Plasma concentration of trimethylamine-*N*-oxide and risk of gestational diabetes mellitus

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ABSTRACT

Background: The microbiota-dependent metabolite trimethylamine -*N*-oxide (TMAO) has been reported as a novel and independent risk factor for the development of cardiovascular and metabolic diseases, but the association with gestational diabetes mellitus (GDM) remains unclear.

Objective: The aim of this study was to investigate the association between plasma TMAO concentration and GDM in a 2-phase study.

Design: A 2-phase design was used in the current study. An initial phase included 866 participants (433 GDM cases and 433 matched controls) with fasting blood samples collected at the time of GDM screening (24–32 wk of gestation). An independent-phase study, with 276 GDM cases and 552 matched controls who provided fasting blood samples before 20 wk of gestation and who had GDM screened during 24–32 wk of gestation, was nested within a prospective cohort study. These 2 studies were both conducted in Wuhan, China, and the incidence of GDM in the cohort study was 10.8%. Plasma TMAO concentrations were determined by stable isotope dilution liquid chromatography–tandem mass spectrometry. GDM was diagnosed according to the American Diabetes Association criteria by using an oral-glucose-tolerance test.

Results: In the initial case-control study, the adjusted OR of GDM comparing the highest TMAO quartile with the lowest quartile was 1.94 (95% CI: 1.28, 2.93). Each SD increment of ln-transformed plasma TMAO was associated with 22% (95% CI: 5%, 41%) higher odds of GDM. In the nested case-control study, women in the highest quartile also had increased odds of GDM (adjusted OR: 2.06; 95% CI: 1.28, 3.31) compared with women in the lowest quartile, and the adjusted OR for GDM per SD increment of ln-transformed plasma TMAO was 1.26 (95% CI: 1.08, 1.47).

Conclusions: Consistent findings from this 2-phase study indicate a positive association between plasma TMAO concentrations and GDM. Future studies are warranted to elucidate the underlying mechanisms. This trial was registered at www.clinicaltrials.gov as NCT03415295. *Am J Clin Nutr* 2018;108:603–610.

Keywords: trimethylamine-*N*-oxide, gestational diabetes mellitus, nested case-control study, nutrition, metabolite

INTRODUCTION

Gestational diabetes mellitus (GDM), characterized by glucose intolerance with onset or first diagnosis during pregnancy, is one of the most common complications of pregnancy (1). According to data from the International Diabetes Federation, nearly 1 in 6 or 21.3 million live births were affected by high blood glucose in pregnancy in 2017 (2). GDM can lead to short- and long-term serious health risks for both the mother and child. Hyperglycemia increases the risk of a range of adverse perinatal outcomes, including cesarean delivery, induction of labor, macrosomia, large-for-gestational age, and shoulder dystocia (3). Women who have been previously diagnosed have a higher risk of developing GDM in subsequent pregnancies and type 2 diabetes later in life, and their children are more likely to be obese and develop diabetes as teenagers or as young adults (4, 5). Thus, the prevention of GDM is clearly imperative, and identifying modifiable risk factors and understanding their mechanisms

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Abbreviations used: d9-TMAO, TMAO-trimethyl-d_9; FMO3, flavin monooxygenase 3; GDM, gestational diabetes mellitus; OGTT, oral-glucose-tolerance test; TMAO, trimethylamine-*N*-oxide.

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should be of high priority. Although the pathogenesis of GDM is not well understood, many studies indicate that diet and lifestyle factors could contribute to the prevention of GDM (6, 7).

Trimethylamine-*N*-oxide (TMAO), a metabolite derived from dietary phosphatidylcholine and L-carnitine, was identified as a novel and independent risk factor for the development of atherosclerosis and cardiovascular diseases (8–10). Choline and its structural analogs, found in dietary sources such as red meat, eggs, and cheese, can be converted by gut microbes to form trimethylamine, which is further oxidized by hepatic flavin monooxygenase 3 (FMO3) to form TMAO. In animal models, dietary TMAO has been shown to exacerbate impaired glucose tolerance and hyperglycemia by blunting insulin signal transduction and causing adipose tissue inflammation (11). Consistently, reducing plasma TMAO could improve glucose and lipid homeostasis in mice by inhibition of FMO3 (12). Several studies have explored the relation between TMAO and diabetes (13–19), and some found that diabetes was associated with a significantly higher concentration of plasma TMAO (13–17). In our recent study, higher plasma TMAO was also found to be associated with increased odds of newly diagnosed type 2 diabetes (20). In addition, epidemiologic studies suggest that red meat consumption is related to an increased risk of diabetes and TMAO may be a strong candidate molecule mediating the risk (21). However, to date, the role of choline as well as its metabolites in the pathogenesis of GDM has not been extensively studied. Only one exploratory nuclear magnetic resonance metabonomics study with 54 samples explored the relation between TMAO and GDM, which indicated that plasma TMAO concentrations were lower in GDM subjects (19).

Thus, our aim was to investigate the association between plasma TMAO and GDM in a case-control study with a cross-sectional design and an independent case-control study nested within a prospective cohort study.

METHODS

Study population and design

In the current study, we used a 2-phase design. The flow charts of participant recruitment and case-control selection are shown in **Supplemental Figure 1**. The initial-phase study with a case-control design included 866 pregnant women (433 GDM cases and 433 matched controls) in Wuhan, China. Study participants were recruited from pregnant women who attended the outpatient clinics of the Department of Endocrinology, Tongji Hospital, to screen for GDM at 24–32 wk of gestation between August 2012 and April 2015 or pregnant women who visited the Hubei Maternal and Child Health Hospital or the Central Hospital of Wuhan for a routine antenatal checkup from May 2014 to November 2016. The inclusion criteria of participants were as follows: age ≥20 y, gestational age at blood sample collection between 24 and 32 wk, no history of a diagnosis of diabetes or gestational diabetes, and no history of receiving pharmacologic treatment known to affect glucose metabolism. Women with clinically significant neurological, endocrinological, or other systemic diseases were excluded from the study. The response rate of the study was 82.7%.

To clarify the temporal relation of the association between plasma TMAO and odds of GDM, we conducted a nested case-control study within an ongoing prospective cohort study, namely the Tongji Maternal and Child Health Cohort (TMCHC). The TMCHC was designed to investigate maternal dietary and lifestyle effects on the outcomes of both mothers and their offspring in Wuhan, China (22). Briefly, 8649 women who received prenatal care before 16 wk of gestation were recruited between January 2013 and May 2016. The response rate was 65.7%. Exclusion criteria included prepregnancy diabetes; clinically significant neurological, endocrinological, or other systemic diseases; and multiple pregnancies. All of the enrolled pregnant women received a regular prenatal checkup in the hospital and a fasting blood sample was collected if women consented. All of the participants were also invited to undergo an oral-glucose-tolerance test (OGTT) during 24–32 wk of gestation to screen for GDM. A total of 5667 (65.5%) participants screened for GDM within the prescribed period of time, and the incidence of GDM was 10.8%. On this basis, we excluded 3101 participants who did not provide fasting blood samples before 20 wk of gestation. Finally, 276 members who developed GDM and provided enough fasting blood samples were included as cases in this analysis. Two controls were individually matched to each case from among women without GDM.

Data on socioeconomic status, demographic and lifestyle characteristics, and health information were collected by using a self-administered, structured questionnaire. To improve the reliability of the data, the questionnaire survey was performed by trained investigators through face-to-face interviews. Maternal fasting blood samples were collected in anticoagulative tubes and centrifuged at 1620 × *g* at 4°C for 5 min. Then, the plasma was separated and stored at –80°C for subsequent analysis of TMAO and other blood variables.

These 2 studies were approved by the ethics committee of Tongji Medical College. All of the participants gave informed written consent before they were included in the study. The trial was registered at www.clinicaltrials.gov as NCT03415295.

GDM case definition

GDM was diagnosed according to the American Diabetes Association criteria (23), which is based on the "one-step" approach recommended by the International Association of Diabetes and Pregnancy Study Groups. All women underwent a 75-g OGTT in the morning after an overnight fast, with measurement of fasting plasma glucose and at 1 and 2 h. The criteria for GDM diagnosis were to have ≥1 abnormal value for fasting glucose (≥5.1 mmol/L; 92 mg/dL), 1-h glucose (≥10.0 mmol/L; 180 mg/dL), or 2-h glucose (≥8.5 mmol/L; 153 mg/dL).

Control selection and matching

In the initial case-control study with a cross-sectional design, 1 control subject was selected for each GDM case according to age (±2 y), gestational age at blood sample collection (±2 wk), and parity.

In the nested case-control study, a random sample of 552 control subjects were selected among women without an indication of GDM and matched 2:1 to GDM cases ($n = 276$) on

the basis of age (±2 y), gestational age at blood sample collection (±2 wk), and parity.

Assessment of plasma TMAO concentrations

Plasma TMAO concentrations were determined by stable isotope dilution liquid chromatography with online electrospray ionization tandem mass spectrometry (AB SCIEX Q-Trap 4500; Applied Biosystems) in the Ministry of Education Key Laboratory of Environment and Health at Tongji Medical College of Huazhong University of Science and Technology. Samples (50 μL plasma) were mixed with 20 μL 500-μg/L internal standard composed of TMAO-trimethyl-d_9 (d9-TMAO) in acetonitrile and then extracted with the use of 200 μL acetonitrile by vortex for 1 min. The supernatant was recovered after centrifugation at 15,000 rpm at 4°C for 5 min. The extraction procedure was repeated twice. Afterward, the combined supernatant was evaporated to dryness at 45°C in a vacuum concentration system and reconstituted with 50 μL acetonitrile/water (vol:vol, 1:1), followed by mixing on a vortex for 1 min. The chromatographic separation was performed on a BETASIL Phenyl Column (2.1 × 150 mm, 3 μm; Thermo Scientific). The column temperature was set to 30°C, and the flow rate was 0.4 mL/min. The gradient elution consisted of 0.1% acetic acid in water (solvent A) and 0.1% acetic acid in acetonitrile (solvent B). TMAO and d9-TMAO were monitored in positive-ion mode with multiple reaction monitoring of precursor and characteristic product-ion transitions of *m/z* 76→58 and 85→66, respectively (24). Various concentrations of non–isotopically labeled TMAO were mixed with a fixed amount of internal standard d9-TMAO to prepare the calibration curves for quantification of plasma TMAO. For quality assurance, 4 different quality-control samples with TMAO concentrations ranging between 4 and 400 μg/L were used for the evaluation of accuracy and precision. The accuracy of quality-control samples was within the range of 85–105% of the nominal values, the intra- and interassay CVs were <3%, and the absolute recovery was between 88% and 109%. All of the assays were performed without knowledge of GDM status.

Assessment of covariates

Maternal demographic, lifestyle, and health information was obtained from self-administered structured questionnaires. Prepregnancy BMI was calculated as self-reported prepregnancy weight divided by the square of height (kg/m^2). Fasting plasma glucose and serum lipid profile, including total cholesterol, triglycerides, HDL cholesterol, and LDL cholesterol, were analyzed with the use of commercial assay kits (Biosino Bio-Technology and Science, Inc.). ELISA kits (Mercodia Company) were used to measure the concentrations of fasting plasma insulin. Insulin resistance was evaluated according to the HOMA-IR index using the following equation: fasting glucose (mmol/L) × fasting insulin (μU/mL)/22.5.

Statistical analysis

Continuous variables are summarized as means ± SDs if normally distributed and medians with IQRs if not normally distributed. Differences of characteristics between cases and controls were assessed by Student's *t* test (normal distribution) or Mann-Whitney *U* test (nonnormal distribution) for continuous variables and chi-square test for categorical variables. Conditional logistic regression was used to estimate ORs of GDM by quartiles of plasma TMAO, with cutoffs defined by the distribution of plasma TMAO concentrations among control subjects. Adjustments were made for potential confounding variables, including age, prepregnancy BMI, gestational age at blood sample collection, parity, family history of diabetes, drinking habits, and smoking status. Tests for linear trend were conducted by using the median value for each quartile and treating it as a continuous variable in the conditional logistic regression model. Then, a logarithmic transformation was used to improve the normality of the plasma TMAO distributions and ORs (95% CIs) of GDM associated with an SD increment in the ln-transformed TMAO were calculated. To further explore the potential nonlinearity of the relation between plasma TMAO and odds of GDM, a restricted cubic spline model was used with 4 knots at the 5th, 35th, 65th, and 95th percentiles of plasma TMAO concentration via Stata version 13 (StataCorp). All of the other statistical analyses were conducted with SPSS 20.0 software package (SPSS, Inc.). *P* values presented are 2-tailed with a significance level of 0.05.

RESULTS

Case-control study with a cross-sectional design

Characteristics of the participants

General anthropometric and metabolic characteristics of the 866 participants (433 cases of GDM and 433 controls) are summarized in **Table 1**. There were no significant differences in age, parity, and gestational age between groups. Compared with control subjects, womenwho developed GDM were more likely to have a family history of diabetes, higher prepregnancy BMI, higher 1-h OGTT and 2-h OGTT, and higher concentrations of triglycerides, fasting plasma glucose, insulin, and plasma TMAO. With regard to insulin sensitivity, higher HOMA-IR was noted in women with GDM.

Association between plasma TMAO concentration and GDM

Higher plasma TMAO concentrations were associated with increased odds of GDM. The adjusted OR of GDM comparing the highest with the lowest quartile of plasma TMAO was 1.94 (95% CI: 1.28, 2.93) after adjustment for age, prepregnancy BMI, gestational age at blood sample collection, parity, family history of diabetes, drinking habits, and smoking (**Table 2**). The multivariable-adjusted OR for GDM per SD increment of ln-transformed plasma TMAO was 1.22 (95% CI: 1.05, 1.41).

In the restricted cubic spline model, potential nonlinearity of the relation between plasma TMAO and odds of GDM was found (**Supplemental Figure 2**A).

Nested case-control study with a prospective design

Characteristics of the participants

There were no relevant differences in age, parity, and gestational age between cases and control subjects (**Table 3**). Women

TABLE 1
Characteristics among women with GDM and their matched controls: initial case-control study[1]

Characteristics	GDM ($n = 433$)	Non-GDM ($n = 433$)	P
Age, y	29.81 ± 4.05	29.43 ± 3.72	0.157
Parity, *n* (%)			1.000
1	350 (80.83)	350 (80.83)	
2	76 (17.55)	76 (17.55)	
≥3	7 (1.62)	7 (1.62)	
Gestational age at blood sample collection, wk	27.00 (25.50–29.00)	27.00 (25.35–29.00)	0.623
Prepregnancy BMI, kg/m^2	22.04 ± 3.18	20.85 ± 2.63	<0.001
Family history of diabetes, *n* (%)	102 (23.56)	47 (10.85)	<0.001
Alcohol consumers, *n* (%)	18 (4.16)	14 (3.23)	0.590
Smokers, *n* (%)	13 (3.00)	11 (2.54)	0.837
Fasting plasma glucose, mmol/L	5.16 (4.78–5.40)	4.63 (4.38–4.81)	<0.001
OGTT, mmol/L			
1 h	9.78 (8.71–10.80)	7.38 (6.50–8.36)	<0.001
2 h	8.51 (7.38–9.24)	6.60 (5.87–7.37)	<0.001
Fasting plasma insulin, μU/mL	9.25 (6.68–12.98)	7.30 (5.00–9.87)	<0.001
HOMA-IR	2.19 (1.48–3.09)	1.47 (1.00–2.01)	<0.001
Total cholesterol, mmol/L	5.28 (4.53–6.10)	5.18 (4.55–5.92)	0.208
Triglycerides, mmol/L	2.61 (2.00–3.31)	2.25 (1.73–3.01)	<0.001
LDL cholesterol, mmol/L	3.04 (2.34–3.84)	2.96 (2.35–3.63)	0.191
HDL cholesterol, mmol/L	1.39 (1.20–1.68)	1.43 (1.19–1.70)	0.849
TMAO, μg/L	83.00 (60.30–121.00)	76.90 (54.00–107.50)	0.008

[1] Values are *n* (%) for categorical data, means ± SDs for normally distributed data, or medians (IQRs) for nonnormally distributed data. Differences in characteristics between cases and controls were assessed by chi-square test for categorical variables and Student's *t* test (normal distribution) or Mann-Whitney *U* test (nonnormal distribution) for continuous variables. GDM, gestational diabetes mellitus; OGTT, oral-glucose-tolerance test; TMAO, trimethylamine-*N*-oxide.

who subsequently developed GDM had higher prepregnancy BMI; higher concentrations of fasting plasma glucose, insulin, and triglycerides; higher HOMA-IR index; and higher alcohol consumption. Meanwhile, plasma concentrations of TMAO before 20 gestational weeks were significantly higher among women with GDM, which was consistent with the results of the initial case-control study.

Plasma TMAO concentration in relation to subsequent odds of GDM

We observed similar results in the independent case-control study within a prospective cohort to findings from the former study. After adjustment for age, prepregnancy BMI, gestational age at blood sample collection, parity, family history of diabetes, drinking habits, and smoking, women in the highest quartile had increased odds of GDM (adjusted OR: 2.06; 95% CI: 1.28, 3.31) compared with women in the lowest quartile, and the multivariable OR for GDM per SD increment of ln-transformed plasma TMAO was 1.26 (95% CI: 1.08, 1.47) (**Table 4**).

Both the independent validation study and the initial case-control study showed a similar pattern of nonlinear association between plasma TMAO and GDM, which is depicted in Supplemental Figure 2B.

DISCUSSION

Our study, which included an initial phase and a validation phase in 2 independent populations, provides consistent evidence of a positive relation between plasma TMAO concentration

TABLE 2
Association between plasma TMAO concentration and GDM: initial case-control study[1]

	Quartile of plasma TMAO concentration					
	1 (≤54.10 μg/L)	2 (>54.10–76.95 μg/L)	3 (>76.95–108.00 μg/L)	4 (>108.00 μg/L)	P-trend[2]	Per SD increment of ln(TMAO)
GDM cases/controls, *n*/*n*	81/108	106/109	106/108	140/108		
Crude model	1	1.26 (0.86, 1.83)	1.26 (0.86, 1.85)	1.69 (1.16, 2.46)	0.008	1.19 (1.04, 1.36)
Model 1	1	1.29 (0.87, 1.92)	1.30 (0.86, 1.95)	1.79 (1.20, 2.65)	0.005	1.19 (1.04, 1.37)
Model 2	1	1.34 (0.89, 2.03)	1.20 (0.78, 1.84)	1.94 (1.28, 2.93)	0.003	1.22 (1.05, 1.41)

[1] Values are ORs (95% CIs). Model 1 was a conditional logistic regression model that adjusted for age (years) and prepregnancy BMI (kg/m^2). Model 2 was a conditional logistic regression model that adjusted as for model 1 plus gestational age at blood sample collection (weeks), parity (1, 2, or ≥3), family history of diabetes (yes or no), drinking habits (yes or no), and smoking (yes or no). GDM, gestational diabetes mellitus; TMAO, trimethylamine-*N*-oxide.

[2] Tests for linear trend were conducted by using the medium value for each quartile and treating it as a continuous variable in the conditional logistic regression.

TABLE 3
Baseline characteristics among women with GDM and their matched controls: nested case-control study within a prospective cohort[1]

Characteristics	GDM ($n = 276$)	Non-GDM ($n = 552$)	P
Age, y	29.51 ± 3.25	29.33 ± 3.11	0.463
Parity, *n* (%)			1.000
1	228 (82.61)	456 (82.61)	
2	47 (17.03)	94 (17.03)	
≥3	1 (0.36)	2 (0.36)	
Gestational age at blood sample collection, wk	16.50 (16.10–17.30)	16.50 (16.10–17.20)	0.572
Prepregnancy BMI, kg/m^2	21.85 ± 3.15	20.84 ± 2.51	<0.001
Family history of diabetes, *n* (%)	28 (10.14)	44 (7.97)	0.217
Alcohol consumers, *n* (%)	6 (2.17)	3 (0.54)	0.030
Smokers, *n* (%)	5 (1.81)	16 (2.90)	0.525
Fasting plasma glucose, mmol/L	4.58 (4.16–5.22)	4.40 (4.02–4.83)	<0.001
Fasting plasma insulin, μU/mL	6.47 (4.13–11.49)	4.86 (3.38–7.49)	<0.001
HOMA-IR	1.28 (0.84–2.48)	0.93 (0.63–1.46)	<0.001
Total cholesterol, mmol/L	4.85 (4.24–5.45)	4.80 (4.31–5.36)	0.490
Triglycerides, mmol/L	1.76 (1.35–2.38)	1.58 (1.29–2.02)	<0.001
LDL cholesterol, mmol/L	2.72 (2.16–3.40)	2.62 (2.14–3.20)	0.113
HDL cholesterol, mmol/L	1.55 (1.26–1.79)	1.52 (1.26–1.83)	0.992
TMAO, μg/L	67.00 (45.00–103.25)	59.20 (40.85–86.33)	0.002

[1] Values are *n* (%) for categorical data, means ± SDs for normally distributed data, or medians (IQRs) for nonnormally distributed data. Differences in characteristics between cases and controls were assessed by chi-square test for categorical variables and Student's *t* test (normal distribution) or Mann-Whitney *U* test (nonnormal distribution) for continuous variables. GDM, gestational diabetes mellitus; TMAO, trimethylamine-*N*-oxide.

and GDM. In the case-control study with a cross-sectional design, we found that higher plasma TMAO was associated with increased odds of GDM, and the association remained rather consistent after multivariable adjustment. In order to clarify the temporal relation of the observed association, we conducted an independent nested case-control study, which also showed that plasma TMAO concentrations in early and midpregnancy were significantly and positively related to subsequent odds of GDM.

According to previous studies, the mean/median concentrations of plasma TMAO in humans were in the range of 1.43–3.70 μmol/L (108.68–281.20 μg/L) in healthy individuals (9, 16, 24), 2.39–7.50 μmol/L (181.64–570.00 μg/L) in diabetic patients (13, 16, 25), and 1.80–5.00 μmol/L (136.80–380.00 μg/L) in patients with cardiovascular disease (9, 15, 26), which were higher than our measurements in pregnant women in China. The difference may be attributed to several factors. First, the majority of the aforementioned studies were conducted in the United States and Europe where the consumption of red meat and fat is much higher than in China (27–29). As has been noted previously, dietary factors might be the most important regulators of circulating TMAO concentrations (30). Therefore, the low average intake of foods rich in choline and L-carnitine among our participants may be the leading cause of lower plasma TMAO in our study. Second, racial differences and the diversity of the gut microbiota or the activity of hepatic FMO3 may contribute to the difference (31, 32). Third, during physiologic human pregnancy, plasma volume increases by >1 L, on average, compared with

TABLE 4
Risk of GDM according to quartiles of plasma TMAO concentration measured before 20 wk of gestation: nested case-control study within a prospective cohort[1]

	Quartile of plasma TMAO concentration					
	1 (≤40.85 μg/L)	2 (>40.85–59.20 μg/L)	3 (>59.20–86.32 μg/L)	4 (>86.32 μg/L)	P-trend[2]	Per SD increment of ln(TMAO)
GDM cases/controls, *n*/*n*	48/138	65/137	70/139	93/138		
Crude model	1	1.41 (0.90, 2.20)	1.52 (0.96, 2.40)	2.05 (1.32, 3.17)	0.002	1.27 (1.10, 1.47)
Model 1	1	1.39 (0.88, 2.22)	1.56 (0.96, 2.51)	2.14 (1.36, 3.39)	0.001	1.29 (1.11, 1.50)
Model 2	1	1.42 (0.88, 2.29)	1.56 (0.95, 2.55)	2.06 (1.28, 3.31)	0.004	1.26 (1.08, 1.47)

[1] Values are ORs (95% CIs). Model 1 was a conditional logistic regression model that adjusted for age (years) and prepregnancy BMI (kg/m^2). Model 2 was a conditional logistic regression model that adjusted as for model 1 plus gestational age at blood sample collection (weeks), parity (1, 2, or ≥3), family history of diabetes (yes or no), drinking habits (yes or no), and smoking (yes or no). GDM, gestational diabetes mellitus; TMAO, trimethylamine-*N*-oxide.

[2] Tests for linear trend were conducted by using the median value for each quartile and treating it as a continuous variable in the conditional logistic regression.

nonpregnant conditions (33), which could lead to the dilution of plasma TMAO. Furthermore, women are prone to alter their diets during pregnancy (34). Loss of appetite and morning sickness could also lead to lower intakes of dietary choline and L-carnitine, particularly in the first and second trimesters. Therefore, future investigations in other study populations are needed to confirm our findings.

To our knowledge, our study is the first to prospectively examine the relation between plasma TMAO and GDM. As a microbiota-dependent metabolite, TMAO was first identified to be strongly associated with the risk of cardiovascular disease in a large independent clinical cohort by Wang et al. (8), and studies in mice indicated a causal relation. Since then, TMAO has attracted attention and increasing studies have found that TMAO is associated with diabetes (13–16), kidney failure (35), and cancer (36). In addition, increased plasma TMAO has been linked to higher mortality risk independent of glycemic control in patients with type 2 diabetes (17). Our findings with regard to the positive association between plasma TMAO concentration and subsequent odds of GDM are in line with previous observations of type 2 diabetes among nonpregnant individuals (13–16, 20, 25, 26). Only one exploratory nuclear magnetic resonance metabonomics study that aimed to correlate biofluid metabolic changes with prenatal disorders indicated that plasma TMAO concentrations were lower in subjects with GDM (19), which is inconsistent with our findings. Considering that the sample size of this study was small (29 GDM cases and 25 controls) and the GDM cases and controls were not well matched, the results should be interpreted with caution. The nonlinear dose-response associations between TMAO and GDM risk in this study warrant further investigation.

The exact biological mechanisms that explain how plasma TMAO concentrations are involved in glucose metabolism still need to be clarified. First, TMAO was elucidated to exacerbate impaired glucose tolerance, obstruct hepatic insulin signaling, and promote adipose tissue inflammation (11). Consistently, the inhibition of FMO3, which resulted in reduced TMAO concentrations, was observed with significant decreases in plasma glucose and insulin (12). Second, one study, conducted by Miao et al. (37), identified the enzyme FMO3 to be a target of insulin. They found that liver insulin receptor knockout mice were unable to respond to insulin and subsequently had markedly elevated FMO3 expression along with plasma TMAO concentrations. Then, knockdown of FMO3 in insulin-resistant mice entirely normalized glucose tolerance and improved insulin tolerance through the suppression of forkhead box O1, a central node for metabolic control. This report suggests that the FMO3-TMAO pathway may play an essential part in the pathophysiology of diabetes and other metabolic dysfunction. In addition, an increase in plasma TMAO could lead to significantly lower expression of the key bile acid synthetic enzymes and multiple bile acid transporters. Current studies have suggested that bile acids are signaling molecules and could regulate glucose metabolism and inflammation (38), and lowering bile acid pool size with nuclear farnesoid X receptor agonist could induce obesity and diabetes through reduced energy expenditure (39).

Although our data provide evidence of an association between higher plasma TMAO concentrations and GDM, the involvement of TMAO in any causal or compensatory pathway has not been proven. There might also be noncausal explanations of the observed association between TMAO and GDM. For example, altered TMAO concentrations could simply be an indicator of changes in gut microbiota composition. Accumulating evidence suggests that an altered gut microbiota may be linked to cardiometabolic diseases including type 2 diabetes (40), GDM (41), obesity (42), and cardiovascular diseases (43). Examination of the proportion of specific bacterial genera and plasma TMAO concentrations showed that several taxa were simultaneously significantly associated with both vegetarian compared with omnivore status and plasma TMAO concentrations (10). These results indicated that the gut microbiota and its composition might play an important role in the association between plasma TMAO and GDM. In addition, the potential confounding due to the consumption of meat products or dietary patterns could elevate the risk of GDM in a mechanism independent of TMAO, which needs further exploration in future studies.

Our study has several notable strengths. First, to our knowledge, this is the first population-based study systematically exploring the relation between plasma TMAO and GDM. Second, we conducted 2 case-control studies in different populations including one case-control study with a cross-sectional design followed by a nested case-control study with a prospective design, which allowed us to clarify the robustness and temporal sequence of the association. Third, all of our GDM cases were defined by an OGTT test, and each case was well matched with 1 (in the initial case-control study) or 2 (in the nested case-control study) controls in order to better control the confounders.

Although we observed very consistent findings in the 2-phase studies in independent populations, several limitations of our study should also be acknowledged. First, in China, the typical diet contains less red meat and other animal products than in some countries, which leads to lower TMAO concentrations in Chinese populations. Thus, the potentially limited generalizability is noteworthy, and further studies are needed to determine if the findings are generalizable to other populations. Second, our studies were conducted only in a specific city in China, and generalizability may even be limited to other cities in China. Third, we did not have enough dietary intake data on the subjects to perform the analysis between dietary factors and plasma TMAO. Future studies are suggested to take this into account. Fourth, although we controlled for multiple GDM risk factors, such as age, prepregnancy BMI, gestational age, and parity, we could not rule out the possibility of residual confounding by other unmeasured factors such as data on plasma choline and carnitine and changes during pregnancy that may affect diet, such as morning sickness.

In conclusion, our study suggests a positive association between plasma TMAO concentrations and GDM. Future studies are warranted to confirm our findings in other populations and to elucidate the underlying mechanisms.

The authors' responsibilities were as follows—PL, NY, and LL: designed the research; PL, CZ, SL, TS, HH, XC, YZ, XH, XP, XZ, and JC: contributed to the data collection; PL and CZ: analyzed the data; PL and LL: wrote the manuscript; WB, ZS, and FBH: edited the manuscript; LL and NY: are the guarantors of this work and have full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data

analysis; and all authors: read and approved the final manuscript. None of the authors declared a competing interest.

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