervals (CIs) of levels of bile acids for the risk of GDM in univariable and multivariable analyses. Ryan-Holm step-down Bonferroni procedure was used to adjust *P* values and 95%CIs of the ORs for multiple comparisons [22, 23].

We plotted the frequency distribution for the bile acids (Appendix Fig. S1) and found that most of bile acids were non-linearly associated with risk of GDM. Therefore, restricted cubic spline (RCS) analysis was used in univariable and multivariable analyses to examine full-range associations of bile acid levels with GDM [24]. Given the small sample size, we used 3 knots as suggested [25]. After careful visual checking of the shapes of the OR curves of bile acids for GDM, we stratified these bile acid species into categorical variables at specific cutoff points where the risk of GDM started to increase steeply. This method of selection of cutoff points had been used in many of our previous studies [26, 27], including analyses of this GDM cohort [28].

A structured adjustment scheme was used to control for confounding factors. First, we performed univariable analysis to obtain unadjusted ORs; Second, we adjusted for traditional GDM risk factors and liver function to control for their possible residual confounding effects [2], including BMI at registration, family history of diabetes in first-degree relatives, systolic and diastolic BP at registration, habitual smoking and drinking before and during pregnancy, gestational age at registration, parity (≥1), educational attainment (>12 years of school education), Han Chinese ethnicity, weight gain up to the time of GCT and alanine aminotransferase (ALT); Third, we performed stepwise (forward as well as backward) selection to identify significant bile acid species that had predictive values in addition to the traditional risk factors ($p = .05$ for entry and/or exit). Finally, we linear-transformed the optimal bile acids and tested the effect sizes of these bile acids as a continuous variable and compared the areas under the receiver operating characteristic curves (AUC) for the traditional risk factor model, the bile acid model and the risk factor plus bile acid model to check the potential improvement of inclusion of bile acid metabolites in the traditional risk factor model in discrimination of GDM. In this analysis, p values < .05 were considered to be statistically significant.

3. Results

3.1. Characteristics of the study population

Compared with women free of GDM, those who developed GDM had higher body weight, BMI, higher BPs and alanine aminotransferase (ALT) at their first antenatal care visit. Women who developed GDM were more likely to be overweight or obese and have family history of diabetes in first degree relatives. Body height, gestational week at registration, weight gain from registration to GCT, proportions of ethnic Han Chinese, educational levels, parity, and habitual smoking and alcohol drinking were similar between the two groups.

3.2. Differences in individual bile acids between GDM and non-GDM

Nine bile acids, CA, CDCA, DCA, glycoursodeoxycholic acid (GUDCA), glycochenodeoxycholic acid (GCDCA), glycodeoxycholic acid (GDCA), taurochenodeoxycholic acid (TCDCA), glycocholic acid (GCA) and taurocholic acid (TCA) were detectable in ≥97% of the serum samples and were used in the analysis (Table 1) and others are listed in Appendix Table S3. Although CA, CDCA and TCA were similar in GDM and non-GDM, CA ≤ 0.155 nmol/mL, CDCA < 0.160 nmol/mL and TCA ≤ 0.1 nmol/mL were more frequent in GDM women than in non-GDM women. DCA, GUDCA, GCDCA, GDCA, TCDCA and GCA were lower in GDM than in non-GDM (Table 1).

Table 1
Clinical and biochemical characteristics of the study women.

Characteristic	Non-GDM	GDM	P value
No. subjects	243	243	
Variables at registration			
Age, year	29.2 ± 3.3	29.2 ± 2.7	1.000
Height, cm	163.2 ± 4.6	163.1 ± 5.0	0.280
Weight, kg	58.2 ± 9.6	63.7 ± 10.5	<0.001
BMI, kg/m^2	21.8 ± 3.6	23.9 ± 3.6	<0.001
BMI in category			<0.001
≥24.0- < 28.0 kg/m^2	45(18.5)	77(31.7)	
≥28.0 kg/m^2	12(4.9)	31(12.8)	
Gestational age, week	10.1 ± 2.0	10.1 ± 2.1	0.943
DBP, mmHg	67.9 ± 7.7	70.6 ± 8.0	<0.001
SBP, mmHg	104.0 ± 10.5	108.3 ± 10.5	<0.001
Han Nationality	234(96.3)	238(97.9)	0.285
Family history of diabetes in first-degree relatives	14(5.8)	30(12.4)	0.014
Education>12 years	132(54.3)	135(55.6)	0.780
Parity ≥1	12(4.9)	14(5.8)	0.683
Habitual smoker*	13(5.4)	15(6.2)	0.695
Alcohol drinker	57(23.5)	72(29.6)	0.742
Alanine aminotransferase, U/L	16.0 (10.7–21.0)	19.0 (14.0–26.0)	<0.001
Bile acid species			
CA, nmol/mL	0.10 (0.08–0.15)	0.10 (0.09–0.13)	0.146
≤ 0.155 nmol/mL	184(76.7)	196(84.5)	0.017
CDCA, nmol/mL	0.09 (0.05–0.21)	0.08 (0.04–0.13)	0.198
≤0.160 nmol/mL	159(65.4)	194(80.5)	<0.001
DCA, nmol/mL	0.26 (0.15–0.45)	0.20 (0.10–0.32)	0.002
<0.280 nmol/mL	129(53.1)	161(66.8)	0.003
GUDCA, nmol/mL	0.03 (0.02–0.06)	0.02 (0.01–0.03)	<0.001
≤0.070 nmol/mL	190(78.5)	220(95.65)	<0.001
GCDCA, nmol/mL	0.36 (0.17–0.71)	0.20 (0.12–0.39)	<0.001
≤0.800 nmol/mL	189(78.8)	232(95.5)	<0.001
GDCA, nmol/mL	0.12 (0.06–0.27)	0.08 (0.04–0.14)	<0.001
≤0.200 nmol/mL	168(69.1)	206(85.1)	<0.001
TCDCA, nmol/mL	0.10 (0.05–0.20)	0.06 (0.04–0.10)	<0.001
≤0.200 nmol/mL	183(75.3)	221(91.0)	<0.001
GCA, nmol/mL	0.08 (0.04–0.14)	0.05 (0.03–0.09)	0.010
≤0.160 nmol/mL	193(79.4)	222(91.4)	<0.001
TCA, nmol/mL	0.05 (0.04–0.09)	0.06 (0.05–0.08)	0.325
≤0.10 nmol/mL	190(79.5)	209(88.6)	0.005
Variables at GCT			
Habitual smoker during pregnancy	1(0.4)	2(0.8)	1.000
Alcohol drinker during pregnancy	3(1.2)	2(0.8)	1.000
GCT glucose, mmol/L	6.3(5.4–7.2)	9.0(8.4–10.0)	<0.001
Weight, kg	66.7 ± 9.7	71.9 ± 10.8	<0.001
Weight gain up to GCT, kg	8.7(3.2)	8.4(3.6)	0.1123

Data are presented as means ± SD or n (%).
Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure; GCT: glucose challenge test; CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; GUDCA, glycoursodeoxycholic acid; TCA, taurocholic acid.
* Defined as having continuously smoked one or more cigarette per day for at least 6 months before pregnancy.

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3.3. Associations between individual bile acids and the risk of GDM

CA was inversely associated with risk of GDM in a linear manner and TCA was associated with risk of GDM in an A-shaped manner. Both did not have a clear threshold for GDM. On the other hand, all the other bile acids were inversely associated with risk of GDM in a non-linear manner (Fig. 2 & Appendix Fig. S2). GUCDA had a clear threshold effect and decreasing levels of GUDCA at ≤0.07 nmol/mL were associated a rapid rise in the risk of GDM. Women with GUDCA < 0.07 nmol/mL were at markedly increased risk of GDM in univariable analysis (OR: 8.80, 95%CI: 1.24–62.64) and multivariable analysis (OR: 6.42, 95%CI: 1.31–31.37). Decreasing DCA was associated with increased risk of GDM with a threshold at 0.280 nmol/mL. The OR of DCA≤ versus >0.280 nmol/mL was significant in univariable (OR: 1.77, 95%CI: 1.07–2.92) and multivariable analysis (OR: 2.10, 95%CI: 1.10–3.99). In traditional risk factor model, the stepwise procedure identified that GUDCA ≤ 0.07 nmol/mL and DCA ≤ 0.280 nmol/mL were still predictive of GDM (OR: 6.84, 95%CI: 1.10–42.48 & 2.06, 1.26–3.37). Used as continuous variables, GUDCA decrease per nmol/L was associated with a 3% (95%CI: 0%–6%) increase in the risk of GDM and DCA decrease per $\log_{10}$ nmol/L was associated with 40% (17%–57%) increase in the risk of GDM (Table 2).

Using the same method to select cutoffs of these bile acids, CA ≤ 0.155 nmol/mL, CDCA ≤ 0.160 nmol/mL, GCDCA ≤ 0.800 nmol/mL, TCDCA ≤ 0.200 nmol/mL, GCA ≤ 0.160 nmol/mL and TCA ≤ 0.1 nmol/mL (versus their higher levels) were significantly associated with increased risk of GDM in univariable analysis and multivariable analysis (except for CA that was borderline significance). However, these bile acids apart from GUDCA and DCA were not selected into the model by stepwise procedures at $P < .05$ for entry and/or exit (Table 2).

3.4. Potential increase in predictive values of bile acids for GDM

The DCA and GUDCA model (AUC: 0.69, 95%CI: 0.64–0.73) and the traditional risk factor model (0.69, 0.64–0.74) had a similar AUC.

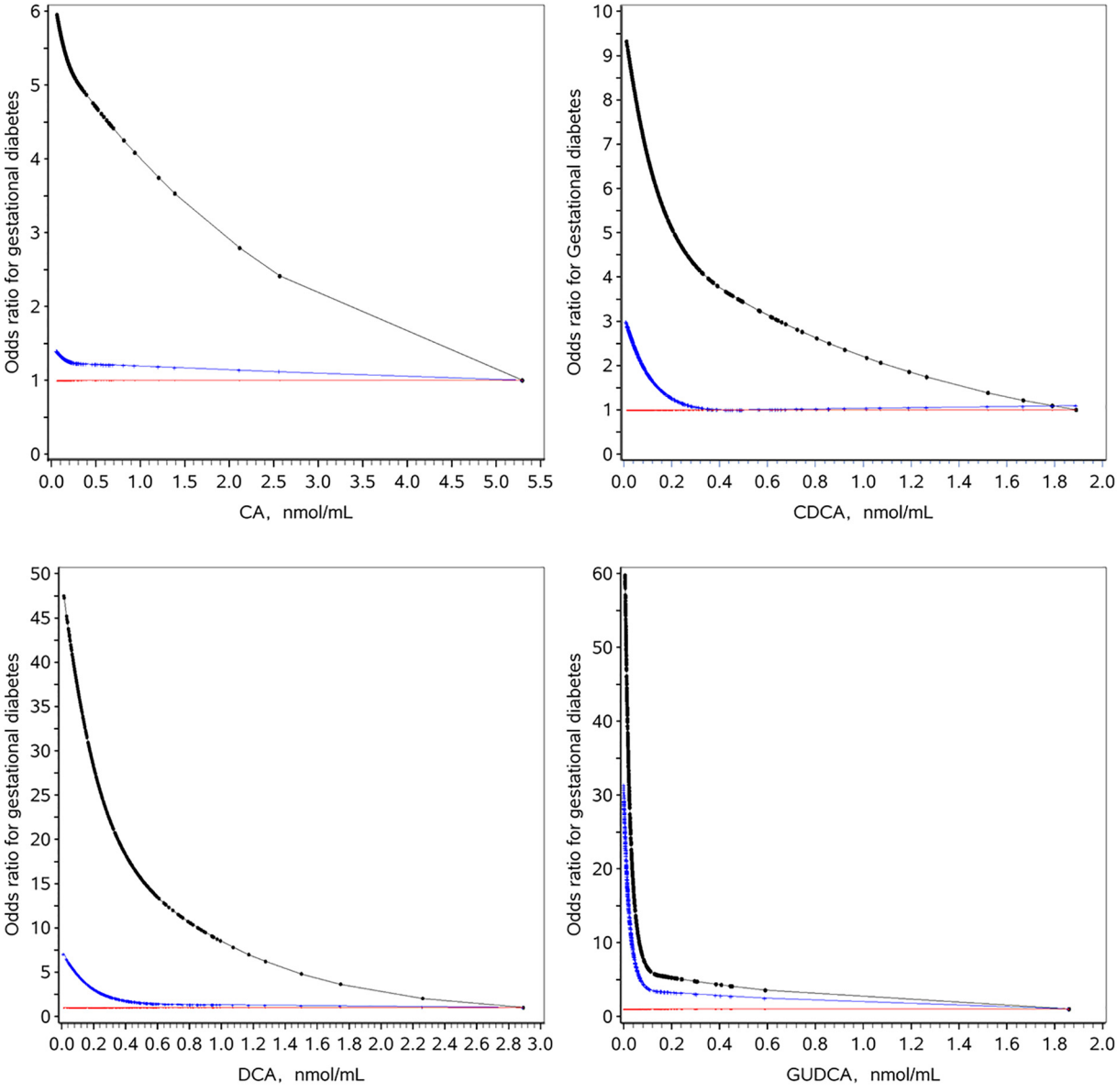

Fig. 2. Associations between individual bile acids and the risk of gestational diabetes mellitus (GDM) in Chinese women. Abbreviations: CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; GUDCA, glycoursodeoxycholic acid; OR, odds ratio. The upper (dotted, black) lines were derived from univariable analyses, the middle (crossed, blue) lines were derived from multivariable analyses (See Table 2, multivariable model 1 for the list of adjusted variables) and the bottom (red, straight) lines were the reference line at OR = 1. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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Table 2
Odds ratios of individual bile acid species for the risk of GDM in Chinese women.

	OR (95% CI)[a]	*P*-value[a]
Univariable Model 1		
CA≤ vs. > 0.155 nmol/mL	1.86 (1.11–3.13)	0.019
CDCA≤ vs. > 0.160 nmol/mL	2.17 (1.10–4.28)	0.002
DCA≤ vs. >0.280 nmol/mL	1.77 (1.07–2.92)	0.008
GUDCA≤ vs. >0.070 nmol/mL	8.80 (1.24–62.64)	<0.001
GCDCA≤ vs. >0.800 nmol/mL	6.38 (1.18–34.49)	<0.001
GDCA≤ vs. >0.200 nmol/mL	2.46 (1.11–5.47)	0.001
TCDCA≤ vs. >0.200 nmol/mL	3.24 (1.14–9.22)	<0.001
GCA≤ vs. >0.160 nmol/mL	3.07 (1.16–8.16)	0.002
TCA≤ vs. >0.1 nmol/mL	2.16 (1.07–4.36)	0.011
Multivariable Model 1		
CA≤ vs. >0.155 nmol/mL	1.81 (0.96–3.42)	0.068
CDCA≤ vs. >0. 160 nmol/mL	2.30 (1.10–4.81)	0.006
DCA≤ vs. >0.280 nmol/mL	2.10 (1.10–3.99)	0.005
GUDCA≤ vs. >0.070 nmol/mL	6.42 (1.31–31.37)	0.002
GCDCA≤ vs. >0.800 nmol/mL	5.25 (1.26–21.81)	0.001
GDCA≤ vs. >0.200 nmol/mL	2.52 (1.15–5.55)	0.005
TCDCA≤ vs. >0.200 nmol/mL	3.32 (1.15–9.56)	0.001
GCA≤ vs. >0.160 nmol/mL	4.17 (1.25–13.97)	0.001
TCA≤ vs. >0.1 nmol/mL	2.65 (1.08–6.54)	0.008
Multivariable Model 2		
DCA≤ vs. >0.280 nmol/mL	2.06 (1.26–3.37)	0.004
GUDCA≤ vs. >0.070 nmol/mL	6.84 (1.10–42.48)	<0.001
Multivariable Model 3		
Log_{10}DCA, nmol/L	0.60 (0.43–0.83)	0.002
GUDCA (coded to 70 nmol/L if GUDCA ≥70 nmol/L), nmol/L	0.97 (0.94–1.00)	<0.001

Abbreviations: GDM, gestational diabetes mellitus; OR, odds ratio; CI: confidence interval; CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; GUDCA, glycoursodeoxycholic acid; GCDCA, Glycochenodeoxycholic acid; GDCA, Glycodeoxycholic acid; TCDCA, Taurochenodeoxycholic acid; GCA, Glycocholic acid; TCA, Taurocholic acid.
Multivariable Model 1, adjusted for body mass index at registration, family history of diabetes in first-degree relatives, systolic blood pressure and diastolic blood pressure at registration, habitual smoking and drinking before and during pregnancy as well as gestational weeks at registration, parity (≥1) and education attainment (>12 years of school education), Han nationality, alanine aminotransferase at the first antenatal care visit and weight gain up to the time of glucose challenge test.
Multivariable Model 2, stepwise (forward) regression was performed to select bile acids with enter of the traditional risk factors listed in multivariable model 1 ($P < .05$ for entry and exit).
Multivariable Model 3, adjusted for the variables listed in multivariable model 1 and the two bile acid species listed in multivariable model 2.
[a] Adjusted for multiple comparison using Ryan-Holm step-down Bonferroni procedure.

Inclusion of GUDCA and DCA in the traditional risk factor model significantly increased the AUC to 0.76 (95%CI: 0.71–0.80) ($P < .0001$) (Fig. 3).

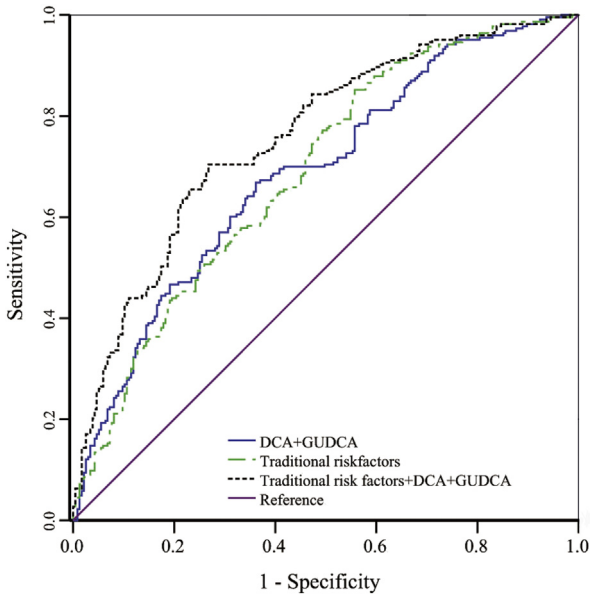

Fig. 3. Receiver operating characteristic curves of traditional risk factors, bile acids and traditional risk factors plus bile acids for gestational diabetes mellitus in Chinese women. Abbreviations: DCA, deoxycholic acid; GUDCA, glycoursodeoxycholic acid; ROC: receiver operating characteristic curve. Legends: The blue (solid) curve stands for the DCA and GUDCA model; the green (dash-dot) curve for the traditional risk factor model (Multivariable Model 1 in Table 2 for the list of variables), the black (dashed) curve for the traditional risk factor plus DCA and GUDCA model. The area under the operating characteristic curve (AUC) was 0.69 (95% CI: 0.64–0.73) for the DCA and GUDCA model, 0.69 (95% CI: 0.64–0.74) for the traditional risk factors model and 0.76 (95% CI: 0.71–0.80) for the traditional risk factor plus DCA and GUDCA model ($P < .0001$ for comparison of the traditional risk factor plus DCA and GUDCA model with either of the other two models). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

Our study has generated intricate findings regarding the associations between individual bile acids and the risk of GDM. GUDCA and DCA had threshold effects for GDM and low GUDCA levels at ≤0.070 nmol/mL and low DCA levels at ≤0.280 nmol/mL were associated with markedly increased risks of GDM. Both associations were independent of traditional risk factors and the other bile acid.

Basic science research has unveiled important roles of bile acids in the regulation of glucose, energy metabolism, inflammation and various cellular processes [8]. Some small studies have also found associations of individual bile acids with T2DM. A study of 15 T2DM patients and 15 healthy controls showed that T2DM patients had elevated postprandial levels of DCA and UDCA as compared with non-T2DM controls [29]. Two small studies did not find significant differences in the two bile acid species between GDM women and controls in the 28th gestational week [13, 14]. Similarly, Hou et al. [15] performed a nested case-control study of 131 women with GDM and 138 controls in early pregnancy to examine global metabolic changes in GDM. The study did not detect significant changes in DCA, UDCA and GUDCA but did observe that primary bile acid (CA) was down-regulated, and some other secondary bile acids such as LCA and HDCA were up-regulated in GDM as compared with non-GDM in univariable analysis. Our study findings provided further evidence that low secondary bile acids, in particular, low DCA and GUDCA, were independently associated with the increased risk of GDM.

In the past 20 years, bariatric surgery has proven to be the most effective method to delay or prevent incident T2DM, which can rapidly normalize blood glucose levels in obese people with T2DM far before body weight loss [30]. A recent study [31] documented that longitudinal changes of 17 bile acid species in 21 morbidly obese patients before and after Roux-en-Y gastric bypass and found that fasting total plasma bile acids after the operation increased in a bimodal manner at 1 and 24 months. Sharp increases in UDCA, GUDCA (the glycine conjugates of UDCA) and TUDCA (the taurine conjugates of UDCA) were responsible for the one-month surge in the total bile acids while gradual rises in unconjugated bile acids, such as DCA and its glycine conjugate, were

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responsible for the 12-month rise in the total bile acids. The rise in UDCA and DCA was respectively associated with improved 1-month and 12-month hepatic sensitivity. In this connection, our study found that GUDCA ≤ 0.07 nmol/mL and DCA ≤ 0.280 nmol/mL were independently associated with greatly elevated risks of GDM in Chinese women in early pregnancy.

Normal pregnancy is characterized by an increase in serum bile acid levels with increasing gestational age [32]. In some cases, serum bile acid levels rise sharply, leading to intrahepatic cholestasis of pregnancy (ICP) [33], which was associated with elevated risk of GDM [34, 35]. Ursodeoxycholic acid (UDCA) is effective and safe in the treatment of ICP, with significantly modulated levels of total bile acids (TBA), alanine aminotransferase, aspartate aminotransferase and total bilirubin and without adverse drug reactions [36]. On the other hand, UDCA treatment for morbid obesity was shown to lead to decreased intestinal expression of FGF19 and farnesoid X receptor (FXR) activation which resulted in increased bile acid synthesis and cholesterol uptake from blood [37], thus enhancing hepatic and muscle insulin sensitivity [38]. Besides, experimental data showed that UDCA had antioxidative properties of preventing pancreatic damage [12].

Primary bile acids, i.e., CA and CDCA are secreted into duodenum after meals and further transformed into secondary bile acids by gut microbiota [8]. In the large intestine, bacterial 7-dehydroxylase converts CA to DCA, and CDCA to lithocholic acid (LCA) and 7-epimerases convert CDCA to the secondary bile acids, including UDCA [8, 39]. As humans lack the 7-epimerase [39], UDCA may only stem from gut microbiome in humans. A randomized placebo-controlled trial demonstrated that enrichment of gut microbiota (i.e., lactobacillus reuteri) resulted in increased secretion of glucagon-like peptides-1 and -2, and higher insulin levels, but did not alter peripheral and hepatic insulin resistance [40]. Our findings support the notion that abnormal gut microbiome may be associated with increased risk of GDM via decreased conversion of primary bile acids to secondary bile acids, in particular, GUDCA and DCA, which may play a causal role in the etiology of GDM.

Our findings have important potential public health implications. The etiology and pathogenesis of GDM are still unclear. Lifestyle modifications even in early pregnancy can only result in a small reduction in GDM risk [3]. Given the close association between gut microbiota and secondary bile acids, it is warranted to conduct randomized controlled trials to test the effect of interventions that change gut microbiota for prevention of GDM in early pregnancy. In addition, our study shows that inclusion of GUDCA and DCA in the prediction model consisting of traditional GDM risk factors significantly increased the predictive accuracy of traditional risk factors. So GUDCA and DCA may be useful risk markers for GDM.

Our study has several strengths. First, our study was a case-control study nested in a population-based prospective cohort of pregnant women, and thus had a good representativeness although some variables were slightly different from the entire cohort. Second, traditional risk factors for GDM were carefully collected in our cohort and were available to the current analysis and thus, unadjusted bias was minimal. Our study also had limitations. First, some lifestyle factors such as dietary habits were not collected due to busy clinical setting. Second, we used a two-step procedure to identify incident GDM and some GDM cases might have been missed. Misclassification of GDM as non-GDM is more likely to lead to underestimation of the effect size. Third, we did not exclude women with liver or bile duct diseases in our analysis. Nevertheless, only 10 had positive hepatitis B surface antigen and ALT was measured and adjusted in the multivariable analysis. The very small changes in the ORs of bile acids for GDM after the adjustment suggest that potential confounding effects by liver or bile duct diseases if any were small (data not shown). Fourth, the AUC was not derived from a representative cohort and further replication studies are needed in representative cohorts in our and other populations.

In conclusion, using a matched case-control study nested in a prospective study of pregnant women, we found that GUDCA and DCA were inversely associated with increased risk of GDM. GUDCA and DCA had threshold effects; GUDCA levels ≤ 0.07 nmol/mL and DCA ≤ 0.028 nmol/mL were associated with markedly elevated risks of GDM. Given the close link between gut microbiota and secondary bile acids, randomized controlled trials are warranted to test whether methods to change gut microbiota can prevent GDM.

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Declaration of interests

The authors declared no conflict of interest.

Contribution statements

X.Y. & Z.F. conceived the idea and designed the study. P.S., J.L. & C.Z. collected the data; Y.C., S.L., Z.D., & X.S., conducted the measurement of bile acids; J.L. & X.H. analyzed the data; X.Y., J.L. & X.H. wrote the first draft; R.M. gave critical comments and edited the manuscript; All authors gave comments and contributed to the writing of the manuscript. All authors agreed to submit and publish the manuscript. X.Y. & Z.F. (the corresponding authors) and J.L. (the first author) take full responsibility for the work as a whole, including the study design, access to the data, and decision to submit.

Details of ethics approval

Ethics approval was obtained from the Ethics Committee for Clinical Research of Tianjin Women and Children's Health Centre on 1 December 2009 (ref. no. 2009-02).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ebiom.2018.08.015.

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