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ORIGINAL RESEARCH

Maternal Plasma Betaine in Middle Pregnancy Was Associated with Decreased Risk of GDM in Twin Pregnancy: A Cohort Study

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Purpose: Although previous studies have shown that choline-related metabolites in one carbon metabolism (OCM) were related to gestational diabetes mellitus (GDM) risk in singleton pregnancy, their role in twin gestations remains unclear. We aimed to investigate the associations between choline, betaine, methionine, dimethylglycine (DMG), trimethylamine N-oxide (TMAO) and GDM risk among women with twin gestations.

Patients and Methods: This hospital-based cohort study included 187 women with dichorionic twin gestations. Blood samples were collected during pregnancy at a median of 16.1 weeks of gestation (IQR: 13.9 −17.9). Concentrations of plasma metabolites were measured by HPLC-triple quadrupole MS. Log-binomial regression models were applied to estimate the associations between plasma metabolites and the risk of GDM.

Results: A total of 57 (30.5%) GDM cases were diagnosed over the study follow-up. Eighty-seven percent of women conceived through ART. Plasma betaine had an inverse association with GDM risk, and the adjusted RR of GDM comparing the highest tertile with the lowest tertile was 0.41 (95% CI: 0.19–0.86, P_{trend}=0.015). Women with a high betaine/choline ratio or a low DMG/betaine ratio were at decreased GDM risk (P_{trend}=0.031 or 0.001, respectively). Plasma choline, methionine, DMG and TMAO were not associated with GDM risk.

Conclusion: Among women with dichorionic twin gestations, higher plasma level of betaine in the second trimester was associated with lower risk of GDM. This finding needs further confirmation.

Keywords: one-carbon metabolism, betaine, gestational diabetes mellitus, twin pregnancy

Introduction

Gestational diabetes mellitus (GDM), one of the most common complications of pregnancy, is estimated to affect approximately 16.9% of pregnancies worldwide.[1] Factors such as pre-pregnant body weight and body mass index (BMI),[2] advanced age[3] and dietary patterns[4] are associated with the prevalence of GDM. It is not only associated with adverse pregnancy outcomes including macrosomia, fetal dystocia, and cesarean delivery but also with increased diabetes risk for both mother and child in their later life,[5,6] for example, children with intrauterine exposed to GDM have higher risk of adiposity.[7] Twin gestations itself is an independent risk factor for GDM due to increased insulin resistance.[8] Although available evidence is limited, several studies have shown that GDM in twin pregnancy is associated with similar adverse maternal and neonatal outcomes than that in singleton pregnancy.[9–11]

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Choline is an essential nutrient necessary for the integrity of cell membranes, cholinergic neurotransmission and one-carbon metabolism.[12] In one-carbon metabolism, choline is irreversibly oxidized to betaine, which donates its methyl group in the remethylation of homocysteine to methionine, a reaction generating dimethylglycine (DMG)[13] (Figure 1). Trimethylamine N-oxide (TMAO) is a gut microbial-derived metabolite of choline, betaine and L-carnitine. Emerging evidence suggests that choline and its related metabolites may contribute to the pathogenesis of insulin resistance.[14–16] Betaine supplementation has been shown to reduce fasting glucose and improve insulin resistance in mouse models.[14,16] An animal study found that dietary TMAO increased fasting insulin levels and exacerbated impaired glucose tolerance.[15] A metabonomic study in pregnant women showed that higher urine choline, lower plasma betaine and TMAO were associated with increased risk of GDM, respectively.[17] So far, two epidemiologic studies have examined the association between choline and its related metabolites and GDM risk in singleton pregnancies.[18,19] A U-shaped association of choline and an inverse association of betaine with GDM risk were reported in a nested case–control study.[18] For TMAO, these two studies showed inconsistent results, one showed an inverse association but the other revealed a positive association with GDM risk.[18,19] To the best of our knowledge, no previous studies have explored the relationship between choline-related metabolites and GDM risk in twin pregnancy.

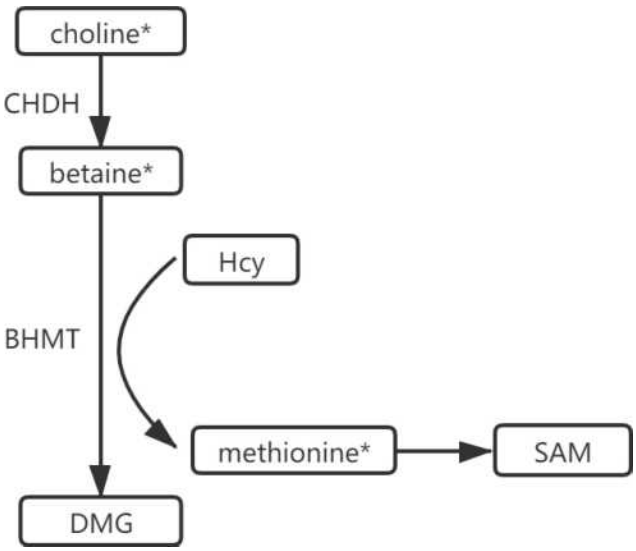

Figure 1 Choline oxidation metabolites in one carbon metabolism. *They can be obtained from dietary.
Abbreviations: CHDH, choline dehydrogenase; DMG, dimethylglycine; Hcy, homocysteine; SAM, S-adenosylmethionine.

Considering the increasing number of twin pregnant women as a result of the widespread use of reproductive techniques and advanced maternal age and the increasing incidence of GDM worldwide,[1,20] identifying the potential risk factors of GDM in this high-risk group would provide important public health implications. Our aim is to investigate the relationship between plasma concentrations of choline, betaine, methionine, DMG, TMAO and GDM risk in twin pregnancy in a hospital-based cohort. The association between the ratio of betaine to choline or DMG to betaine with GDM risk are also explored because the ratios were considered as stronger predictors of metabolic disturbances.[21]

Methods

Study Participants

A hospital-based cohort of women with twin pregnancy was established between March 2017 and December 2018 in Peking University Third Hospital in Beijing, China. Twin pregnant women who received regular antenatal care and planned to delivery at this hospital were recruited. Participants were excluded as follows: those who underwent fetal reduction, those who were pregnant with monochorionic-monoamniotic (MCMA) twins or monochorionic diamniotic (MCDA) twins, and those who presented chromosomal and other congenital abnormalities. A total of 314 women with twin gestations were recruited, 127 of whom were excluded due to the exclusion criteria, resulting in 187 women with dichorionic twin gestations included in the study eventually.

Collection of Covariates

When participants underwent the first antenatal care visit, nurses measured their height and the value was accurate to 0.01m. Pre-pregnancy BMI was calculated as pre-pregnancy weight (kg, self-reported data from women) divided by height squared (m^2). Gestational weight gain was calculated by subtracting the weight before pregnancy from the weight before delivery. Information on age at childbirth, ethnicity, occupation, parity, mode of conception, coexisted diseases (complications) and infant sex were extracted from questionnaires completed during antenatal care visits or medical records after admission.

Gestational age at birth was calculated using the last menstrual period and was further confirmed or revised by

early ultrasound. Gestational age based on ultrasound findings was used when there was a discrepancy larger than seven days. For women used assisted reproduction techniques (ART) for conception, gestational age was calculated from embryo transfer day plus 17 or 19 days in pregnancy for cryopreserved embryos transfer pregnancy. Chorionicity was determined by sonographic findings (placental sites and lambda or T-signs) and was further confirmed by examination of the placenta after delivery.

Diagnosis of GDM

At 24 to 28 weeks of gestational age, DCDA women underwent the 75 g 2 h One Step test to identify GDM which was recommended by the International Association of the Diabetes and Pregnancy Study Groups (IADPSG) and the American Diabetes Association (ADA).[22] Blood was drawn on an empty stomach in the morning; 75 g of glucose was then dissolved in 200 to 300 mL of water and drunk within five min. The time was recorded when the first mouthful of sugar water was drunk, and blood was drawn every hour. GDM was diagnosed if fasting plasma glucose was ≥5.1 mmol/L, 1-h after oral glucose tolerance test (OGTT) plasma glucose was ≥10.0 mmol/L or 2-h after OGTT plasma glucose was ≥ 8.5 mmol/L. The median gestational age at OGTT test was 24 weeks (range: 22–28 weeks), and most women (91.9%) took the OGTT test between 24 and 25 weeks.

Blood Samples Collection and Preparation

Early morning fasting blood samples were collected in vacuum blood tubes (EDTA-K2) with routine pregnancy check-ups, after centrifugation, plasma was separated and stored at –80°C refrigerator until tested. All blood samples were collected before 23 weeks of gestation.

In brief, 50 μL plasma was precipitated by adding 300 μL methanol containing internal standards (choline-d9, betaine-d3, DMG-d6, methionine-d3 and TMAO-d9). The mixture was further vortexed for 30 seconds and then centrifuged at 15,000 g for 15 min (4°C). The supernatant was analyzed by using HPLC-MS/MS analysis.

Measurement of Plasma Metabolites

We applied HPLC (Shimadzu)- Triple Quadrupole MS (API 4000+; AB Sciex, USA) to analyze the concentrations of plasma choline, betaine, DMG, methionine and TMAO. A XBridge BEH Amide column (1.7 μm, 2.1×100 mm, Waters, US) was used for metabolites separation. The column flow rate was 0.3 mL/min, and the sample injection volume was 1 μL. The column temperature was 35°C. The mobile phases consist of phase A (0.1% formic acid in acetonitrile) and phase B (water with 0.1% formic acid and 5 mM ammonium formate). The gradient program commenced at 15% B at 0.01min, then slowly increased to 45% B at 8.5 min, followed by increased to 80% B at 8.5 min and maintained for 2 min. The targeted metabolites and their internal standards were monitored positive ion and in multiple reaction monitoring (MRM) mode. MS parameters were optimized for each metabolite and internal standard. The inter-day CVs of quality control samples (at the low, medium and high concentration) during analyses were as follows: 1.5% −7.4% for choline, 2.8–5.6% for betaine, 3.7% −6.5% for DMG, 2.2% −5.2% for methionine, 2.2–5.9% for TMAO. All sample measurements were conducted by LC-MS technicians who were blinded to GDM status of our participants.

Statistical Analysis

Concentrations of all metabolites were presented as medians with interquartile ranges (IQRs) because of non-normal distributions. Differences of participant characteristics and plasma metabolite concentrations between women with and without GDM were compared using Mann–Whitney *U*-test or *t* test or Chi-square test as appropriate.

We tested nonlinear associations between plasma metabolites and GDM risk using restricted cubic spline models with three knots at the 10th, 50th, and 90th centiles, and no nonlinear relationship was detected (all $P_{nonlinearity} > 0.1$). Since odds ratios often overestimate risk ratios when the occurrence of outcome events is not rare,[23] we applied log-binomial regression models to directly estimate relative risks (RRs) of GDM in relation to plasma metabolites given the high GDM rate (30.5%) in our study. Robust Poisson regression models were used if log-binomial models did not converge. A log-transformation was performed to improve the normality of metabolites distributions, and a standard deviation score (SDS) was then created for each of the metabolites before further analyses. The effect size is interpreted as changes in GDM risk associated with per SD change of log-transformed metabolite concentration. Besides, Concentrations of plasma metabolites and ratios of metabolites were categorized into tertiles based on the cutoffs defined among all participants. RRs were estimated from models with adjustment for pre-pregnancy BMI, age,

parity, ART, gestational age at blood sampling, ethnicity, occupation, infant sex, hypertensive disorders in pregnancy, gestational weight gain (Model 1) and from models adjusted for covariates in Model 1 plus the five mutually adjusted plasma metabolites (Model 2). All participants were not smokers and were not drinking during pregnancy in our study. Thus, smoking and drinking were not considered in multivariable models. Mutual adjustment of each metabolite was conducted to obtain the independent association of each metabolite with GDM risk. P_{trend} was obtained by entering the categorized tertiles into a regression model as a continuous variable.

To explore the potential modification effect by pre-pregnancy BMI, age, parity or infant sex for GDM, stratified analyses were conducted. $P_{interaction}$ was obtained by adding multiplicative interaction terms in the multivariable models. We performed sensitivity analyses by assessing the association between metabolite and GDM risk after excluding ethnic minorities or women conceived naturally. All statistical tests were 2-tailed and P values <0.05 were considered as significant. Statistical analyses were performed by SPSS software (version 24.0) and R statistical software (version 3.4.0).

Results

The characteristics of study population were showed in Table 1. Among 187 participants, 57 developed GDM during the follow-up. The majority of mothers were Han Chinese (94.1%), white-collar workers (69%), nulliparous (78.1%), and were conceived by ART (87.2%). Nearly half of mothers were older than 35 years old (44.9%). The incidence of hypertensive disorders in pregnancy was 26.7%. Women of advanced reproductive age were more common in GDM group than non-GDM group (56.1% vs 40.0%, P=0.041), whereas all other characteristics were similar among two groups. For plasma concentration of metabolites, the median betaine concentration was significantly lower in GDM group compared with the non-GDM group (13.8μmol/l vs 15.3umol/l, P=0.023).

We investigated the associations between metabolites and GDM risk. Plasma betaine had an inverse association with GDM risk in all three models, and stronger association was observed with the inclusion of more variables in regression models (Table 2). In the fully adjusted model, the RRs of GDM with the increase of plasma betaine tertiles were 1.00 (ref.), 0.54 (95% CI: 0.28, 1.06) and 0.41 (95% CI: 0.19, 0.86), respectively (P_{trend}=0.015). Each SD increase in betaine concentration was associated lower risk of GDM with an RR of 0.68 (95% CI 0.47, 1.00). Plasma choline, DMG, methionine or TMAO were not related to GDM risk in all three models.

Furthermore, we also examined the ratio of upstream and downstream metabolites of betaine (Table 3). The RR of GDM for the highest (vs lowest) tertile of the plasma betaine: choline ratio was 0.48 (95% CI: 0.25, 0.91, P_{trend}=0.031) in the adjusted model. Notably, with regard to the plasma DMG:betaine ratio, highest tertile was selected as reference group. Women in the lowest tertile of DMG:betaine ratio had lower risk of GDM compared with the highest tertile (RR: 0.42 95% CI:0.24, 0.74, P_{trend}=0.001) in the adjusted model.

The reference model included traditional risk factors produced a receiver operating characteristic curve (ROC) area under the curve (AUC) of 0.67 (Table 4, Figure 2, Curve 1). The ROC AUC reached 0.74 after adding choline, DMG, methionine and TMAO in model 1 (Curve 2). After further adding betaine, the ROC AUC was significantly improved to 0.76 (P=0.042 vs the reference model). Model which additionally included betaine: choline and DMG: betaine performed best in predicting GDM risk (AUC=0.79, P=0.006 vs the reference model).

The association between betaine and GDM risk was not modified by pre-pregnancy BMI, age, parity or infant sex; consistent associations were observed in each strata of these risk factors and all P values for interaction were not statistically significantly (data were not shown). In sensitivity analyses after excluding ethnic minorities or women conceived naturally, the relationship between plasma betaine and GDM risk still existed although the sample sizes were much smaller (data were not shown).

Discussion

To our knowledge, this is the first study to investigate the associations between choline-related metabolites and GDM risk in twin pregnancy. We observed that plasma levels of betaine during the second trimester was negatively related to the risk of GDM. However, plasma choline, DMG, methionine and TMAO were not significantly associated. Besides, we found that high betaine: choline ratio or low DMG: betaine ratio, which contained upstream or downstream metabolites of betaine, was related to the decreased risk of GDM.

Our finding that plasma betaine was inversely associated with GDM risk was in line with findings from a nested case–control study among Chinese women with singleton pregnancy,[18] a markedly elevated risk of GDM was observed for women with lower betaine status (≤200

Table 1 Characteristics of Mother and Child Pairs in This Cohort[a]

	Total (n=187)	Non-GDM (n=130)	GDM (n=57)	*P* value
Age, n (%), years				0.041
<35	103 (55.1)	78 (60.0)	25 (43.9)	
≥35	84 (44.9)	52 (40.0)	32 (56.1)	
Ethnicity, n (%)				0.266
Han Chinese	176 (94.1)	124 (95.4)	52 (91.2)	
Ethnic minorities	11 (5.9)	6 (4.6)	5 (8.8)	
Occupation, n (%)				0.908
Administrator/other white-collar worker	129 (69.0)	90 (69.2)	39 (68.4)	
Blue-collar worker	11 (5.9)	7 (5.4)	4 (7.0)	
Unemployed/freelancer/student/other	47 (25.1)	33 (25.4)	14 (24.6)	
Pre-pregnancy BMI, n (%), kg/m^2				0.079
<18.5	13 (7.0)	11 (8.5)	2 (3.5)	
18.5–24.9	124 (66.3)	90 (69.2)	34 (59.6)	
≥25	50 (26.7)	29 (22.3)	21 (36.8)	
Parity, n (%)				0.849
Nulliparous	146 (78.1)	101 (77.7)	45 (78.9)	
Multiparous	41 (21.9)	29 (22.3)	12 (21.1)	
Mode of conception, n (%)				0.881
ART	163 (87.2)	113 (86.9)	50 (87.7)	
Spontaneous	24 (12.8)	17 (13.1)	7 (12.3)	
Hypertensive disorders in pregnancy, n (%)				0.177
Yes	50 (26.7)	31 (23.8)	19 (33.3)	
No	137 (73.3)	99 (76.2)	38 (66.7)	
Infant sex, n (%)				0.148
Male-male	62 (33.2)	35 (26.9)	8 (14.0)	
Female-female	43 (23.0)	40 (30.8)	22 (38.6)	
Female-male	82 (43.9)	55 (42.3)	27 (47.4)	
Gestational weight gain, mean (sd), kg	15.0 (5.1)	16.5 (5.4)	15.3 (4.4)	0.126
Gestational age at blood sampling, median (95% range), weeks	16.1 (12.6–20.7)	16.2 (12.6–20.3)	16.0 (12.4–21.3)	0.509
Plasma concentrations, median (25th–75th percentile), μmol/l				
Choline	12.7 (10.9, 15.0)	12.5 (10.4, 14.9)	12.9 (11.7, 15.4)	0.252
Betaine	15.2 (12.8, 17.7)	15.3 (13.1, 19.0)	13.8 (12.0, 17.0)	0.023
Dimethylglycine	1.6 (1.3, 1.9)	1.6 (1.2, 1.9)	1.7 (1.4, 2.0)	0.218
Methionine	14.2 (12.0, 16.8)	14.0 (11.9, 16.8)	14.4 (12.0, 16.3)	0.820
Trimethylamine-N-oxide	1.5 (1.1, 2.1)	1.5 (1.1, 2.3)	1.4 (1.1, 1.9)	0.480

Notes: [a]*P* values for differences of baseline characteristics and plasma metabolites concentration between pregnant women with and without GDM were obtained from Mann–Whitney *U*-test or *t* test or Chi-square test.

Abbreviations: BMI, body mass index; ART, assisted reproductive technology; GDM, gestational diabetes mellitus.

nmol/mL) in this study (RR: 4.44, 95% CI: 2.19, 9.00). A metabonomic study conducted in Portugal reported an inverse association of plasma betaine with GDM risk.[17] A recent meta-analysis has shown that women with a history of GDM have a nearly 10-fold increased risk of developing type 2 diabetes mellitus (T2DM).[24] T2DM and GDM share many of the same risk factors and pathogenesis. We are aware of several epidemiologic studies

Table 2 Associations of Metabolites Concentrations with GDM Risk[a]

	GDM/Subtotal	Unadjusted	Model 1	Model 2
		RR (95% CI)	RR (95% CI)	RR (95% CI)
Choline				
Tertile1	13/62	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Tertile2	26/63	1.97 (1.11, 3.47)	1.81 (0.92, 3.54)	1.81 (0.88, 3.70)
Tertile3	18/62	1.39 (0.74, 2.58)	1.18 (0.57, 2.48)	1.44 (0.63, 3.30)
P_{trend}		0.349	0.734	0.431
Per 1 SD increase		1.05 (0.87, 1.27)	0.99 (0.80, 1.22)	1.07 (0.78, 1.45)
Betaine				
Tertile1	27/62	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Tertile2	16/63	0.58 (0.35, 0.97)	0.61 (0.37, 1.00)	0.54 (0.28, 1.06)
Tertile3	14/62	0.52 (0.30, 0.89)	0.52 (0.30, 0.88)	0.41 (0.19, 0.86)
P_{trend}		0.011	0.010	0.015
Per 1 SD increase		0.80 (0.64, 0.99)	0.79 (0.62, 1.00)	0.68 (0.47, 1.00)
DMG				
Tertile1	16/62	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Tertile2	20/62	1.25 (0.72, 2.18)	1.31 (0.75, 2.29)	1.63 (0.79, 3.39)
Tertile3	21/63	1.29 (0.75, 2.23)	1.32 (0.77, 2.27)	1.32 (0.65, 2.64)
P_{trend}		0.366	0.321	0.189
Per 1 SD increase		1.11 (0.93, 1.33)	1.05 (0.88, 1.26)	1.02 (0.86, 1.21)
Methionine				
Tertile1	18/62	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Tertile2	20/63	1.09 (0.64, 1.86)	1.15 (0.69, 1.93)	0.97 (0.50, 1.89)
Tertile3	19/62	1.06 (0.62, 1.81)	1.06 (0.62, 1.80)	1.10 (0.56, 2.14)
P_{trend}		0.846	0.835	0.911
Per 1 SD increase		0.95 (0.76, 1.19)	0.95 (0.77, 1.17)	0.92 (0.69, 1.22)
TMAO				
Tertile1	18/62	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Tertile2	24/62	1.33 (0.81, 2.20)	1.38 (0.85, 2.24)	1.23 (0.66, 2.29)
Tertile3	15/63	0.82 (0.46, 1.48)	0.85 (0.47, 1.52)	0.74 (0.37, 1.49)
P_{trend}		0.533	0.617	0.421
Per 1 SD increase		1.00 (0.79, 1.25)	1.02 (0.82, 1.26)	1.02 (0.78, 1.33)

Notes: [a]Model 1: adjusted for gestational age at blood sampling, pre-pregnancy BMI, age, parity, mode of conception, ethnicity, occupation, infant sex, hypertensive disorders in pregnancy, gestational weight gain. Model 2: adjusted for covariates in model 1 plus mutually adjusted five plasma metabolites.
Abbreviations: DMG, dimethylglycine, TMAO, trimethylamine N-oxide; 95% CI, 95% confidence interval; RR, relative risk.

examining plasma betaine in relation to T2DM risk; the results were consistent.[25–29] Two large prospective cohort studies and two nested case–control studies, conducted in Norway,[28] Netherlands,[25] Spain[26] and United States,[29] respectively, consistently showed an inverse relation between plasma betaine and T2DM risk. Besides, a cross-sectional study of older adults in the United States also showed that subjects with higher levels of betaine had lower odds of T2DM.[27] The consistent result from different ethnic groups or different study designs, suggesting that plasma betaine have a strong relationship with T2D or GDM.

After all, the mechanism and causality were still uncertain since our study was an observational study. Betaine has been reported to improve pathological processes related to GDM. Betaine supplementation to mice with nonalcoholic fatty liver reverses hepatic insulin resistance by increasing the activation of insulin receptor substrate 1 (IRS1).[16] In mouse models, betaine supplementation improved glucose homeostasis via increasing the levels

Table 3 Associations of Metabolites Ratios with GDM Risk[a]

	GDM/ Subtotal	Unadjusted	Adjusted[a]
		RR (95% CI)	RR (95% CI)
Betaine: choline ratio			
Tertile1	24/63	1.00 (ref.)	1.00 (ref.)
Tertile2	23/61	0.99 (0.63, 1.55)	1.17 (0.74, 1.85)
Tertile3	10/63	0.42 (0.22, 0.80)	0.48 (0.25, 0.91)
P_{trend}		0.010	0.031
DMG: betine ratio			
Tertile3	29/62	1.00 (ref.)	1.00 (ref.)
Tertile2	16/63	0.54 (0.33, 0.90)	0.54 (0.32, 0.90)
Tertile1	13/62	0.42 (0.23, 0.73)	0.42 (0.24, 0.74)
P_{trend}		0.001	0.001

Notes: [a]adjusted for gestational age at blood sampling, pre-pregnancy BMI, age, parity, use of ART, ethnicity, occupation, infant sex, hypertensive disorders in pregnancy, gestational weight gain.
Abbreviations: DMG, dimethylglycine; 95% CI, 95% confidence interval; RR, relative risk.

of hepatic and circulating fibroblast growth factor 21 (FGF21), a hormone is secreted by the liver and regulates whole-body glucose and lipid metabolism.[14] Also, betaine-modulated improvements in metabolic health may also involve its osmolyte functions and the reduction of oxidative stress.[14,30]

We noted a significant inverse association between betaine: choline ratio and GDM risk in the present study. The ratios of product: substrate may reflect the activities of involved enzymes. Choline can be synthesized through the hepatic phosphatidylethanolamine N-methyltransferase (PEMT). Enhanced hepatic PEMT activity would increase choline, while depleting betaine, a source for the PEMT reaction, resulting in a lower betaine: choline ratio.[21] This result was supported by an animal study in which lack of PEMT (*Pemt*$^{-/-}$) showed a protective effect against diet-induced obesity and insulin resistance.[31] Betaine can be catalyzed by betaine-homocysteine methyltransferase (BHMT) to form methionine while generate DMG. Enhanced BHMT activity would decrease plasma betaine while increase plasma DMG. Our observed protective role of lower DMG: betaine ratio, suggesting less active of BHMT, in GDM was strengthened by a study which showed *Bhmt* deletion resulted in enhanced insulin sensitivity and glucose tolerance.[32] Besides, ratios of metabolites performed better in predicting GDM risk than metabolites alone in our study. These results suggest that activities of enzymes involve betaine metabolism might also contribute to the pathogenesis of GDM in women with twin pregnancy.

TMAO is a metabolite derived from L-carnitine, choline, and betaine by gut microbiota. An increasing number of epidemiologic studies have investigated the associations of TMAO with the risk of GDM or T2DM. Most of previous studies,[28,33,34] but not all,[26] reported a positive association between levels of TMAO and T2DM risk. As for GDM, two studies showed that GDM group had lower levels of plasma TMAO,[17,18] while another study from

Table 4 AUC and the Comparison[a]

	AUC	95% CI	*P*
Curve1	0.67	0.60, 0.74	ref.
Curve2	0.74	0.67, 0.80	0.067
Curve3	0.76	0.69, 0.82	0.042
Curve4	0.79	0.72, 0.84	0.006

Notes: [a]Curve 1 represents the basic risk factors (gestational age at blood sampling, pre-pregnancy BMI, age, parity, use of ART, ethnicity, occupation, infant sex, hypertensive disorders in pregnancy, gestational weight gain). Curve 2 represents basic factors plus choline, methionine, DMG and TMAO. Curve 3 indicates covariates in Curve 2 plus betaine. Curve 4 indicates basic risk factors, DMG: betaine, betaine: choline, TMAO, methionine and DMG.
Abbreviations: 95% CI, 95% confidence interval; AUC, area under the curve.

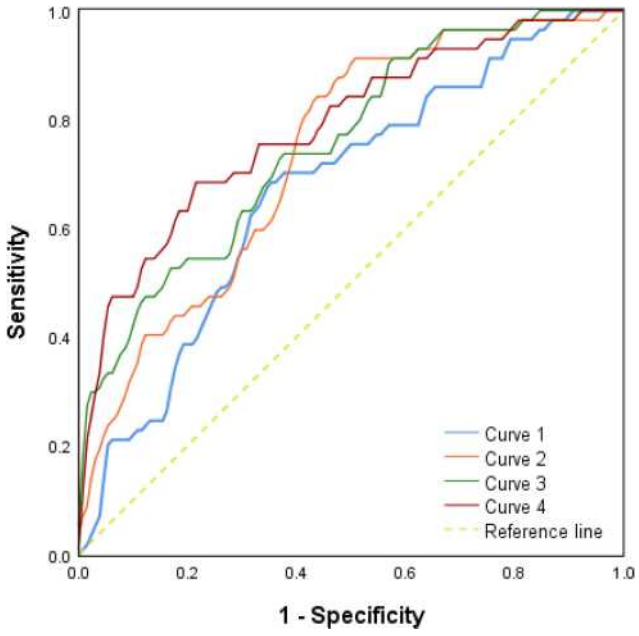

Figure 2 ROC curve analysis. Curve 1 (blue) represents the basic risk factors (Table 2: covariates in model 1). Curve 2 (orange) represents basic factors plus choline, methionine, DMG and TMAO. Curve 3 (green) indicates covariates in Curve 2 plus betaine. Curve 4 (red) indicates basic risk factors, DMG: betaine, betaine: choline, TMAO, methionine and DMG.

China observed inverse results.[19] A U-shaped association of choline status in early pregnancy with GDM risk were reported in a nested case–control study in China.[18] Additionally, a recent metabonomic study in Finland observed an increased risk of T2DM in relation to DMG.[35] However, in our study, TMAO, choline, DMG or methionine was not associated with GDM risk. Differences in characteristics and disease status of population across studies might partly explain such inconsistency. Also, these metabolites may have differential associations with GDM among women with singleton or twin pregnancy. In addition, a fluctuation in the levels of one metabolite may alter the levels of other metabolites because they share the same metabolic pathway. However, mutual adjustments of these metabolites were not conducted in previous studies when assessing their relations with GDM or T2DM.

The main strength of our study was the prospective design. The relatively long follow-up time between the blood sampling and GDM diagnosis (median: 8.1 weeks, 90% range: 5.4–11.4) might reduce the possibility of reverse causation. Extensive collections and full adjustments of multiple risk factors and related metabolites were also considered as strengths of this study. However, there were some limitations in our study. First, small sample size limits the power of our study. Second, only one time point assessment may not represent the metabolites status during whole pregnancy. Also, our participants were recruited in a large tertiary referral hospital and 69% of them are white collar workers, which may decrease the generalizability of our results.

Conclusion

Higher levels of plasma betaine in the second trimester of pregnancy were associated with lower GDM risk among Chinese women with twin pregnancy. Moreover, the well performance of the ratio of betaine: choline and DMG: betaine in predicting GDM risk is worthy of attention. Our results need to be confirmed in larger cohorts of twin gestations.

Ethics Approval and Informed Consent

This study was conducted in accordance with the Declaration of Helsinki and approved by the Peking University Third Hospital Medical Ethics Committee (IRB00006761-2016145), and all participants provided informed consent.

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Author Contributions

All authors made contributions to conception and design, acquisition of data, or data analysis, and took part in drafting the article or revising it critically, have agreed to submit to the current journal, gave final approval of the version to be published, and agreed to be accountable for all aspects of the work.

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Disclosure

The authors have no conflicts of interest to disclose.

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