Association of Maternal Folate and Vitamin B_{12} in Early Pregnancy With Gestational Diabetes Mellitus: A Prospective Cohort Study

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OBJECTIVE

To investigate the association of folate and vitamin B_{12} in early pregnancy with gestational diabetes mellitus (GDM) risk.

RESEARCH DESIGN AND METHODS

The data of this study were from a subcohort within the Shanghai Preconception Cohort Study. We included pregnancies with red blood cell (RBC) folate and vitamin B_{12} measurements at recruitment (between 9 and 13 gestational weeks) and those with three samples available for glucose measurements under an oral glucose tolerance test. GDM was diagnosed between 24 and 28 weeks' gestation. Odds ratio (OR) and 95% CI of having GDM was used to quantify the association.

RESULTS

A total of 1,058 pregnant women were included, and GDM occurred in 180 (17.01%). RBC folate and vitamin B_{12} were significantly higher in pregnancies with GDM than those without GDM (P values were 0.045 and 0.002, respectively) and positively correlated with 1-h and 2-h serum glucose. Daily folic acid supplementation in early pregnancy increases the risk of GDM; OR (95% CI) was 1.73 (1.19–2.53) ($P = 0.004$). Compared with RBC folate <400 ng/mL, pregnancies with RBC folate ≥600 ng/mL were associated with ~1.60-fold higher odds of GDM; the adjusted OR (95% CI) was 1.58 (1.03–2.41) ($P = 0.033$). A significant trend of risk effect on GDM risk across categories of RBC folate was observed ($P_{trend} = 0.021$). Vitamin B_{12} was significantly associated with GDM risk (OR 1.14 per 100 pg/mL; $P = 0.002$). No significant association of serum folate and percentile ratio of RBC folate/vitamin B_{12} with GDM was observed.

CONCLUSIONS

Higher maternal RBC folate and vitamin B_{12} levels in early pregnancy are significantly associated with GDM risk, while the balance of folate/vitamin B_{12} is not significantly associated with GDM.

As one of the most common pregnancy complications, gestational diabetes mellitus (GDM) affects ~17% of pregnancies worldwide (1). In China, ~2.9 million pregnant women suffer from this disorder (2). GDM has long-term adverse outcomes in both mothers and offspring (3). Despite its serious complications, the diagnosis of GDM is

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not performed until the late second or early third trimester (4). Investigating modifiable risk factors in early pregnancy stage would more significantly contribute to the early prevention of GDM.

Folate and vitamin B_{12}, metabolically entwined during one-carbon metabolism, are both key nutrients in early pregnancy and involved in the DNA methylation and cell metabolism (5–7). Folic acid supplementation (FAS) of 0.4 mg/day is conventionally recommended for women of the childbearing age before and during the first trimester of pregnancy for the essential role of folate in the prevention of neural tube defects (NTDs) (8,9). The mandatory folic acid fortification aimed to alleviate micronutrient deficiencies has been implemented by >50 countries (10). Nevertheless, the relationship between folate and GDM risk with inconsistent findings has emerged as a field of interest. The Nurses' Health Study II including 14,533 women has observed that FAS before pregnancy is associated with a lower risk of GDM (11); in contrast, another cohort study gave opposite conclusions that daily folic acid intake in early pregnancy increased the risk of GDM (12), reminding us that the association of folate with GDM is still equivocal.

Compared with serum folate, red blood cell (RBC) folate responds slower to changes in folate intake and represents the long-term folate status, because the erythrocytes have a 120-day life span and only accumulate folate during erythropoiesis (13). As serum folate is an indicator of recent folate intake and is dramatically affected by FAS, these case-control studies are subject to bias derived from dietary folic acid (13). As such, investigating the association of RBC folate with GDM will be more helpful to elucidate the long-term exposure of folate and GDM risk. A 15-year national cohort observed higher RBC folate is associated with an increased risk of death among adults with diabetes (14). Xie et al. (15) found maternal RBC folate concentrations during the second trimester significantly in association with an increased risk of GDM, which is diagnosed in the same period of gestation. Whether maternal RBC folate exposure in early pregnancy associated with the incidence of GDM has not been investigated.

Vitamin B_{12} deficiency has been associated with risk of metabolic abnormalities in pregnant women, such as insulin resistance, fatty acids, and GDM (16).

Imbalanced levels of folate and vitamin B_{12} increase the risk of being born small for gestational age (17). Increasing concerns have risen about supplementing high doses of folic acid to women of reproductive age with low vitamin B_{12} intake, although this issue has not been well investigated yet (5). Several case-control studies demonstrated that higher serum folate/vitamin B_{12} ratio was positively associated with GDM risk (18,19). However, to our knowledge, no studies have investigated the relationship between maternal vitamin B_{12} in early pregnancy and GDM. In this study, we aimed to evaluate the association of maternal folate, vitamin B_{12}, and percentile ratio of folate/vitamin B_{12} in early pregnancy with GDM using a prospective cohort study. The findings of this study would be useful to provide a theoretical basis for early prevention of GDM.

RESEARCH DESIGN AND METHODS

Study Population
This study was conducted within the Shanghai Preconception Cohort (SPCC; NCT02737644) that aims to investigate the associations between periconceptional maternal key nutrients and neonatal health outcomes, including congenital heart disease (20). Two subcohorts consisting of 938 and 458 subjects were derived for studying the associations of RBC folate with congenital heart diseases and infant atopic dermatitis. These two subcohorts thus formed the source of our study population. We restricted our study sample to pregnancies with folate and vitamin B_{12} measurements examined at recruitment between 9 and 13 gestational weeks and with three glucose measurements under an oral glucose tolerance test (OGTT), resulting in 1,058 pregnancies included in the final analysis.

Baseline characteristics were obtained by a standardized self-reported questionnaire via face-to-face interviewers. The preconceptional BMI was calculated through self-report prepregnancy weight and height. Smoking exposure was defined as self-smoker or exposed to second-hand cigarette smoking during pregnancy. Alcohol drinking was defined as drinking any alcoholic beverages 3 months before or during pregnancy. FAS was defined as a regular intake of folic acid tablets or compound vitamin supplements after the last menstrual period and during pregnancy. The folic acid tablet contains

400 μg/pill of folic acid, and the compound vitamin supplements contain 400 μg/pill or 800 μg/pill depending on the brands. Written informed consent was obtained from each study subject before recruitment. This study was approved by the Ethics Committee of the Children's Hospital of Fudan University (Shanghai, China).

Diagnosis of GDM
All the pregnant women underwent a 75-g OGTT during 24–28 gestational weeks, and diagnosis of GDM was made when any of the following criteria were met according to the recommendations of the International Association of the Diabetes and Pregnancy Study Groups Consensus Panel (4): fasting glucose $\geq$5.1 mmol/L, 1-h glucose $\geq$10.0 mmol/L, or 2-h glucose $\geq$8.5 mmol/L.

Blood Sample Collection and Nutritious Biomarker Measurements
The details of blood sample collection and nutritious biomarker measurements were described elsewhere (20). Briefly, the remaining blood samples after routine examinations at the first antenatal visit were collected by trained staff. These samples were temporarily stored in a refrigerator at 4°C for dispensing within 6 h and were transferred to another refrigerator at −20°C. Serum and whole blood were then transported by trained investigators to the central laboratory for storage in freezers at −80°C. Given the effect of light on folate measurements, lightproof tubes completely protected from light were used when transported and stored. RBC folate, serum folate, and vitamin B_{12} levels were measured in an ETDA anticoagulated blood sample according to a standard protocol. These biomarkers were measured using electrochemiluminescence assays (ARCHITECT i2000SR Analyzer; Abbott Laboratories) by professional staff. Three standard solutions (quality controls $[QC]_1$, QC_2, and QC_3) were used as daily QC. The interassay coefficient of variation of QC_1–QC_3 for serum folate, vitamin B_{12}, and RBC folate was <7.5%, and the intra-assay coefficient of variation was <6.5% for the entire study population.

Statistical Analysis
Continuous data were summarized as mean (SD) or median (interquartile range),

and categorical data were displayed as percentages. An unpaired Student t test was used to test the differences in quantitative variables with a normal distribution. Otherwise, the equivalent nonparametric test was used. Categorical variables were compared with χ^2 tests. RBC folate concentrations >400 ng/mL (906 nmol/L) have been recommended as the optimal cutoff level for preventing NTDs in women of reproductive age (21). Additionally, RBC folate concentrations >600 ng/mL have been chosen as a cutoff for high RBC folate according to the Canadian Health Measures Survey (22). Accordingly, RBC folate concentrations were categorized as <400, 400–600, and ≥600 ng/mL in the current study. Vitamin B_{12} and serum folate were categorized into tertiles because there is no specified cutoff for pregnancy. We used multivariable logistic regression analyses to investigate the associations of RBC folate, serum folate, and vitamin B_{12} with GDM and treated these biomarkers as continuous and categorical variables, with adjustment for age, preconceptional BMI, family history of diabetes, smoking exposure, and drinking status. To further examine the effects of combinations of folate and vitamin B_{12} status on the risk of GDM, we used percentiles of RBC folate, serum folate, and vitamin B_{12} to derive ratios of RBC folate/vitamin B_{12} and serum folate/vitamin B_{12}. Percentile ratios were categorized into tertiles for the association analyses with the GDM risk. According to previous studies (19), the folate/vitamin B_{12} ratio was also calculated based on their raw data. Moreover, the restricted cubic spline (RCS) regression model with assumed three knots was used to outline the potential nonlinear relations among continuous RBC folate, vitamin B_{12}, and GDM risk. The association of serum folate at the time of OGTT with GDM based on a subgroup of our study subjects ($n = 458$) was also conducted. Serum folate change was calculated by the levels in early pregnancy subtracted by the levels in midgestation. Odds ratio (OR) and 95% CI were reported. Correlation analysis was used to evaluate the relationship among RBC folate, vitamin B_{12}, fasting serum glucose, and 1-h and 2-h serum glucose. Stata version 16.0 and R package (version 3.6.1) were used for all statistical analyses. A two-tailed P value of 0.05 was defined as statistical significance.

RESULTS

Baseline Characteristics

The demographic characteristics and nutrient levels of the study population are summarized in Table 1. The mean ($\pm$ SD) age and preconceptional BMI of the pregnant women were 30.24 ($\pm$ 3.97) years and 21.03 ($\pm$ 2.81) kg/m^2, respectively. Among the pregnant women, 38.75%, 15.60%, and 13.42%, respectively, had a family history of diabetes, smoking exposure, and alcohol consumption during gestation. FAS was found in 63.70% (674 out of 1,058) of the pregnant women, of whom 96.43% (650 out of 674) took compound vitamin supplements. The median (interquartile range) concentrations of RBC folate, serum folate, and vitamin B_{12} were 385.92 (289.97–529.68), 14.39 (12.60–16.80), and 405.93 (298.00–485.00) (Supplementary Fig. 1).

Comparison of RBC Folate, Serum Folate, and Vitamin B_{12}

Among 1,058 pregnancies, GDM occurred in 180 women (17.01%) with a mean follow-up time of 11.1 $\pm$ 1.5 gestational weeks. Compared with the group without GDM, median RBC folate and vitamin B_{12} levels in early pregnancy were significantly higher in the group with GDM (for RBC folate: 426.44 vs. 380.95 ng/mL, $P = 0.045$; for vitamin B_{12}: 421.00 vs. 364.00 pg/mL, $P = 0.002$). No significant differences in serum folate concentration between GDM and non-GDM were found (Supplementary Fig. 2).

Associations of Maternal Folate Status and Vitamin B_{12} With GDM Risk

FAS was associated with an increased risk of GDM (adjusted OR [aOR] 1.73 [95% CI 1.19–2.53]; $P = 0.004$). No significant association was found between RBC folate and serum folate levels as continuous variables with GDM (for RBC folate: aOR 1.07 [95% CI 0.99–1.15]; $P = 0.08$; for serum folate: aOR 1.01 [95% CI 0.97–1.05]; $P = 0.43$) (Table 2). Compared with pregnant women who had RBC folate levels <400 ng/mL, those with RBC folate ≥600 ng/mL were associated with ~1.60-fold higher odds of GDM (aOR 1.58 [95% CI 1.03–2.41]; $P = 0.033$). An obvious increased risk trend

Table 1—Demographic characteristics of the study population (N = 1,058)

Characteristics	Summary
Age (years)	30.2 ± 4.0
Preconception BMI (kg/m^2)	21.0 ± 2.8
<18.5	170 (16.1)
18.5–24	748 (70.7)
>24	140 (13.2)
Family history of diabetes [n (%)]	
Yes	410 (38.7)
No	648 (61.3)
Smoking exposure [n (%)]	
Yes	165 (15.6)
No	893 (84.4)
Alcohol consumption [n (%)]	
Yes	142 (13.4)
No	916 (86.6)
FAS [n (%)]	
Compound vitamin supplements	650 (61.4)
Folic acid tablet	24 (2.3)
No	384 (36.3)
RBC folate (ng/mL)	385.92 (290.0–529.7)
<400	557 (52.6)
400–600	301 (28.5)
≥600	200 (18.9)
Serum folate (ng/mL)	14.39 (12.6–16.8)
<25.5	1,045 (98.8)
≥25.5	13 (1.2)
Vitamin B_{12} (pg/mL)	405.93 (298.0–485.0)
<200	113 (10.7)
≥200	945 (89.3)

Continuous variables are presented as means ± SD or median (interquartile range), and categorical variables are listed as n (%). No missing data existed in the current analysis.

across RBC folate groups with GDM risk was observed (P_{trend} = 0.021).

Vitamin B$_{12}$ level was significantly associated with GDM risk (OR 1.14 per 100 pg/mL [95% CI 1.04–1.24]; P = 0.002). Of note, pregnant women in the upper tertile of vitamin B$_{12}$ had approximately twofold higher odds of GDM (aOR 2.00 [95% CI 1.35–2.96]; P = 0.001). We also investigated the nonlinear relationship among RBC folate, vitamin B$_{12}$, and GDM risk. The RCS regression model revealed that higher levels of RBC folate and vitamin B$_{12}$ were associated with an increased risk of GDM in a nonlinear fashion (Fig. 1).

Moreover, RBC folate concentration in early pregnancy was positively correlated with OGTT 1-h and 2-h serum glucose levels, with the correlation coefficient r values being 0.19 and 0.11, respectively (both P < 0.001). Similar correlations were observed between vitamin B$_{12}$ and 1-h and 2-h serum glucose level (both r values were 0.13; P < 0.001).

Associations of Folate/Vitamin B$_{12}$ Balance With GDM Risk

A greater serum folate/vitamin B$_{12}$ ratio was associated with a decreased GDM risk (aOR 0.98 [95% CI 0.97–0.99]; P = 0.009) (Supplementary Table 1). The risk of GDM became significantly lower in the Q3 tertile compared with that in the Q1 tertile (aOR 0.60 [95% CI 0.41–0.98]; P =

0.042) and showed a significant trend (P_{trend} = 0.041). However, no significant association was found between RBC folate/vitamin B$_{12}$ ratio and GDM. When analyzed as percentile ratios, neither RBC folate/vitamin B$_{12}$ nor serum folate/vitamin B$_{12}$ was significantly associated with GDM (Table 3).

Associations of Serum Folate in Midgestation With GDM Risk

Among a subgroup of our study cohort with available serum folate examined at midgestation, we found that higher serum folate levels at the time of OGTT were associated with a higher risk of GDM (aOR 1.08 [95% CI 1.03–1.14]; P = 0.002) (Supplementary Table 2). More pronounced effects were found when analyzed using serum folate tertiles (highest vs. lowest tertiles: aOR 2.54 [95% CI 1.28–5.03]; P = 0.007). However, the change in serum folate was negatively associated with GDM risk (aOR 0.95 [95% CI 0.90–0.99]; P = 0.019), indicating an association of greater decrease of serum folate from early to midgestation with a lower risk of GDM.

CONCLUSIONS

In this prospective cohort, we investigated the association of maternal folate, vitamin B$_{12}$, and percentile ratios of folate/vitamin B$_{12}$ in early pregnancy

with the incidence of GDM. We found an increased risk of GDM in a dose-response manner across RBC folate and vitamin B$_{12}$ concentrations and across their corresponding categories during early pregnancy. Supportive findings include positive correlations between the two biomarkers with OGTT 1-h and 2-h serum glucose levels. However, we did not find a significant impact of the balance of the two biomarkers measured by percentile ratio with GDM.

Folate is one of the key nutrients for pregnant women for its protective effect in preventing birth defects. Two large studies have assessed the association of periconceptional FAS with subsequent GDM risk (11,12) but with inconsistent findings. While preconception FAS was associated with reduced GDM risk in the Nurses' Study (11), opposite findings were found for FAS during early pregnancy in the China-Anhui Birth Cohort (12). However, due to variations in individual folate metabolism, periconceptional folate status evaluated by FAS via questionnaire cannot directly reflect the folate levels in the body. In a case-control study of 2,282 Chinese pregnancies, Xie et al. (15) found that RBC folate during midpregnancy (19–24 weeks' gestation) was linked with a 1.16-fold increased risk of GDM. Nevertheless, it is hard to infer that higher folate levels result in GDM, because both the RBC folate and GDM diagnosis were achieved in almost the same period of gestation. In the current study, we were able to directly measure RBC folate levels in early pregnancy. We found that the relationship between RBC folate status with GDM was nonlinear based on our RCS model, and RBC folate of >400 ng/mL, especially >600 ng/mL, conferred as much as a 1.6-fold higher odds of GDM. The findings were supported by the positive correlations between RBC folate with 1-h and 2-h serum glucose, which was consistent with recently published results (15,19). To our knowledge, our study provides the first evidence that the higher RBC folate concentration in early pregnancy may confer subsequent GDM risk. Given the protective role of RBC folate for NTDs and other folate-sensitive defects (21), it is justifiable to maintain an optimal level of ~400 ng/mL for women with baseline RBC levels less than this value. However, for those with a RBC folate level of >400 ng/mL,

Table 2—Association of maternal FAS, RBC folate, and vitamin B$_{12}$ in early pregnancy with GDM risk

Variables	GDM/total (%)	Model 1†			Model 2‡		
		OR	95% CI	P	OR	95% CI	P
FAS							
No	43/340 (12.7)		Reference			Reference	
Yes	137/718 (19.1)	1.62	1.12–2.35	0.010	1.73	1.19–2.53	0.004
RBC folate (ng/mL)*		1.08	1.00–1.17	0.033	1.07	0.99–1.15	0.08
<400	80/557 (14.4)		Reference			Reference	
400–600	56/301 (18.6)	1.36	0.93–1.98	0.10	1.39	0.94–2.04	0.09
≥600	44/200 (22.0)	1.68	1.11–2.53	0.013	1.58	1.03–2.41	0.033
Trend test				0.009			0.021
Serum folate (ng/mL)		1.01	0.97–1.04	0.53	1.01	0.97–1.05	0.43
Q1 (<13.9)	64/401 (16.0)		Reference			Reference	
Q2 (13.9–16.0)	36/255 (14.1)	0.86	0.55–1.34	0.52	0.91	0.58–1.44	0.71
Q3 (≥16.0)	80/402 (20.0)	1.30	0.91–1.87	0.14	1.36	0.94–1.99	0.10
Trend test				0.13			0.09
Vitamin B$_{12}$ (pg/mL)*		1.11	1.02–1.20	0.012	1.14	1.04–1.24	0.002
Q1 (<330)	51/397 (12.9)		Reference			Reference	
Q2 (330–429)	45/254 (17.7)	1.46	0.94–2.25	0.08	1.49	0.95–2.33	0.07
Q3 (≥429)	84/407 (20.6)	1.76	1.20–2.57	0.003	2.00	1.35–2.96	0.001
Trend test				0.003			0.001

*RBC folate and vitamin B$_{12}$ were divided by 100. †Univariate model. ‡Adjusted for age, preconceptional BMI, family history of diabetes, smoking exposure, and drinking status.
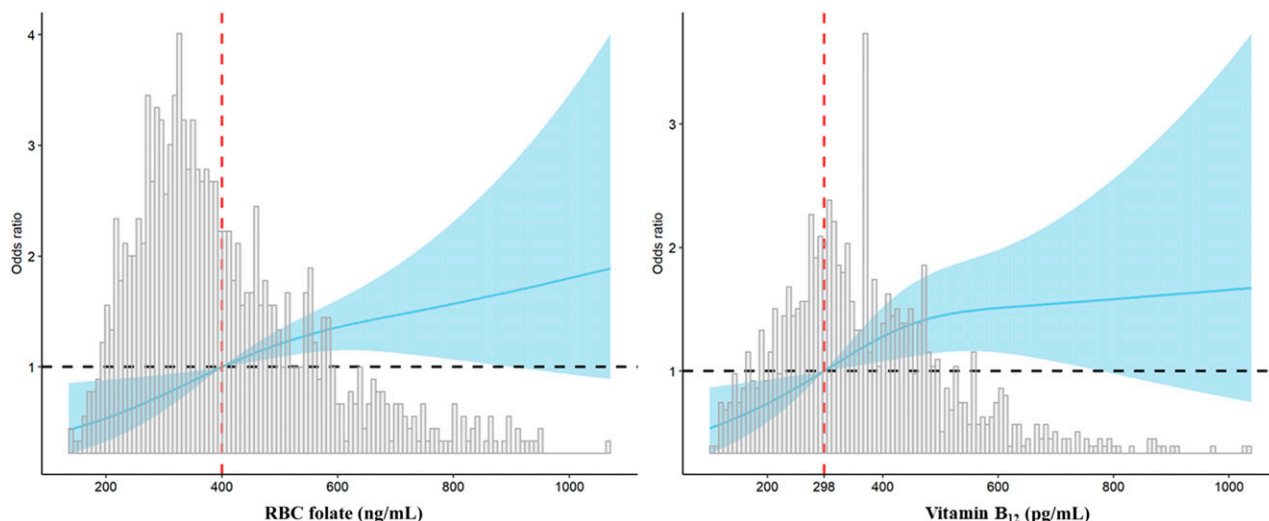
Figure 1—RCS regression analysis of RBC folate and vitamin B_{12} with GDM risk. *A*: RBC folate 400 ng/mL was selected as the reference level. *B*: The median of vitamin B_{12} 298 pg/mL was selected as the reference level. The lines indicate estimated ORs, and the light blue areas represent 95% CI.

attention should be paid to the potential increased risk of GDM as well as other health outcomes (23).

The association between serum vitamin B_{12} levels and GDM has been explored in two small prospective studies (24,25), in which vitamin B_{12} was measured after 24 weeks of gestation. In both studies, lower vitamin B_{12} levels were associated with an increased risk of GDM at 28–30 gestational weeks. On the contrary, we observed a positive association between vitamin B_{12} concentration in early pregnancy and GDM risk. Although the reason for this discrepancy is unknown, it may be because of the differences in the gestational time points when

determinations of vitamin B_{12} were made between the studies. Research has documented physiological changes influencing maternal vitamin B_{12} requirements and status during pregnancy, including a gradual decrease in vitamin B_{12} concentrations as pregnancy processed from preconception to midgestation (26,27). In addition, the proper preparation and storage of vitamin B_{12} are critical to obtaining accurate results. In the study by Krishnaveni et al. (24), vitamin B_{12} was examined in samples that were stored for 8 years and under no lightproof conditions, which might affect the association of vitamin B_{12} deficiency and GDM risk.

The mechanisms by which folate and vitamin B_{12} may confer an increased GDM risk are not well understood. Evidence from an animal model revealed that higher maternal folate concentration was positively associated with insulin resistance in offspring (28). A positive correlation between RBC folate and insulin resistance was observed in the obese population (29). In addition, folate was reported to play a role in the progression of type 1 diabetes via NK cell dysfunction in human subjects (30). Regarding vitamin B_{12}, higher levels of its circulating form in our study may be partially due to liver function impairment. About 50% of absorbed vitamin B_{12} is stored in the liver, and elevated serum vitamin B_{12} levels have been found in chronic viral hepatitis and cirrhosis and severe alcoholic liver disease (31,32). In our additional subgroup analysis, we found that higher γ-glutamyl transferase levels, which were an indicator of mild liver dysfunction and were associated with GDM risk (33), were higher in our group with GDM (Supplementary Fig. 3). We thus assumed pregnancies with GDM might have concurrent mild liver dysfunction. Consequently, the observed association of serum vitamin B_{12} in early pregnancy with GDM might reflect differences in liver function between the pregnancies with or without GDM.

Among a subgroup of the study sample, we also observed associations of higher serum folate levels at the time of the OGTT with a higher risk of GDM

Table 3—Association of percentile folate/vitamin B_{12} ratio in early pregnancy with GDM risk

Variables	GDM/total (%)	Model 1†			Model 2‡		
		OR	95% CI	P	OR	95% CI	P
RBC folate/vitamin B_{12}*		0.99	0.97–1.00	0.39	0.99	0.97–1.00	0.33
Q1 (<0.68)	72/409 (17.60)		Reference			Reference	
Q2 (0.68–1.62)	59/354 (16.67)	0.93	0.64–1.36	0.73	0.89	0.60–1.31	0.56
Q3 (≥1.62)	49/295 (16.61)	0.93	0.62–1.38	0.73	0.84	0.55–1.26	0.41
Trend test				0.71			0.40
<1	111/613 (18.10)		Reference			Reference	
≥1	69/445 (15.50)	0.82	0.59–1.15	0.26	0.77	0.55–1.09	0.15
Serum folate/vitamin B_{12}*		0.99	0.94–1.03	0.70	0.98	0.94–1.03	0.51
Q1 (>0.76)	63/336 (18.75)		Reference			Reference	
Q2 (0.76–1.24)	61/322 (18.94)	1.01	0.68–1.49	0.94	1.01	0.68–1.51	0.93
Q3 (≥1.24)	56/400 (14.00)	0.70	0.47–1.04	0.08	0.66	0.44–1.01	0.05
Trend test				0.07			0.05
<1	98/529 (18.52)		Reference			Reference	
≥1	82/529 (15.50)	0.80	0.58–1.11	0.19	0.78	0.56–1.09	0.15

*Based on percentile of concentrations. †Univariate model. ‡Adjusted for age, preconceptional BMI, family history of diabetes, smoking exposure, and drinking status.

(Supplementary Table 2), which confirmed the findings of previous studies (19). Furthermore, greater decrease of serum folate from early to midgestation was associated with a lower risk of GDM, supporting our hypothesis that high folate levels in early pregnancy could be a risk factor for GDM. Our novel findings raise an important question regarding the potential mechanism or causality for the development of GDM in subjects with vitamin insufficiency. Future studies longitudinally assessing the relationship of early pregnancy vitamin status in women with low folate and B$_{12}$ levels late in gestation with GDM may be helpful to explore the mechanism behind the positive associations of folate and vitamin B$_{12}$ with GDM.

Considering that both folate and vitamin B$_{12}$ are essential for nucleic acid synthesis, methyl group generation, and conversion of homocysteine to methionine, their balance may have important implications for maternal and neonatal health (5–7). A recent case-control study of 406 pregnant women reported that a higher serum folate/vitamin B$_{12}$ ratio midgestation (24–28 weeks) calculated based on raw values was associated with a higher risk of GDM (19). We extended their work to using RBC folate/vitamin B$_{12}$ ratio at early gestation by using a percentile ratio that is robust to differences in measurement scales. Intriguingly, we did not find any significant association between this ratio at early pregnancy and GDM. Unlike the work by Li et al. (19), in which directionally opposite associations were found for folate and vitamin B$_{12}$, these two markers showed directionally similar associations with GDM in our study. Besides, >95% of the pregnant women took multivitamins in which proportions of the two nutrients were fixed, and the levels of these two biomarkers correlated significantly in our population ($r = 0.12$; $P < 0.001$; data not shown). It is thus likely that the effects of RBC folate and vitamin B$_{12}$ on GDM may be confounded, and this may partially explain the disappeared association for GDM when the percentile ratio of RBC folate and vitamin B$_{12}$ was used.

This study has several limitations. First, selection bias may exist because we only included pregnancies with measurements of RBC folate, serum folate, and vitamin B$_{12}$ in early pregnancy from the large-scale SPCC, and the generalizability of our findings to other regions needs further validation. Second, residual confounding cannot be ruled out, as we did not have data on covariates, including physical activities, diet, medication use, and lipid levels. Third, the exploratory nature of our study without a prespecified power calculation precludes us from drawing any confirmative conclusions regarding the association among folate, vitamin B$_{12}$, and GDM.

In conclusion, we report that higher maternal RBC folate and vitamin B$_{12}$ levels in early pregnancy are significantly associated with GDM risk, while the balance of folate/vitamin B$_{12}$ is not significantly associated with GDM. Given the exploratory nature of this study, more investigations are needed to figure out the appropriate levels of folate and vitamin B$_{12}$ in early pregnancy that are essential for maternal and offspring health.

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