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Circulating Lysophosphatidylcholines in Early Pregnancy and Risk of Gestational Diabetes in Chinese Women

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Objectives: This study aimed to explore associations of lysophosphatidylcholines (LPCs) in early pregnancy with gestational diabetes mellitus (GDM), and whether LPCs mediated the associations of bile acids with GDM risk or had interactive effects with bile acids on GDM risk.

Design: We conducted a 1:1 nested case-control study (n = 486) from a large prospective pregnant women cohort in urban Tianjin, China. Blood samples were collected at their first antenatal care visit (median at 10th gestational week). LPCs were measured by liquid chromatography-tandem mass spectrometry analysis. Conditional binary logistic regression and restricted cubic spline analysis were used to identify cutoff points of these metabolites for GDM risk.

Results: Of the 6 detectable LPCs, LPC14:0 less than 0.24 nmol/mL, LPC15:0 at 0.45 nmol/mL or greater, and LPC18:0 at 18.00 nmol/mL or greater were independently associated with GDM risk. Adjustment for LPC18:0 slightly attenuated odds ratios (ORs) of deoxycholic acid (DCA, ≤ 0.36 nmol/mL) and glycoursodeoxycholic acid (GUDCA, ≤ 0.07 nmol/mL) for GDM, and the correlations of DCA and GUDCA with LPC18:0 were weak. However, the presence of DCA at 0.36 nmol/mL or less greatly amplified the adjusted OR of LPC18:0 at 18.00 nmol/mL or greater alone for GDM from 8.18 (2.51-26.7) up to 17.7 (6.64-47.1), with significant additive interaction. Similarly, the presence of GUDCA at 0.07 nmol/mL or less also greatly amplified the adjusted OR of LPC18:0 at 18.00 nmol/mL or greater alone for GDM from 17.2 (1.77-168) up to 73.8 (12.7-429), with significant additive interaction.

Conclusions: LPCs in early pregnancy were associated with GDM risk. Low DCA or GUDCA greatly amplified the effect of high LPC18:0 on GDM, and its molecular mechanism is worth further investigations. (***J Clin Endocrinol Metab*** **105: e982–e993, 2020)**

Key Words: bile acids, Chinese women, gestational diabetes mellitus, lysophosphatidylcholines, metabolism

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Abbreviations: AP, attributable proportion due to the interaction; AUC, area under the curve; BMI, body mass index; DBP, diastolic blood pressure; DCA, deoxycholic acid; FFA, free fatty acid; FXR, farnesoid X receptor; GCT, glucose challenge test; GDM, gestational diabetes mellitus; GUDCA, glycoursodeoxycholic acid; IL, interleukin; LPC, lysophosphatidylcholine; OGTT, oral glucose tolerance test; OR, odds ratio; PG, plasma glucose; ROC, receiver operating characteristic; SBP, systolic blood pressure.

The International Diabetes Federation has estimated that about 20.4 million women had hyperglycemia during pregnancy in 2019, with 83.6% of cases being gestational diabetes mellitus (GDM) (1). GDM increases risks of both short-term and long-term health outcomes in mothers and their offspring (2, 3). Lifestyle intervention can reduce GDM risk by 20% if initiated within 15 gestational weeks, but are generally ineffective if initiated after the 15th gestational week (4). Hence, it is critically important to explore novel biomarkers of GDM in early pregnancy for a better understanding of the etiology of GDM, as well as identifying at-risk women for effective intervention.

Lysophosphatidylcholines (LPCs), a class of lipid biomolecules, play important roles in several physiological processes, such as vascular development, reproduction, and myelination (5). Besides the function of membrane phospholipid metabolites, their role as intracellular signal molecules is increasingly appreciated, including regulating cellular proliferation, inflammation, and tumor cell invasion (6, 7). Increasing evidence suggests that abnormal LPC levels are associated with many diseases, including diabetes, cardiovascular diseases, and cancers (8-10). However, findings from those lipidomics studies were inconsistent. For example, a study found that plasma LPCs were positively associated with the risk of diabetes (11), but another study showed that plasma LPCs were inversely associated with diabetes risk (12). Our group reported that decreased bile acids, i.e., deoxycholic acid (DCA) and glycoursodeoxycholic acid (GUDCA), were independently associated with the risk of GDM (13). In this connection, several metabolomics studies found that abnormal bile acid metabolism was associated with phospholipid disorders and both of them can be used as biomarkers of diabetes (14, 15). An animal study further showed that increased bile acids might improve high-fat diet–induced diabetes by reducing LPC, phosphatidycholine, sphingomyeline, and ceramide levels (16). Therefore, LPCs are more likely to lie downstream of bile acids. However, it remains unknown how bile acids and LPCs work together to increase the risk of GDM. There are two possibilities about the working patterns of DCA/GUDCA and LPCs for GDM. One is that low DCA/GUDCA increases GDM risk via increasing levels of LPCs. The other is that low DCA/GUDCA enhances the effect of LPCs on GDM, that is, there is an interactive effect of DCA/GUDCA and LPCs on GDM risk.

Using a nested case-control study within a prospective population-based cohort of pregnant women in Tianjin, China, we aimed to explore 1) the associations between LPCs in early pregnancy and the risk of GDM; 2) whether LPCs mediated the association of low DCA/GUDCA with GDM risk or had an interactive effect with low DCA/GUDCA on the risk of GDM; and 3) the predictive values of LPCs for GDM.

Materials and Methods

Study design and participants

The study design, participants, and methods of this prospective cohort have been described in detail previously (17). Briefly, 22 302 pregnant women were recruited through the universal GDM screening and management system from the 6 central urban districts of Tianjin, China, between October 2010 and August 2012. This study was approved by the ethics committee of Tianjin Women and Children's Health Center (TWCHC) and written informed consent was obtained from all participants before data collection.

A two-step procedure was used to identify GDM among pregnant women. First, pregnant women at the 24th to 28th weeks of pregnancy were offered a 50-g, 1-hour glucose challenge test (GCT) in a nonfasting status at a primary care hospital close to their residence. Then, pregnant women with GCT greater than or equal to 7.8 mmol/L were referred to TWCHC for a 75-g, 2-hour oral glucose tolerance test (OGTT) after at least an 8-hour fasting status. GDM was diagnosed by the International Association of Diabetes and Pregnancy Study Group's criteria: meeting fasting plasma glucose (PG) 5.1 mmol/L or greater, 1-hour PG 10.0 mmol/L or greater, or 2-hour PG 8.5 mmol/L or greater (18).

The selection procedure of the nested case-control study has been published in a previous study (13). In brief, 2991 women were enrolled and provided overnight fasting venous blood samples at their first antenatal care visit (median [interquartile range]: 10 [9-11] gestational weeks). Among them, we excluded 227 women without GCT results or OGTT results if their GCT was 7.8 mmol/L or greater at the 24th to 28th gestational weeks. In the remaining 2764 women, 243 women who developed GDM during pregnancy were selected as the cases and 243 women without GDM were selected as the controls matched by maternal age (±1 year). The 1:1 nested case-control study (n = 486) was used to address our research questions in the current study.

Data collection procedures

All nurses or obstetricians were trained in a series of workshops before the fieldwork to standardize all the procedures. Data were collected using a series of questionnaires or retrieved from the medical records at the first antenatal care visit and GCT time till postpartum. Information on maternal characteristics included maternal age, height, weight, gestational weeks, systolic blood pressure (SBP), diastolic blood pressure (DBP), nationality, education attainment, parity, personal or family history of diabetes, current smoker before or during pregnancy, alcohol drinker before or during pregnancy, etc. Prepregnancy body mass index (BMI) was calculated as weight in kilograms at the first antenatal care visit divided by the squared height in meters and categorized as underweight (< 18.5 kg/m^2), normal weight (≥ 18.5 to < 24 kg/m^2), overweight (≥ 24 to < 28 kg/m^2) and obesity (≥ 28 kg/m^2) according to the criteria of the Working Group on Obesity in China (19). Weight gain to GCT was calculated as the difference of body

weight divided by the difference of gestational weeks between the first registration and GCT time.

Measurement of plasma lysophosphatidylcholines

Sample pretreatment. Each blood sample was separated from the venous blood immediately and stored at –80°C until used. Stored plasma was thawed at 4°C. Each sample (10 μL) was mixed with an internal standard solution (10 μL), 0.9% NaCl (10 μL), and chloroform:methanol (2:1) (100 μL). The mixture was vortexed for 20 seconds, and then stood at 4°C for 30 minutes. After being centrifuged at 7800 *g* for 3 minutes, 20 μL of the supernatant was transferred and concentrated to dry under nitrogen. Before being injected for liquid chromatography–tandem mass spectrometry analysis, the dried supernatant was dissolved with acetonitrile:isopropanol (1:1) (20 μL) and vortexed for 60 seconds.

Liquid chromatography–tandem mass spectrometry analysis. The LPC components were identified and quantified using Eksigent Ultra liquid chromatography 100 coupled with a Triple TOF 5600 system (AB SCIEX), and separated using a 2.1 × 100mm XBridge Peptide BEH C18 column (Waters) with a 4 × 2.0mm guard column (Phenomenex). The separation of LPCs was achieved under a column temperature of 40°C using ammonium acetate (10 mM), formic acid (0.1%), and water (99.9%) as mobile phase A, and ammonium acetate (10 mM), formic acid (0.1%), acetonitrile (49.95%) and isopropanol (49.95%) as mobile phase B. The step gradient was as follows: 0.01 min, 35% (v/v) B; 0.01 to 2 minutes, 35% to 80% (v/v) B; 2 to 9 minutes, 80% to 100% (v/v) B; 9 to 15 minutes, 100% (v/v) B; 15 to 16 minutes, 100% to 35% (v/v) B; 16 to 20 minutes, 35% (v/v) B. The injection volume was 2 μL and the total run time was 20 minutes at a flow rate of 0.4 mL/min. The parameters, under a negative model, were set as follows: curtain gas, ion source gas 1, and ion source gas 2 at 30, 50, and 50 psi, respectively; source temperature at 550°C and ion spray voltage floating at –4500 V. In automatic information-dependent acquisition, the m/z range for time of flight mass spectrometry scan and production of ion scan were 100 to 1200 Da and 50 to 1200 Da, respectively. The collision energy of the production ion scan was set at –35 ± 15 V and the declustering potential was set at –80 V.

Data processing. The Peak View 1.2 was used to identify LPCs, and Multi Quant 2.1 was used to quantity LPCs based on the m/z value and sample retention time.

Measurement of plasma bile acids

The detailed measurement method of bile acids has been described previously (13). Briefly, Eksigent ultral liquid chromatography 100 coupled with a Triple TOF 5600 system (AB SCIEX), and a 2.1 × 100mm XBridge Peptide BEH C18 column (Waters) with a 4 × 2.0mm guard column (Phenomenex) were used to quantify plasma bile acids.

Statistical analysis

All statistical analyses were performed using Statistical Analysis System Release 9.4 (SAS Institute Inc, Cary, NC, USA), unless specified. A two-tailed *P* value less than .05 was considered to be statistically significant. A Q-Q plot was used to test the normal distribution of continuous variables. Differences between the GDM and non-GDM group were compared using a paired Student t test for the continuous variables with normal distribution, Wilcoxon signed-rank test for the continuous variables without normal distribution, and McNemar test or Fisher exact test for categorical variables.

Restricted cubic spline analysis nested in conditional binary logistic regression was used to examine the full-range associations of LPCs with the risk of GDM in univariate and multivariable analyses. Continuous LPCs were stratified into categorical variables at the points where GDM risk started to change steeply. A structured adjustment plan was implemented to control for potential confounding factors. First, we performed conditional logistic regression to obtain the unadjusted odds ratio (OR) and 95% CI. Second, we adjusted for traditional risk factors including prepregnancy BMI, SBP, nationality, family history of diabetes in first-degree relatives, parity, education attainment, current smoker and alcohol drinker before pregnancy, and weight gain from registration to GCT. Finally, we performed a stepwise forward selection in the conditional logistic regression to identify LPC species that had effects on GDM risk independent of these traditional risk factors ($P < .05$ for entry and exit).

In addition, the mediating effect of LPCs on the association of low DCA/GUDCA on GDM risk was tested by the change in ORs of DCA/GUDCA for GDM before and after adjustment for particular LPC species. The separating point of DCA/GUDCA before and after the adjustments was used to recategorize DCA/GUDCA as we did before (20). Partial Spearman correlation analysis was used to test correlations between bile acids and LPCs while adjusting for maternal age and prepregnancy BMI. Additive interaction was used to test interactive effects between low DCA/GUDCA and high LPC species at the selected cutoff points for GDM. Significant relative excess risk due to interaction greater than 0, attributable proportion due to interaction (AP) greater than 0 or synergy index greater than 1 suggested an additive interaction (21). Finally, a receiver operating characteristic (ROC) curve was used to check whether the inclusion of LPCs was able to increase the predictive value for GDM, with inclusion of traditional risk factors only and traditional risk factors plus DCA and GUDCA.

Results

Characteristics of the study participants

Clinical and biomedical characteristics of the participating pregnant women are shown in Table 1. The mean age of participants was 29.2 years (SD, 3.1 years) and the median gestational age at first registration was 10 weeks (interquartile range, 9-11 weeks). There were no significant differences in body height, gestational age at registration, nationality, education attainment, parity, current smoker and alcohol drinker before or during pregnancy, and weight gain from registration to GCT between GDM and non-GDM groups. However,

Table 1. Clinical and biochemical characteristics of GDM and non-GDM women

Characteristic	Non-GDM (N = 243)	GDM (N = 243)	*P*
Variables at registration			
Age, y	29.2 ± 3.3	29.2 ± 2.7	–
Height, cm	163.2 ± 4.6	163.1 ± 5.0	.28[a]
Weight, kg	58.2 ± 9.6	63.7 ± 10.5	< .001[a]
BMI, kg/m^2	21.8 ± 3.4	23.9 ± 3.6	< .001[a]
BMI in category			< .001[b]
≥ 24.0 to < 28.0	45 (18.5)	77 (31.7)	
≥ 28.0	12 (4.9)	31 (12.8)	
Gestational age, wks	10.1 ± 2.0	10.1 ± 2.1	.94[a]
Systolic blood pressure, mm Hg	104.0 ± 10.5	108.3 ± 10.5	< .001[a]
Diastolic blood pressure, mm Hg	67.9 ± 7.7	70.6 ± 8.0	< .001[a]
Han nationality	234 (96.3)	238 (98.0)	.29[b]
Education > 12 y	132 (54.3)	135 (55.6)	.78[b]
Parity ≥ 1	12 (4.9)	14 (5.8)	.68[b]
Family history of diabetes in first-degree relatives	14 (5.8)	30 (12.4)	.01[b]
Current smoker before pregnancy	9 (3.7)	10 (4.1)	.81[b]
Alcohol drinker before pregnancy	57 (23.5)	72 (29.6)	.12[b]
LPC species			
LPC egg, nmol/mL	76.77 (62.41-97.32)	125.80 (97.55-157.59)	< .001[a]
≥ 95.00	63 (25.93)	187 (76.95)	< .001[b]
LPC14:0, nmol/mL	0.21 (0.17-0.28)	0.20 (0.16-0.30)	.85[a]
< 0.24	84 (34.57)	59 (24.28)	.05[b]
≥ 0.24 to < 0.42	143 (58.85)	157 (64.61)	
≥ 0.42	16 (6.58)	27 (11.11)	
LPC15:0, nmol/mL	0.32 (0.28-0.39)	0.37 (0.30-0.48)	< .001[a]
≥ 0.45	30 (12.35)	69 (28.40)	< .001[b]
LPC17:0, nmol/mL	0.70 (0.52-0.93)	1.03 (0.79-1.31)	< .001[a]
≥ 0.80	89 (36.63)	178 (73.25)	< .001[b]
LPC18:0, nmol/mL	13.06 (10.76-17.19)	22.77 (19.02-28.93)	< .001[a]
≥ 18.00	52 (21.40)	199 (81.89)	< .001[b]
LPC18:1, nmol/mL	4.19 (3.50-5.29)	5.84 (4.89-7.19)	< .001[a]
≥ 4.50	101 (41.56)	202 (83.13)	< .001[b]
Variables during pregnancy			
Current smoker during pregnancy	1 (0.4)	2 (0.8)	1.00[b]
Alcohol drinker during pregnancy	2 (0.8)	2 (0.8)	1.00[b]
Weight gain to GCT, kg/wk	0.6 ± 0.2	0.6 ± 0.2	.16[a]
GCT glucose, mmol/L	6.3 (5.4-7.2)	9.0 (8.4-10.0)	< .001[a]

Data are reported in mean ± SD or number (percentages) or median (25th-75th percentile).
Abbreviations: BMI, body mass index; GCT, glucose challenge test; GDM, gestational diabetes mellitus; LPC, lysophosphatidylcholine.
[a]Derived from paired t test or Wilcoxon signed-rank test.
[b]Derived from McNemar test or Fisher exact test.

women with GDM had higher BMI, SBP/DBP, and GCT glucose levels. The percentage of women with a family history of diabetes in a first-degree relative was also higher in the GDM group than in the non-GDM group. The levels of LPC egg, LPC15:0, LPC17:0, LPC18:0, and LPC18:1 were higher in the GDM group compared to the non-GDM group, whereas LPC14:0 was similar in both groups.

Associations of lysophosphatidylcholines with gestational diabetes mellitus

LPC egg, LPC15:0, LPC17:0, LPC18:0, and LPC18:1 were positively associated with GDM risk in a nonlinear manner with clear threshold effects (Fig. 1). LPC egg at 95.00 nmol/mL or greater, LPC15:0 at 0.45 nmol/mL or greater, LPC17:0 at 0.80 nmol/mL or greater, LPC18:0 at 18.00 nmol/mL or greater, and LPC18:1 at 4.50 nmol/mL or greater were associated with increased risk of GDM in univariate and multivariable analyses (Table 2). LPC14:0 was associated with GDM risk in a U-shaped manner. Using 0.24 to 0.42 nmol/mL as the reference, LPC14:0 less than 0.24 nmol/mL was inversely associated with GDM risk in univariate and multivariable analyses, but LPC14:0 at 0.42 nmol/mL or greater for GDM was no longer significant in multivariable analysis. The stepwise selection procedure identified that LPC14:0 less than 0.24 nmol/mL, LPC15:0 at 0.45 nmol/mL or greater, and LPC18:0 at 18.00 nmol/mL or greater were significantly associated with increased risk of GDM, independent of traditional risk factors (OR of LPC14:0: 3.85, 95% CI: 1.50-9.84; LPC15:0: 3.55, 1.15-10.9; and LPC18:0: 18.8, 7.88-44.7).

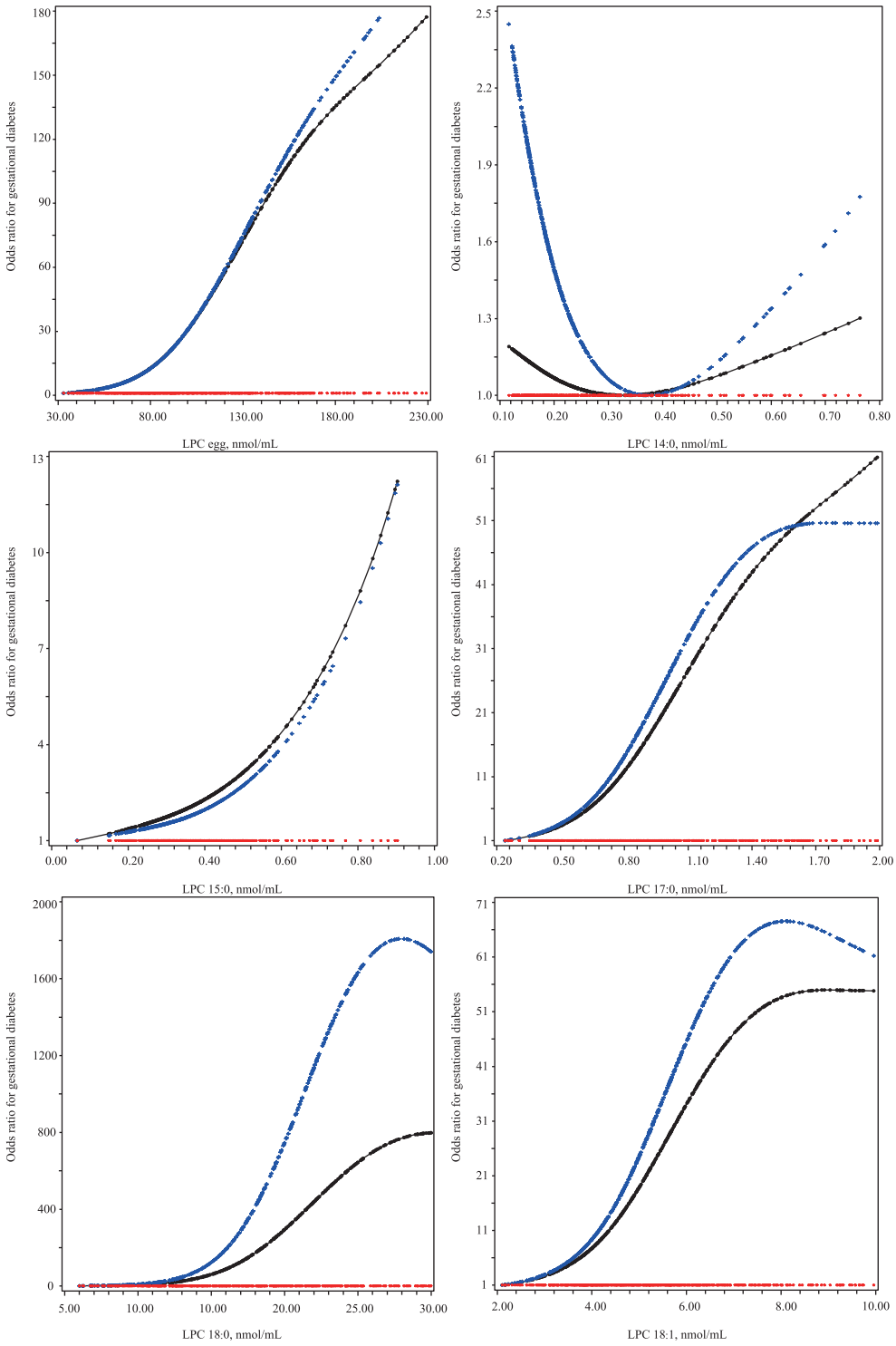

Figure 1. The association of individual lysophosphatidylcholines (LPCs) with gestational diabetes risk. The dotted (black, bottom) lines were derived from univariate analyses, the crossed (blue, upper) lines were derived from multivariable analyses with adjustment for traditional risk factors, included prepregnancy body mass index, systolic blood pressure, Han-nationality, family history of diabetes in first-degree relatives, parity, education attainment, current smoker before pregnancy, alcohol drinker before pregnancy, and weight gain to glucose challenge test, and the straight (red) lines are the reference line at odds ratio equal to 1.

Mediating effects of lysophosphatidylcholines on associations between bile acids and gestational diabetes mellitus risk

Adjustment for LPC18:0 slightly attenuated the effects of low DCA (≤ 0.36 nmol/mL) and low GUDCA (≤ 0.07 nmol/mL) on GDM risk (Table 3 and Fig. 2). Using the two cutoff points, the OR of DCA less than or equal to vs greater than 0.36 nmol/mL for GDM was reduced from 2.09 (1.27-3.46) before adjustment for LPC18:0 to 1.29 (0.62-2.67) after the adjustment.

Table 2. Odds ratios of LPCs for risk of GDM

	Unadjusted		Adjusted[a]	
	OR (95% CI)	*P*	OR (95% CI)	*P*
Step 1[b]				
LPC egg ≥ vs < 95.00, nmol/mL	7.53 (4.66-12.2)	< .001	8.55 (4.75-15.4)	< .001
LPC14:0, nmol/mL				
< 0.24	1.53 (1.03-2.29)	.04	1.93 (1.16-3.23)	.01
≥ 0.24 to < 0.42	(Reference)		(Reference)	
≥ 0.42	2.53 (1.19-5.35)	.02	2.37 (1.00-5.66)	.05
LPC15:0 ≥vs <0.45, nmol/mL	3.05 (1.82-5.13)	< .001	3.40 (1.87-6.21)	< .001
LPC17:0 ≥ vs < 0.80, nmol/mL	5.68 (3.49-9.26)	< .001	6.15 (3.43-11.0)	< .001
LPC18:0 ≥ vs < 18.00, nmol/mL	15.7 (8.29-29.8)	< .001	16.4 (7.89-34.0)	< .001
LPC18:1 ≥ vs < 4.50, nmol/mL	6.61 (4.03-10.9)	< .001	7.84 (4.28-14.4)	< .001
Step 2[c]				
LPC14:0, nmol/mL				
< 0.24	1.96 (1.00-3.82)	.05	3.85 (1.50-9.84)	.005
≥ 0.24 to < 0.42	(Reference)		(Reference)	
≥ 0.42	0.58 (0.18-1.85)	.36	0.27 (0.06-1.14)	.07
LPC15:0 ≥ vs < 0.45, nmol/mL	2.29 (0.98-5.32)	.06	3.55 (1.15-10.9)	.03
LPC18:0 ≥ vs < 18.00, nmol/mL	15.0 (7.65-29.5)	< .001	18.8 (7.88-44.7)	< .001

Abbreviations: GDM, gestational diabetes mellitus; LPC, lysophosphatidylcholine; OR, odds ratio.
[a]Traditional risk factors adjusted in the multivariable analysis included prepregnancy body mass index, systolic blood pressure, Han nationality, family history of diabetes in first-degree relatives, parity, education attainment, current smoker before pregnancy, alcohol drinker before pregnancy, weight gain to glucose challenge test, in addition to the variables listed in the model.
[b]Univariate and multivariable odds ratios of individual LPC for the risk of GDM.
[c]Stepwise regression was performed to identify LPC species that had effects on GDM risk independent of these traditional risk factors (*P* < .05 for entry and exit).

Table 3. Odds ratios of low DCA and GUDCA for risk of GDM mediated by LPCs

	OR	95% CI	*P*
Model 1			
DCA ≤ vs > 0.36, nmol/mL	2.09	1.27-3.46	.004
GUDCA ≤ vs > 0.07, nmol/mL	6.32	2.34-17.1	< .001
Model 2			
DCA ≤ vs > 0.36, nmol/mL	2.07	1.24-3.46	.005
GUDCA ≤ vs > 0.07, nmol/mL	7.63	2.68-21.7	< .001
Model 3			
DCA≤ vs > 0.36, nmol/mL	1.89	1.13-3.18	.02
GUDCA ≤ vs > 0.07, nmol/mL	5.31	1.92-14.7	.001
Model 4			
DCA ≤ vs > 0.36, nmol/mL	1.29	0.62-2.67	.50
GUDCA ≤ vs > 0.07, nmol/mL	4.53	1.26-16.3	.02

Model 1 was adjusted for traditional risk factors.
Model 2 was adjusted for variables in model 1 and level of LPC14:0.
Model 3 was adjusted for variables in model 1 and level of LPC15:0.
Model 4 was adjusted for variables in model 1 and level of LPC18:0.
Abbreviations: DCA, deoxycholic acid; GDM, gestational diabetes mellitus; GUDCA, glycoursodeoxycholic acid; LPC, lysophosphatidylcholine; OR, odds ratio.

Similarly, the OR of GUDCA less than or equal to vs greater than 0.07 nmol/mL was attenuated by adjustment for LPC18:0 from 6.32 (2.34-17.1) to 4.53 (1.26-16.3). On the other hand, there were weak correlations of LPC18:0 with DCA and GUDCA after adjustment for maternal age and prepregnancy BMI (Spearman correlation coefficients with DCA: –0.1344; Spearman correlation coefficients with GUDCA: –0.1971, both *P* values < .05) (Table 4).

Additive interactions between bile acids and lysophosphatidylcholines for gestational diabetes mellitus

DCA less than or equal to 0.36 nmol/mL alone for GDM (0.84, 0.32-2.23) was not significant but greatly increased the OR of LPC18:0 greater than or equal to 18.00 nmol/mL alone from 8.18 (2.51-26.7) up to 17.7 (6.64-47.1), that is, for presence of both risk factors, with significant additive interaction

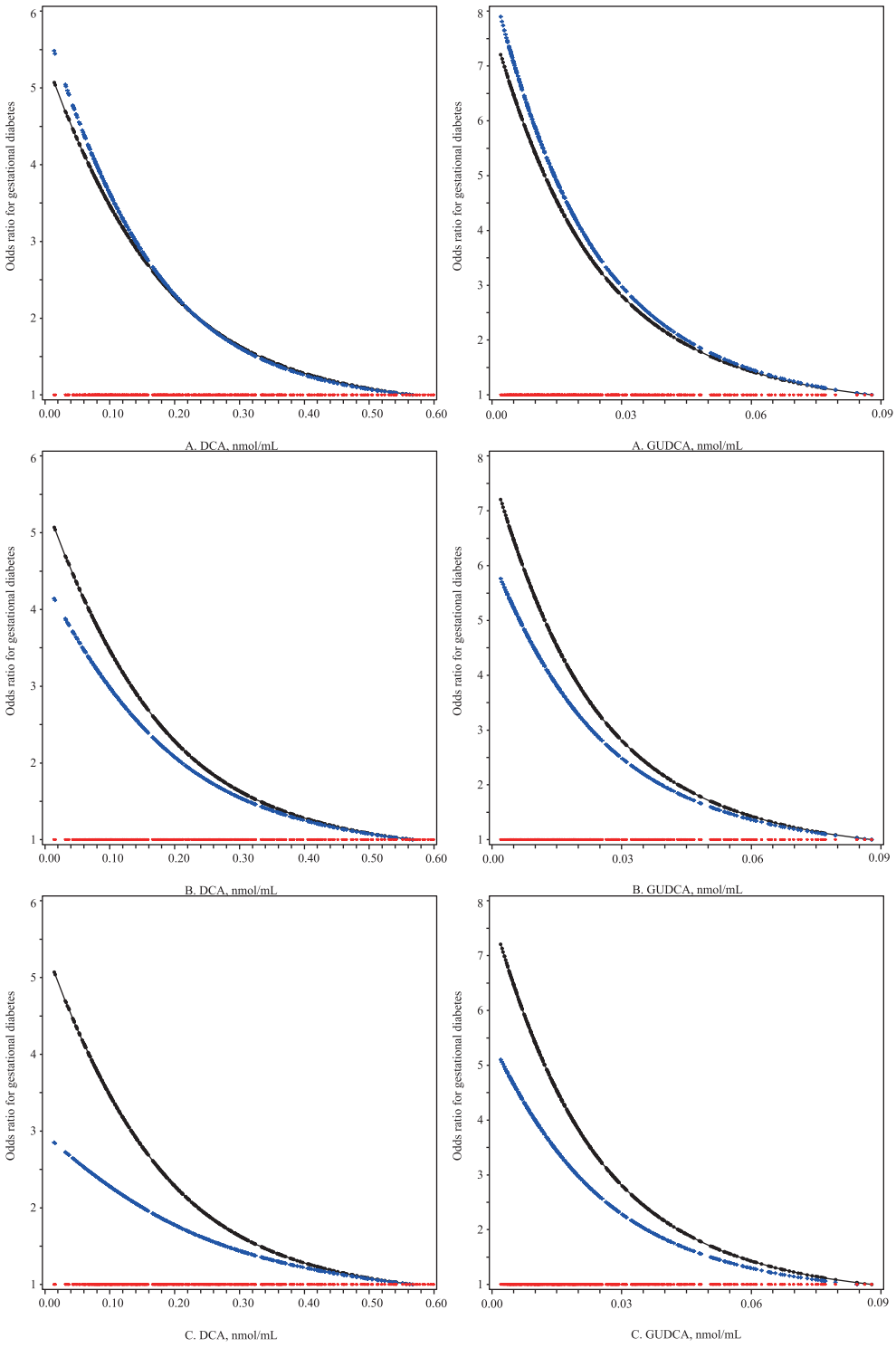

Figure 2. The association of deoxycholic acid (DCA)/glycoursodeoxycholic acid (GUDCA) with gestational diabetes mediated by species lysophosphatidylcholine (LPC). The dotted (black, upper) lines were derived from multivariable analyses with adjustment for traditional risk factors, the crossed (blue, bottom) lines were derived from multivariable analyses with adjustment for traditional risk factors plus individual LPC (A, plus LPC14:0; B, plus LPC15:0; C, plus LPC18:0), and the straight (red) lines are the reference line at odds ratio equal to 1.

(AP: 0.55, 0.05-1.05). In the same vein, GUDCA less than or equal to 0.07 nmol/mL alone for GDM (4.75, 0.90-25.0) was also not significant but it greatly increased the OR of LPC18:0 greater than or equal to 18.00 nmol/mL alone from 17.2 (1.77-168) to 73.8 (12.7-429), that is, for presence of both risk factors, with a significant additive interaction (AP: 0.72, 0.29-1.14) (Table 5).

Table 4. Spearman correlation coefficients of bile acids for LPCs

	DCA		GUDCA	
	Correlation Coefficient	*P*	Correlation Coefficient	*P*
Univariate analysis[a]				
LPC14:0	0.0618	.18	–0.0752	.10
LPC15:0	0.0309	.50	–0.1611	< .001
LPC18:0	–0.1359	.003	–0.2150	< .001
Multivariable analysis[b]				
LPC14:0	0.0779	.09	–0.0706	.13
LPC15:0	0.0504	.28	–0.1665	< .001
LPC18:0	–0.1344	.004	–0.1971	< .001

Abbreviations: DCA, deoxycholic acid; GUDCA, glycoursodeoxycholic acid; LPC, lysophosphatidylcholine.
[a]Not adjusted for any other variables.
[b]Adjusted for maternal age and prepregnancy body mass index.

Table 5. Additive interaction between DCA or GUDCA and LPC18:0 for risk GDM

	Unadjusted[a]		Adjusted[b]	
	OR/Estimate (95% CI)	*P*	OR/Estimate (95% CI)	*P*
DCA and LPC18:0				
Additive interaction models				
DCA > 0.36 and LPC18:0 < 18.00	(Reference)		(Reference)	
DCA > 0.36 and LPC18:0 ≥ 18.00	8.19 (2.99 to 22.4)	< .001	8.18 (2.51 to 26.7)	< .001
DCA ≤ 0.36 and LPC18:0 < 18.00	0.83 (0.35 to 1.95)	.67	0.84 (0.32 to 2.23)	.73
DCA ≤ 0.36 and LPC18:0 ≥ 18.00	16.8 (7.22 to 39.2)	< .001	17.7 (6.64 to 47.1)	< .001
Measures of additive interaction[c]				
RERI	8.81 (–2.84 to 20.5)	–	9.67 (-4.97 to 24.3)	–
AP	0.52 (0.09 to 0.96)	–	0.55 (0.05 to 1.05)	–
S	2.26 (0.81 to 6.26)	–	2.38 (0.69 to 8.22)	–
GUDCA and LPC18:0				
Additive interaction models				
GUDCA > 0.07 and LPC18:0 < 18.00	(Reference)		(Reference)	
GUDCA > 0.07 and LPC18:0 ≥ 18.00	12.0 (1.78 to 81.5)	.01	17.2 (1.77 to 168)	.01
GUDCA ≤ 0.07 and LPC18:0 < 18.00	5.01 (1.10 to 22.8)	.04	4.75 (0.90 to 25.0)	.07
GUDCA ≤ 0.07 and LPC18:0 ≥ 18.00	79.1 (16.3 to 383)	< .001	73.8 (12.7 to 429)	< .001
Measures of additive interaction[c]				
RERI	63.0 (–41.2 to 167)	–	52.8 (–50.2 to 156)	–
AP	0.80 (0.57 to 1.03)	–	0.72 (0.29 to 1.14)	–
S	5.19 (1.56 to 17.2)	–	3.64 (0.76 to 17.5)	–

Abbreviations: AP, attributable proportion due to interaction; DCA, deoxycholic acid; GUDCA, glycoursodeoxycholic acid; LPC, lysophosphatidylcholine; OR, odds ratio; RERI, relative excess risk due to interaction; S, synergy index.
[a]Not adjusted for any other variables.
[b]Adjusted for traditional risk factors.
[c]Statistically significant, with RERI greater than 0, AP greater than 0, or S greater than 1 indicating significant additive interaction.

Predictive values of lysophosphatidylcholines for gestational diabetes mellitus

Inclusion of LPCs, that is, LPC14:0, LPC15:0, and LPC18:0, markedly improved the prediction of GDM. The area under the curve (AUC) increased from 0.69 (0.64-0.74) for the model incorporating only traditional risk factors to 0.87 (0.84-0.90) ($P < .001$). Similarly, inclusion of these LPCs also increased the AUC of a model including traditional risk factors plus bile acids (DCA and GUDCA) from 0.75 (0.70-0.79) to 0.88 (0.85-0.92) ($P < .001$) (Fig. 3).

Discussion

In this nested case-control study, we found that LPC14:0 less than 0.24 nmol/mL, LPC15:0 greater than or equal to 0.45 nmol/mL, and LPC18:0 greater than or equal to 18.00 nmol/mL in early pregnancy were independently associated with increased GDM risk. LPC18:0 had weak correlations with DCA and GUDCA and adjustment for high LPC18:0 slightly attenuated the effects of low DCA and GUDCA on risk of GDM, whereas there was a significant additive interaction between

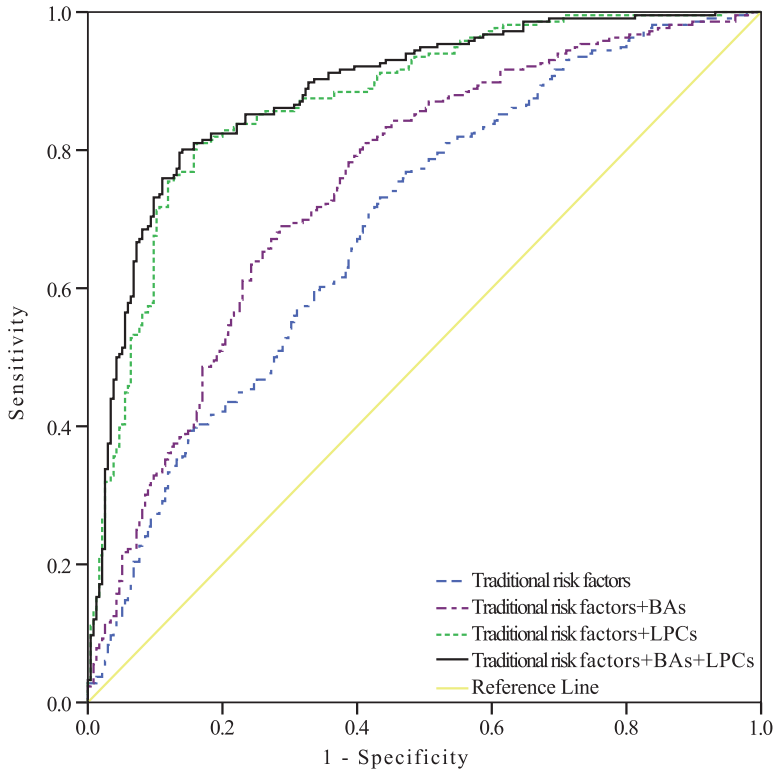

Figure 3. Receiver operating characteristic curves of traditional risk factors, bile acids (BAs) plus lysophosphatidylcholines (LPCs) for gestational diabetes in Chinese pregnant women. BAs include deoxycholic acid and glycoursodeoxycholic acid. LPCs include LPC14:0, LPC15:0 and LPC18:0. The blue (dash, bottom) curve for the traditional risk factors model (model 1), the purple (dashed dot, midbottom) curve for the traditional risk factors plus BAs model (model 2), the green (dot, midupper) curve for the traditional risk factors plus LPCs model (model 3), and the black (solid, upper) curve for the traditional risk factors plus BAs and LPCs model (model 4). The area under curve of those 4 models was 0.69 (95% CI: 0.64-0.74), 0.75 (0.70-0.79), 0.87 (0.84-0.90), and 0.88 (0.85-0.92), respectively. *P* values were less than .001 between model 1 and model 3, between model 2 and model 3, as well as between model 2 and model 4, and .04 between model 3 and model 4.

low DCA/GUDCA and high LPC18:0, that is, low DCA/GUDCA greatly amplified the risk association of LPC18:0 with GDM.

Some small studies have previously investigated the associations between unsaturated LPCs and GDM risk. For example, Zhao et al conducted a 1:1 case-control study (n = 214) during the first and second trimester of pregnancy and observed that the LPC20:4 ratios of second trimester to first trimester were significantly lower in GDM women than in control women, suggesting a role of abnormal LPCs metabolism in the etiology of GDM (22). Dudzik and colleagues also conducted a 1:1 case-control study (n = 40) during the second trimester of pregnancy and reported that LPC18:1, LPC18:2, and LPC20:5 were lower in women with GDM, with LPC18:2 having the best correlation with glycemic status of pregnant women (23). A few earlier studies have shown that plasma LPCs in early pregnancy, particularly saturated LPCs, play a role in GDM. In this case-control study with a larger sample size nested in a large cohort, we found that low LPC14:0, high LPC15:0, and, especially, high LPC18:0 in early pregnancy, had large and independent effects on the risk of GDM.

The mechanism underlying the roles of LPCs in the etiology of GDM remains elusive. Several lines of evidence support the pathway from decreased DCA/GUDCA to increased LPC18:0 could play a role in the etiology of GDM. First, a biological link between decreased DCA/GUDCA and increased LPC18:0 is plausible. In a study of transgenic mice overexpressing cholesterol 7α-hydroxylase (CYP7A1-tg mice), it was shown that increasing intestinal tauro-β-muricholic acid, an antagonist of the farnesoid X receptor (FXR), may reduce a high-fat diet–induced increase in LPCs, possibly via inhibiting activity of the FXR (16). In this regard, another study revealed that GUDCA was an intestinal FXR antagonist and biological links of gut microbiota-GUDCA-FXR to glucose intolerance (24). Hence, a possibility is that low GUDCA can increase the synthesis of LPCs via activity of the FXR. Second, findings from several mechanistic investigations support that high LPCs were associated with insulin resistance and abnormal glucose metabolism. In this connection, higher levels of

LPCs in hepatocytes can upregulate genes of triglyceride biosynthesis and downregulate genes of hepatic fatty acid oxidation via enhancing cytochrome C release and disturbing mitochondrial integrity (25). Increased triglyceride biosynthesis and decreased fatty acid oxidation were both associated with GDM risk (22, 26). Higher LPCs can increase the release of interleukin-1β (IL-1β), IL-6, and tumor necrosis factor-α from adipocytes to induce proinflammatory effects that also increased the risk of GDM (27-29). In addition, higher LPCs not only can modulate inflammatory chemokine expression from endothelical cells involved in the expression of monocyte chemoattractant protein-1, cytotoxicity/apoptosis, and production of IL-8 (30, 31), but also increase oxidative stress via inducing the overproduction of nitric oxide (32). Increased inflammation and oxidative stress may lead to increased GDM risk (33). In this regard, our study found that LPC18:0 only slightly attenuated the effects of DCA and GUDCA on the risk of GDM, and the correlations of DCA and GUDCA with LPC18:0 were quite weak. The weak correlations suggest that LPC18:0 is unlikely to be the direct downstream molecules of DCA/GUDCA. On the other hand, our study also found there was a significant additive interaction between low DCA/GUDCA and high LPC18:0 for GDM risk. The observation supports that low DCA/GUDCA is more likely to play an enhancing role but does not increase the risk of GDM via directly increasing levels of LPC18:0.

It is biologically plausible for low DCA/GUDCA to play an enhancing role in the link between LPC18:0 and GDM. One possibility is that low DCA/GUDCA and high LPC 18:0 work synergistically on downstream molecular pathways, such as the C-Jun N-terminal kinase pathway (34), to increase the risk of GDM. The second possibility is that low DCA/GUDCA enhances the risk association between LPC18:0 and GDM via increasing levels of upstream molecules. In this regard, LPCs were reported to act as a second signal activator and may mediate free fatty acid (FFA)-induced insulin resistance (34). Thus, it is presumed that decreased DCA/GUDCA may further increase levels of FFAs that amplify the effect of LPC18:0 on inducing insulin resistance. Indeed, it is worthwhile to investigate whether FFA and DCA/GUDCA work in a synergistic manner to increase LPC18:0 and consequently to increase the risk of GDM.

Our findings have potential clinical and public health implications. The effectiveness of lifestyle modification for prevention of GDM depends on early initiation of the intervention (4). It is crucial to identify women at high risk of GDM in early pregnancy. The major strength of this study was that pregnant women who provided blood samples were recruited from early pregnancy (median at the 10th gestational week) much earlier than GCT and OGTT (at the 24th-28th gestational weeks), and that further suggests the effect of causation that metabolic changes lead to GDM. In addition, our results also showed that the inclusion of LPCs in models incorporating traditional risk factors greatly increased AUC from 0.69 to 0.87, suggesting that GDM is highly predictable using LPCs in early pregnancy.

This study had several limitations. First, the significance of our findings was in part dependent on the causal relationship between bile acid concentrations and GDM risk. In this regard, we had found that low DCA and low GUDCA in early pregnancy were associated with increased GDM risk in a previous analysis (13). A mechanistic investigation explored biological links of gut microbiota-GUDCA-FXR to glucose intolerance and provided some evidence of a possible causal relationship between low GUDCA and glucose intolerance (24). Although solid evidence from randomized controlled trials is yet to come, the causal associations between low DCA/GUDCA and GDM is highly likely. Second, levels of LPC and bile acid both can be influenced by dietary habits and our study did not collect detailed information on dietary habits from pregnant women. However, diet is associated with many demographic and clinical factors. In this study, we carefully adjusted for potential confounding factors, which may have partially removed the confounding effect of diet if any. Third, the pregnant women in this study were all from urban Tianjin and these findings need to be replicated in other Chinese and non-Chinese populations.

In conclusion, we found that LPC14:0 less than 0.24 nmol/mL, LPC15:0 greater than or equal to 0.45 nmol/mL, and LPC18:0 greater than or equal to 18.00 nmol/mL were independently associated with increased GDM risk. Furthermore, low DCA/GUDCA had a significant additive interaction with high LPC18:0, which greatly amplified the effect of high LPC18:0 on the risk of GDM. The ROC curve analysis for prediction of GDM showed that inclusion of LPCs in the model with traditional risk factors greatly increased the AUC from 0.69 to 0.87. Thus, our study suggests that risk scores with inclusion of LPCs may accurately predict GDM in early pregnancy, so early lifestyle intervention can be conducted in a more cost-effective manner. Future mechanistic studies need to be undertaken to fully understand the underlying molecular mechanisms between DCA/GUDCA and LPC18:0 in the etiology of GDM.

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References

1. International Diabetes Federation. IDF website. *IDF Diabetes Atlas*. 9th ed. 2019. https://diabetesatlas.org/en/. Accessed January 25, 2020.
2. Buchanan TA, Xiang AH, Page KA. Gestational diabetes mellitus: risks and management during and after pregnancy. *Nat Rev Endocrinol*. 2012;**8**(11):639–649.
3. Johns EC, Denison FC, Norman JE, Reynolds RM. Gestational diabetes mellitus: mechanisms, treatment, and complications. *Trends Endocrinol Metab*. 2018;**29**(11):743–754.
4. Song C, Li J, Leng J, Ma RC, Yang X. Lifestyle intervention can reduce the risk of gestational diabetes: a meta-analysis of randomized controlled trials. *Obes Rev*. 2016;**17**(10):960–969.
5. Birgbauer E, Chun J. New developments in the biological functions of lysophospholipids. *Cell Mol Life Sci*. 2006;**63**(23):2695–2701.
6. Fukushima N, Ishii I, Contos JJ, Weiner JA, Chun J. Lysophospholipid receptors. *Annu Rev Pharmacol Toxicol*. 2001;**41**:507–534.
7. Xu Y. Sphingosylphosphorylcholine and lysophosphatidylcholine: G protein-coupled receptors and receptor-mediated signal transduction. *Biochim Biophys Acta*. 2002;**1582**(1-3):81–88.
8. Razquin C, Toledo E, Clish CB, et al. Plasma lipidomic profiling and risk of type 2 diabetes in the PREDIMED trial. *Diabetes Care*. 2018;**41**(12):2617–2624.
9. Schmitz G, Ruebsaamen K. Metabolism and atherogenic disease association of lysophosphatidylcholine. *Atherosclerosis*. 2010;**208**(1):10–18.
10. Kühn T, Floegel A, Sookthai D, et al. Higher plasma levels of lysophosphatidylcholine 18:0 are related to a lower risk of common cancers in a prospective metabolomics study. *BMC Med*. 2016;**14**:13.
11. Rabini RA, Galassi R, Fumelli P, et al. Reduced Na(+)-K(+)-ATPase activity and plasma lysophosphatidylcholine concentrations in diabetic patients. *Diabetes*. 1994;**43**(7):915–919.
12. Barber MN, Risis S, Yang C, et al. Plasma lysophosphatidylcholine levels are reduced in obesity and type 2 diabetes. *PloS One*. 2012;**7**(7):e41456.
13. Li J, Huo X, Cao YF, et al. Bile acid metabolites in early pregnancy and risk of gestational diabetes in Chinese women: a nested case-control study. *EBioMedicine*. 2018;**35**:317–324.
14. Fall T, Salihovic S, Brandmaier S, et al. Non-targeted metabolomics combined with genetic analyses identifies bile acid synthesis and phospholipid metabolism as being associated with incident type 2 diabetes. *Diabetologia*. 2016;**59**(10):2114–2124.
15. Zeng Y, Mtintsilana A, Goedecke JH, Micklesfield LK, Olsson T, Chorell E. Alterations in the metabolism of phospholipids, bile acids and branched-chain amino acids predicts development of type 2 diabetes in black South African women: a prospective cohort study. *Metabolism*. 2019;**95**:57–64.
16. Qi Y, Jiang C, Cheng J, et al. Bile acid signaling in lipid metabolism: metabolomic and lipidomic analysis of lipid and bile acid markers linked to anti-obesity and anti-diabetes in mice. *Biochim Biophys Acta*. 2015;**1851**(1):19–29.
17. Leng J, Shao P, Zhang C, et al. Prevalence of gestational diabetes mellitus and its risk factors in Chinese pregnant women: a prospective population-based study in Tianjin, China. *PLoS One*. 2015;**10**(3):e0121029.
18. International Association of Diabetes and Pregnancy Study Groups Consensus Panel, Metzger BE, Gabbe SG, Persson B, et al. International association of diabetes and pregnancy study groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy. *Diabetes Care*. 2010;**33**(3):676–682.
19. Chen C, Lu FC; Department of Disease Control Ministry of Health, PR China. The guidelines for prevention and control of overweight and obesity in Chinese adults. *Biomed Environ Sci*. 2004;**17(Suppl)**:1–36.
20. Yang X, Ko GT, So WY, et al; Hong Kong Diabetes Registry. Additive interaction of hyperglycemia and albuminuria on risk of ischemic stroke in type 2 diabetes: Hong Kong Diabetes Registry. *Diabetes Care*. 2008;**31**(12):2294–2300.
21. Andersson T, Alfredsson L, Källberg H, Zdravkovic S, Ahlbom A. Calculating measures of biological interaction. *Eur J Epidemiol*. 2005;**20**(7):575–579.
22. Zhao H, Li H, Chung ACK, et al. Large-scale longitudinal metabolomics study reveals different trimester-specific alterations of metabolites in relation to gestational diabetes mellitus. *J Proteome Res*. 2019;**18**(1):292–300.
23. Dudzik D, Zorawski M, Skotnicki M, et al. Metabolic fingerprint of gestational diabetes mellitus. *J Proteomics*. 2014;**103**:57–71.
24. Sun L, Xie C, Wang G, et al. Gut microbiota and intestinal FXR mediate the clinical benefits of metformin. *Nat Med*. 2018;**24**(12):1919–1929.
25. Hollie NI, Cash JG, Matlib MA, et al. Micromolar changes in lysophosphatidylcholine concentration cause minor effects on mitochondrial permeability but major alterations in function. *Biochim Biophys Acta*. 2014;**1841**(6):888–895.
26. Ryckman KK, Spracklen CN, Smith CJ, Robinson JG, Saftlas AF. Maternal lipid levels during pregnancy and gestational diabetes: a systematic review and meta-analysis. *BJOG*. 2015;**122**(5):643–651.
27. Sato A, Kumagai T, Ebina K. A synthetic biotinylated peptide, BP21, inhibits the induction of mRNA expression of inflammatory

substances by oxidized- and lyso-phosphatidylcholine. *Drug Dev Res*. 2014;**75**(4):246–256.
28. Kuniyasu A, Tokunaga M, Yamamoto T, et al. Oxidized LDL and lysophosphatidylcholine stimulate plasminogen activator inhibitor-1 expression through reactive oxygen species generation and ERK1/2 activation in 3T3-L1 adipocytes. *Biochim Biophys Acta*. 2011;**1811**(3):153–162.
29. Kleiblova P, Dostalova I, Bartlova M, et al. Expression of adipokines and estrogen receptors in adipose tissue and placenta of patients with gestational diabetes mellitus. *Mol Cell Endocrinol*. 2010;**314**(1):150–156.
30. Murugesan G, Sandhya Rani MR, Gerber CE, et al. Lysophosphatidylcholine regulates human microvascular endothelial cell expression of chemokines. *J Mol Cell Cardiol*. 2003;**35**(11):1375–1384.
31. Chang MC, Lee JJ, Chen YJ, et al. Lysophosphatidylcholine induces cytotoxicity/apoptosis and IL-8 production of human endothelial cells: related mechanisms. *Oncotarget*. 2017;**8**(63):106177–106189.
32. Kim EA, Kim JA, Park MH, et al. Lysophosphatidylcholine induces endothelial cell injury by nitric oxide production through oxidative stress. *J Matern Fetal Neonatal Med*. 2009;**22**(4):325–331.
33. Rueangdetnarong H, Sekararithi R, Jaiwongkam T, et al. Comparisons of the oxidative stress biomarkers levels in gestational diabetes mellitus (GDM) and non-GDM among Thai population: cohort study. *Endocr Connect*. 2018;**7**(5):681–687.
34. Han MS, Lim YM, Quan W, et al. Lysophosphatidylcholine as an effector of fatty acid-induced insulin resistance. *J Lipid Res*. 2011;**52**(6):1234–1246.