Maternal supplementation with multiple micronutrients containing folic acid increased the risk for Gestational Diabetes Mellitus: Results from a prospective cohort study in China

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Abstract

Objective To investigate the relationship between maternal supplementation with folic acid/ multiple micronutrients containing folic acid (MM-FA) and gestational diabetes mellitus (GDM) risk. Design Prospective cohort study. Setting Haidian Maternal and Child Health Hospital, Beijing, China. Population 3,458 pregnant women and 653 cases of GDM were approached between October 2017 and December 2020. Methods Sociodemographic characteristics, lifestyle data, and information on folic acid supplementation were obtained from a structured questionnaire. GDM was diagnosed according to IADPSG criteria (2010). After adjusting for confounding variables, associations between folic acid/MM-FA supplementation and GDM risk were estimated using binary logistic regression analysis. Main outcome measures Incident GDM. Results Taking MM-FA periconceptionally was associated with a higher GDM risk (aOR 1.33; 95% CI 1.05–1.69) compared to exclusive folic acid supplementation. And this association was observed exclusively in women with a pre-pregnancy BMI < 24kg/m2 (aOR 1.39; 95% CI 1.06–1.82). In separate analysis of pre-conception supplementation, women without folic acid supplementation before conception were more likely to develop GDM than those taking folic acid alone (aOR 1.40; 95% CI 1.01–1.96). Conclusions Maternal MM-FA supplementation may enhance the risk for GDM. These findings indicated that pregnant women should to be mindful of the risk of iron and other micronutrients over-supplementation when using folic acid supplements. It's recommended that women take pure folic acid in preference and begin taking them from pre-conception in accordance with the recommended guidelines. Key words Folic acid; Multiple micronutrients; Gestational diabetes mellitus; Cohort

Title Page

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Running Title : Types of folic acid usage & gestational diabetes

It contains 243 words of abstract, 3225 words of main text, 3 tables, 1 figure and 39 references.

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Conclusions Maternal MM-FA supplementation may enhance the risk for GDM. These findings indicated that pregnant women should to be mindful of the risk of iron and other micronutrients over-supplementation when using folic acid supplements. It's recommended that women take pure folic acid in preference and begin taking them from pre-conception in accordance with the recommended guidelines.

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Tweetable abstract Pregnant women with multiple micronutrients containing folic acid (MM-FA) have higher risk for gestational diabetes mellitus (GDM) compared to exclusive folic acid supplementation.

Introduction

Gestational diabetes mellitus (GDM) refers to diabetes diagnosed in the second or third trimester of pregnancy that was not clearly overt diabetes prior to gestation[1]. GDM is a severe health risk for mothers and infants, as it not only increases the risk for maternal type II diabetes and pre-eclampsia[2,3] but is also associated with preterm birth and large gestational age[4,5]. The updated data from the International Diabetes Federation shows that the standard prevalence of GDM has reached 14.0% globally in 2021[6]. The prevalence of GDM in China is on the rise, and a Meta-analysis found that the prevalence of GDM in mainland China is 14.8%[7]. Given the increasing prevalence and the serious health risks to mothers and infants, identifying controllable risk factors and assisting pregnant women in preventing GDM has become a critical public health concern.

Folate (FA) is a B-vitamin found naturally in vegetables, fruits, beans and other foods. Folate is an indispensable cofactor in carbon metabolism and plays an important role in DNA synthesis, repair and methylation[8-10]. Folic acid supplementation has been shown to effectively reduce the development of fetal neural tube defects (NTDs)[11]. Many countries try to increase the folate intake and folate level of women of childbearing age through food fortification and targeted supplementation policies[12]. China launched the project of "Folic acid supplement to prevent neural tube defects" in 2009, which provides free folic acid supplements to rural women preparing for pregnancy, so as to ensure that they can take 400μg folic acid supplements every day during the periconceptional period to prevent adverse outcomes such as NTDs[13]. Since the implementation of this program, some Chinese women choose to take multiple micronutrients containing folic acid (MM-FA) at their own expense rather than free folic acid tablets[14].

With the widespread application of folic acid supplementation, people are becoming increasingly concern about its safety and potential side effects[15-18]. Several studies have assessed the association between periconceptional folic acid supplementation (including MM-FA) and GDM, but the results were inconsistent[19-25]. In addition, some studies suggest a different effect between taking folic acid alone and MM-FA on the risk for adverse maternal and infant outcomes[26,27], but few studies have explored the difference in the risk for GDM between women using folic acid alone and those using MM-FA. The aim of this study was to assess the association between periconceptional folic acid/ MM-FA supplementation and the risk for GDM.

Methods

Study population and design

This is an ongoing prospective cohort study which was established in 2017 at the Haidian Maternal and Child Health Hospital, Beijing, China. Our earlier study[28,29] covered the survey design, participant eligibility, blood collection procedure, and GDM diagnosis. Briefly, pregnant women who went for a prenatal health checkup or attended the maternity school were recruited in the cohort if they met the following criteria: (1) registered and planning to give birth in this hospital; (2) $<$ 20 gestational weeks; (3) completed the "Informed Consent Form". At the time of recruitment, structured questionnaires were used by trained healthcare workers to collect maternal demographic and perinatal data, as well as lifestyle information (including category and period of folic acid supplementation, smoking status, dietary intake, etc.). Biological samples like blood were also collected at the same time. After recruited in the cohort, pregnant women would undergo a series of prenatal health checkup, such as blood routine examination in first trimester and oral glucose tolerance test (OGTT) blood glucose at 24-28 weeks of pregnancy. And these information from pregnancy examinations and discharge diagnosis would be recorded by hospital data management department.

A total of 4,239 pregnant women were recruited from October 2017 to December 2020. Among them, 781 women were excluded as following criteria: (1) had unclear information of folic acid intake ($n = 213$); (2) [?] 20 gestational weeks at the time of recruitment after verification ($n = 40$); (3) had history of pre-pregnancy diabetes or first-degree family history of diabetes according to the questionnaire and records from hospital ($n = 46$); (4) had no OGTT results ($n = 544$). Therefore, 3458 women were included in the final analysis (Figure S1).

Definition of Major Variable and GDM Diagnosis

Several questions were used to collect information on periconceptional folic acid supplementation for pregnant women, including whether (no, exclusive folic acid, MM-FA); when start (before conception or after conception); and how frequently (24 capsules or more per month, 15–23 capsules per month, or fewer than 15 capsules per month) they had taken folic acid/ MM-FA. In this study, maternal periconceptional folic acid supplementation was defined as a self-report of having ever taken folic acid or MM-FA supplement 3 months before to 3 months after the last menstrual period. In addition, pregnant women who did not take folic acid/MM-FA or began taking folic acid/MM-FA after conception were regarded to have not received folic acid supplementation during the pre-conception period. Finally, pregnant women who took 24 capsules or more per month were considered to have good compliance.

GDM was diagnosed according to criteria recommended by the International Association of Diabetes and Pregnancy Study Groups (IADPSG) (1) Pregnant women between 24 and 28 weeks of gestation were requested to undertake a 2-h 75 g OGTT during their prenatal examination, which was carried out in the morning after an overnight fasting of over 8 hours. The OGTT results were extracted from the medical record, and a pregnant woman was diagnosed with GDM if she met one or more of the following criteria: fasting blood glucose [?] 5.1 mmol/L, 1-h blood glucose [?] 10.0 mmol/L, or 2-h blood glucose [?] 8.5 mmol/L.

Statistical analysis

We calculated baseline groups for age (<30, 30–35, or [?]35 years), pre-pregnancy body mass index (BMI; <24 or [?]24 kg/m2), ethnicity (Han or others), occupation, education (>16, 12-16, [?]12 years), smoking status, parity (nulliparous or multiparous), frequency of meat, egg, milk, vegetable, fruit, soybean and nut consumption ([?]3, 4–6, or >6 times/week) and the category (folic acid alone, or MM-FA), frequency ([?] 24 capsules per month or < 24 capsules per month) and initiation (before conception or after conception) of folic acid intake. The Chi-square test was performed to compare these variables between the GDM and non-GDM groups.

While adjusting for potential confounding variables, binary logistic regression was used to analyze whether the category of folic acid supplementation was associated with the risk for GDM. Women taking folic acid alone will be considered as the reference under China's existing folic acid supplementation policy. Maternal age, pre-pregnancy BMI, ethnicity, education, occupation, parity, and smoking status were among the confounders included in the multivariate models, which were mainly selected based on prior relevant studies[28]. Some food consumption characteristics were also included since they might be a source of dietary folate. Cases with missing data for confounding variables were defined as a new category. The results of logistic analyses are presented as odds ratios (ORs) and their 95% confidence intervals (95% CIs).

Since previous studies[25] observed that the period of folic acid supplementation altered the association between folic acid supplementation and GDM, we further combined the category and period of folic acid supplementation and analyzed the folic acid supplementation only during the pre-conception period. We also tried to explore whether the compliance of supplementation affects the risk for GDM. Moreover, given that pre-pregnancy BMI are associated with GDM, we stratified the pregnant women by pre-pregnancy BMI to further explore the association between folic acid supplementation and GDM.

Finally, we did an exploratory analysis in a subsample to examine the association among Hb levels in early pregnancy, category of folic acid supplementation and GDM, because the hemoglobin (Hb) levels might indicate the iron status in women to some extent[30]. This analysis was restricted to women with informed consent and blood routine examination information. Referring to previous studies[31], Hb in early pregnancy >130 g/L would be considered a high level. Demographic and lifestyle characteristics measured in the study were similar between women with and without blood routine examination information. ORs and 95% CIs were estimated using binary logistic regression models. Multiplicative interaction was used to explore whether early pregnancy Hb levels would modify the effect of MM-FA on GDM. And all analyses in subsample were adjusted for the same covariates.

All statistical analyses were performed with R software, version 4.0.5 (R Development Core Team). A

two-tailed P [?] 0.05 was considered statistically significant.

Results

Participant Characteristics

A total of 3458 women were included in the final analysis, and 653 pregnant women (18.9%) were diagnosed with GDM. Table 1 shows basic characteristics according to GDM and non-GDM groups.

The women ranged in age from 18 to 44 years old ($M = 27.26$,$SD = 4.06$), and 18.4% of them were above normal weight before conception (pre-pregnancy BMI [?] 24 kg/m^2). GDM was more prevelant in older ($P < 0.001$), more obese ($P < 0.001$) and multiparous ($P = 0.015$) women. In addition, the pregnant women with less education ($P = 0.017$) and exposed to smoking ($P = 0.001$) had a much higher risk for GDM. And the risk for GDM in pregnant women was also related to the frequency of egg and milk consumption ($P = 0.018$; $P = 0.021$). However, there were no statistical differences in occupation, ethnicity, education or the frequency of meat, vegetable, fruit, soybean and nut consumption (all $P > 0.05$) (Table 1).

Association Between the Maternal Folic Acid Supplementation and GDM

Only 3.2% of the 3458 women didn't take folic acid periconceptionally, 26.2% took folic acid alone and 70.6% took MM-FA. Taking MM-FA periconceptionally compared to folic acid alone was associated with a significantly increased risk for GDM (aOR 1.33; 95% CI 1.05–1.69). But there were no significant differences in risk for GDM between the women who took folic acid alone and those who did not.

In separate analysis of pre-conception folic acid supplementation, 50.6% of pregnant women didn't take folic acid. When compared to taking folic acid alone, taking MM-FA before conception was associated with a significantly higher risk for GDM (aOR 1.56; 95% CI 1.11–2.20). Besides, compared to those with folic acid alone supplementation before conception, the women who didn't take folic acid had a much higher risk for GDM with crude OR (cOR) 1.33 (95% CI 1.00–1.77) and aOR 1.40 (95% CI 1.01–1.96) (Table 2).

No association was found between the compliance of folic acid supplementation and GDM (Table S1).

Association Between the Maternal Folic Acid Supplementation and GDM Stratified by Pre-pregnancy BMI

Among those with a pre-pregnancy BMI < 24 kg/m^2, the risk for GDM with MM-FA supplementation was 1.39 (95% CI 1.06–1.82) times higher compared to the folic acid alone group, whereas no association was found for those with the pre-pregnancy BMI [?] 24 kg/m^2 (P> 0.05). In addition, among women with a pre-pregnancy BMI < 24 kg/m^2, women who did not take folic acid had a less risk for GDM compared with the folic acid alone group (Figure 1). It should be noted that only 75 pregnant women had no periconceptional folic acid supplementation out of 2680 women with a pre-pregnancy BMI < 24 kg/m^2 (Table S2).

Association among Hb Levels in Early Pregnancy, Category of Folic Acid Supplementation and GDM

In this subsample of 2745 pregnant women, 19.2% were diagnosed with GDM, 1099 (40.0%) reached a high Hb level (>130 g/L) in the first trimester, 2665 (97.1%) had folic acid supplementation, and 695 took MM-FA.

The results suggested that women with high Hb level or MM-FA supplementation were more likely to develop GDM than those with Hb [?] 130 g/L and exclusive folic acid supplementation (all $P < 0.05$). The interaction effect between maternal Hb levels and folic acid supplementation category on the risk for GDM was significant (P -interaction = 0.024) (Table 3).

Discussion

We investigated the relationship between maternal folic acid supplementation and GDM in this prospective cohort analysis and found that taking MM-FA periconceptionally was associated with a significantly increased risk for GDM compared to folic acid alone. In the separate analysis of pre-conception folic acid

supplementation, it showed that women who did not take folic acid before conception were more likely to increase their risk of GDM than those who took folic acid alone.

Several previous observational cohort studies assessed the association between pre-pregnancy/early pregnancy folic acid supplementation and GDM, but the categories of folic acid supplementation were not investigated, described or differentiated in most of these studies[20,23-25]. These researchers considered folic acid to be a key component of the impact while neglecting the effects of other vitamins or minerals. They reported inconsistent results ranging from a protective role[19,20] of folic acid to no effect[21,22] or harmful associations[23-25]. The actual effect of folic acid on GDM is still unclear. The inconsistency is generally considered to be due to a variety of reasons like differences in prevalence of GDM in the study populations, diagnostic criteria for GDM, covariates accounted for in the analysis, and differences in the diet and lifestyle of the populations in which the studies are conducted[32]. However, as shown in this cohort, a total of 96.8% of pregnant women took folic acid periconceptionally, and most of them (70.6%) chose to take MM-FA. Moreover, there was an obvious difference in the risk for GDM between pregnant women who took folic acid alone and those who took MM-FA.

In fact, other micronutrients also play important roles in many cellular and physiological processes, and previous studies have revealed that excessive status of some micronutrients may raise the risk for GDM[30,33]. For example, a dose-response analysis showed a significant U-shaped non-linear association between serum vitamin D concentrations and risk of developing GDM[33]. And a meta-analysis suggested that mean differences in circulating iron, ferritin, hemoglobin, and transferrin saturation were higher in women with GDM than those without GDM[30]. In the subsample, we further analyzed the relationship among hemoglobin levels in early pregnancy, category of folic acid supplementation and GDM, intending to validate the role of other nutrients in the MM-FA with iron as an example. And the results showed the effect of MM-FA on GDM might be modified by hemoglobin levels in early pregnancy. It hinted that excessive iron supplementation may increase the risk for GDM. However, since we cannot obtain and compare the contents of specific nutrients in MM-FA, it is difficult to determine the specific nutrients that raise the risk for GDM.

We also examined whether maternal supplementation with folic acid alone can prevent the occurrence of GDM, and found that taking folic acid alone during the pre-conception period was more likely to reduce the risk for GDM than those without folic acid supplementation. Similarly, a large pregnant women cohort in the United States controlled the other nutrients consumption and found that folic acid supplementation before conception can reduce the risk for GDM[19]. There is evidence that folic acid supplementation can help prevent GDM. For example, folic acid supplementation can reduce homocysteine levels in vivo[34], which in turn reduces the risk of developing GDM[35]. In high-fat diet-fed mice, folic acid supplementation was able to upregulate AMPK which had an ameliorative effect on insulin resistance, according to an animal research[36]. Furthermore, folic acid, an essential supply of one-carbon units for DNA methylation, affects specific DNA methylation levels[10], which in turn impacts the risk of developing GDM[37,38]. Therefore, it is biologically plausible that taking folic acid alone might reduce the risk for GDM.

In this study, we did not observe the difference in GDM risk between women using folic acid alone periconceptionally and those who did not. Firstly, it should be taken into account that the effect of the small sample size may be responsible for the non-significant results of folic acid supplementation periconceptionally on GDM, as only 109 of the 3458 pregnant women in our sample did not take folic acid during the period from 3 months before the last menstrual period to the day of the interview. Secondly, one-carbon metabolites, including betaine, choline, and serine, changed from preconception across gestation[39]. Distinct exposure times to folate (before and/or after conception) might have different effects on one-carbon metabolism. It seems sense that the timing of folate exposure might have a significant impact on how it affects biological processes involving one-carbon metabolism. Additionally, prior research has demonstrated that the periods of folic acid supplementation have varying impacts on the adverse outcomes[14,15].

Our stratified analysis found that the risk for GDM with MM-FA supplementation was higher compared to those taking folic acid alone only in women with a pre-pregnancy BMI < 24 kg/m^2. In this result, the risk for GDM in the group with pre-pregnancy BMI > 24 kg/m^2 was generally higher than those with BMI

< 24 kg/m^2, so we suppose that for the overweight women with GDM, pre-pregnancy BMI may play a more important role and mask the association of MM-FA supplementation. The results suggested that the association between category of folic acid supplementation and GDM might be modified by pre-pregnancy BMI. It may be more effective for women with a pre-pregnancy BMI < 24 kg/m^2 to reduce the risk for GDM by adjusting the category of folic acid supplementation than those with a pre-pregnancy BMI > 24 kg/m^2.

Our study has several strengths. Firstly, conducting a placebo-controlled randomized trial to conclusively prove or disprove the effect of folic acid supplementation on the risk of GDM is neither ethical nor feasible given that folic acid supplementation in early pregnancy has been widely recommended to prevent neural tube defects. Our study was a prospective cohort study so that it could explain the temporal causal reference to some certain extent. Second, by excluding women with a history of diabetes mellitus from the final analysis, we are able to prospectively conclude that folic acid supplementation was primarily responsible for the increased risk of abnormal glucose levels. Besides, although the particular dietary folic acid intake was not specifically examined in our study, the frequency of fruits, vegetables and other food consumption was controlled, which to some extent precluded the impact of dietary folic acid intake on the results. Finally, we stratified the categories and periods of folic acid supplementation, which enabled us to explore the association between specific characteristics of folic acid supplementation and the risk for GDM. To our knowledge, our study firstly found the different effect of taking folic acid alone and MM-FA on the risk for GDM.

Despite these strengths, the population in our study is from Beijing, with a high level of life. The subsample demonstrates that this population has a reasonably good nutritional status. Therefore, it should be cautious when extrapolating the conclusions of this study. In addition, iron in MM-FA may be one of the factors contributing to the higher risk for GDM, according to the results of our stratified analysis based on hemoglobin in the subsamples; however, we are unable to obtain and compare the content of particular nutrients in MM-FA. Further research is required in the future to determine which MM-FA components play a major role.

Conclusion

Maternal periconceptional supplementation with MM-FA may increase risk for GDM in comparison to those with exclusive folic acid supplementation. And pregnant women who take exclusive folic acid during the pre-conception period have a lower risk for GDM. Pregnant women should be aware of the problem of over-supplementation of iron and other micronutrients. Women are recommended to use folic acid alone and start from preconception which would beneficial both for children and pregnant women themselves.

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Disclosure of Interests

None declared. Completed disclosure of interests form available to view online as supporting information.

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Details of ethical approval

This study was approved by the Biomedical Institutional Review Board of Peking University (IRB00001052-17028). Written consent was received from all participants.

Contribution to authorship

SX drafted the first manuscript. SX, YD and ZR analyzed data. JL and ZL contributed to the conception, design of the study, and study supervision. SG, JW and XL were responsible for enrolling participants. SX, YD and JZ were involved in data collection. YD contributed to the graphical abstract. JL and ZL obtained funding. JL and XL contributed to the interpretation of the results and made critical revision to the manuscript. All authors read, reviewed and approved the final manuscript. JL and XL are the guarantors of this work and, as such, had 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.

Data availability statement

The data that support the findings of this study are available from the corresponding authors upon reasonable request.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1 . Flowchart of recruitment and exclusion.

Table S1 . Association between category and compliance of folic acid supplementation and GDM

Table S2 . Association between folic acid supplementation and GDM stratified by pre-pregnancy BMI

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Tables

Table 1. The characteristics of pregnant women between groups of GDM and non-GDM in the cohort, N (%)

Variable	Total (n=3458)[a]	Non-GDM (n=2805) [b]	GDM (n=653) [c]	P [d]
Age (years)				<0.001
<30	1691 (48.9)	1425 (84.3)	266 (15.7)	
30-35	1260 (36.4)	1020 (81.0)	240 (19.0)	
[?]35	507 (14.7)	360 (71.0)	147 (29.0)	
Pre-pregnancy				<0.001
BMI (kg/m^2)				
<24	2680 (81.6)	2241 (83.6)	439 (16.4)	
[?]24	606 (18.4)	416 (68.6)	190 (31.4)	
Occupation				0.231
nonusers	222 (6.5)	171 (77.0)	51 (23.0)	
Famer/industry/business/service	865 (25.3)	694 (80.2)	171 (19.8)	
Public official	1823 (53.4)	1495 (82.0)	328 (18.0)	
others	505 (14.8)	416 (82.4)	89 (17.6)	
Education (years)				0.017
>16	957 (27.7)	803 (83.9)	154 (16.1)	
12-16	2247 (65.1)	1807 (80.4)	440 (19.6)	
[?]12	250 (7.2)	193 (77.2)	57 (22.8)	
Ethnicity				0.875
Han	3242 (93.8)	2629 (81.1)	613 (18.9)	
Others	214 (6.2)	175 (81.8)	39 (18.2)	
Smoking status [e]				0.001
No	2595 (75.8)	2138 (82.4)	457 (17.6)	
Yes	828 (24.2)	640 (77.3)	188 (22.7)	
Parity				0.015
Nulliparous	2492 (80.7)	2042 (81.9)	450 (18.1)	
Multiparous	595 (19.3)	461 (77.5)	134 (22.5)	
Food consumption				
Meat				0.183
[?]3 times/week	1990 (59.9)	1629 (81.9)	361 (18.1)	
4-6 times/week	732 (22.0)	592 (80.9)	140 (19.1)	
>6 times/week	600 (18.1)	471 (78.5)	129 (21.5)	
Egg				0.018
[?]3 times/week	1108 (33.3)	925 (83.5)	183 (16.5)	
4-6 times/week	1127 (33.8)	911 (80.8)	216 (19.2)	
>6 times/week	1097 (32.9)	864 (78.8)	233 (21.2)	
Milk				0.021
[?]3 times/week	1268 (38.1)	1058 (83.4)	210 (16.6)	
4-6 times/week	1003 (30.1)	795 (79.3)	208 (20.7)	
>6 times/week	1059 (31.8)	846 (79.9)	213 (20.1)	
Vegetable				0.155
[?]3 times/week	329 (9.9)	276 (83.9)	53 (16.1)	
4-6 times/week	820 (24.6)	676 (82.4)	144 (17.6)	
>6 times/week	2179 (65.5)	1748 (80.2)	431 (19.8)	
Fruit				0.506
[?]3 times/week	174 (5.2)	147 (84.5)	27 (15.5)	

Variable	Total (n=3458)[a]	Non-GDM (n=2805) [b]	GDM (n=653) [c]	P [d]
4-6 times/week	642 (19.2)	522 (81.3)	120 (18.7)	
>6 times/week	2521 (75.5)	2040 (80.9)	481 (19.1)	
Soybean				0.601
[?]3 times/week	1917 (57.9)	1568 (81.8)	349 (18.2)	
4-6 times/week	872 (26.3)	702 (80.5)	170 (19.5)	
>6 times/week	522 (15.8)	419 (80.3)	103 (19.7)	
Nut				0.560
[?]3 times/week	1634 (49.1)	1336 (81.8)	298 (18.2)	
4-6 times/week	886 (26.6)	719 (81.2)	167 (18.8)	
>6 times/week	808 (24.3)	646 (80.0)	162 (20.0)	
Abbreviations: GDM, gestational diabetes mellitus; BMI, body mass index. [a] Total value is not equal to 3458 because of excluding cases with missing data. [b] Percentage was calculated by dividing the number of cases without GDM by the total number of women in the subcategory. [c] Percentage was calculated by dividing the number of cases with GDM by the total number of women in the subcategory. [d] Differences in percentages of GDM by subcategory were examined with the Chi-square test. [e] Smoking status included exposure to passive smoking.	Abbreviations: GDM, gestational diabetes mellitus; BMI, body mass index. [a] Total value is not equal to 3458 because of excluding cases with missing data. [b] Percentage was calculated by dividing the number of cases without GDM by the total number of women in the subcategory. [c] Percentage was calculated by dividing the number of cases with GDM by the total number of women in the subcategory. [d] Differences in percentages of GDM by subcategory were examined with the Chi-square test. [e] Smoking status included exposure to passive smoking.	Abbreviations: GDM, gestational diabetes mellitus; BMI, body mass index. [a] Total value is not equal to 3458 because of excluding cases with missing data. [b] Percentage was calculated by dividing the number of cases without GDM by the total number of women in the subcategory. [c] Percentage was calculated by dividing the number of cases with GDM by the total number of women in the subcategory. [d] Differences in percentages of GDM by subcategory were examined with the Chi-square test. [e] Smoking status included exposure to passive smoking.	Abbreviations: GDM, gestational diabetes mellitus; BMI, body mass index. [a] Total value is not equal to 3458 because of excluding cases with missing data. [b] Percentage was calculated by dividing the number of cases without GDM by the total number of women in the subcategory. [c] Percentage was calculated by dividing the number of cases with GDM by the total number of women in the subcategory. [d] Differences in percentages of GDM by subcategory were examined with the Chi-square test. [e] Smoking status included exposure to passive smoking.	Abbreviations: GDM, gestational diabetes mellitus; BMI, body mass index. [a] Total value is not equal to 3458 because of excluding cases with missing data. [b] Percentage was calculated by dividing the number of cases without GDM by the total number of women in the subcategory. [c] Percentage was calculated by dividing the number of cases with GDM by the total number of women in the subcategory. [d] Differences in percentages of GDM by subcategory were examined with the Chi-square test. [e] Smoking status included exposure to passive smoking.

Table 2. Maternal periconceptional and pre-conception folic acid supplementation and incidence rate of

GDM.

FA supplementation	Total	Non-GDM	GDM	cOR (95%CI) [a]	aOR(95%CI) [b]	aOR(95%CI) [c]
Periconceptionally						
FA only	906 (26.2)	756 (83.4)	150 (16.6)	reference	reference	reference
Nonusers	109 (3.2)	94 (86.2)	15 (13.8)	0.80 (0.45,1.43)	0.72 (0.37,1.38)	0.80 (0.41,1.55)
MM-FA	2443 (70.6)	1955 (80.0)	488 (20.0)	1.26 (1.03,1.54)	1.29 (1.02,1.62)	1.33 (1.05,1.69)
Pre-conception						
FA only	465 (13.6)	396 (85.2)	69 (14.8)	reference	reference	reference
Nonusers	1732 (50.6)	1406 (81.2)	326 (18.8)	1.33 (1.00,1.77)	1.35 (0.98,1.87)	1.40 (1.01,1.96)
MM-FA	1224 (35.8)	976 (79.7)	248 (20.3)	1.46 (1.09,1.95)	1.54 (1.10,2.14)	1.56 (1.11,2.20)

FA supplementation	Total	Non-GDM	GDM	cOR (95%CI) [a]	aOR(95%CI) [b]	aOR(95%CI) [c]
Abbreviations: GDM, gestational diabetes mellitus; FA, folic acid. [a] cOR: logistic regression, crude odds ratio. No confounders were adjusted; [b] aOR: logistic regression, adjusted odds ratio. Adjusted for maternal age, pre-pregnancy BMI, occupation, education, parity and smoking status. Cases with missing data for confounding variables were defined as a new category. [c] aOR: logistic regression, adjusted odds ratio. Adjusted for maternal age, pre-pregnancy BMI, occupation, education, parity, smoking status, and consumption of eggs, milk, vegetables and fruits. Cases with	Abbreviations: GDM, gestational diabetes mellitus; FA, folic acid. [a] cOR: logistic regression, crude odds ratio. No confounders were adjusted; [b] aOR: logistic regression, adjusted odds ratio. Adjusted for maternal age, pre-pregnancy BMI, occupation, education, parity and smoking status. Cases with missing data for confounding variables were defined as a new category. [c] aOR: logistic regression, adjusted odds ratio. Adjusted for maternal age, pre-pregnancy BMI, occupation, education, parity, smoking status, and consumption of eggs, milk, vegetables and fruits. Cases with	Abbreviations: GDM, gestational diabetes mellitus; FA, folic acid. [a] cOR: logistic regression, crude odds ratio. No confounders were adjusted; [b] aOR: logistic regression, adjusted odds ratio. Adjusted for maternal age, pre-pregnancy BMI, occupation, education, parity and smoking status. Cases with missing data for confounding variables were defined as a new category. [c] aOR: logistic regression, adjusted odds ratio. Adjusted for maternal age, pre-pregnancy BMI, occupation, education, parity, smoking status, and consumption of eggs, milk, vegetables and fruits. Cases with	Abbreviations: GDM, gestational diabetes mellitus; FA, folic acid. [a] cOR: logistic regression, crude odds ratio. No confounders were adjusted; [b] aOR: logistic regression, adjusted odds ratio. Adjusted for maternal age, pre-pregnancy BMI, occupation, education, parity and smoking status. Cases with missing data for confounding variables were defined as a new category. [c] aOR: logistic regression, adjusted odds ratio. Adjusted for maternal age, pre-pregnancy BMI, occupation, education, parity, smoking status, and consumption of eggs, milk, vegetables and fruits. Cases with	Abbreviations: GDM, gestational diabetes mellitus; FA, folic acid. [a] cOR: logistic regression, crude odds ratio. No confounders were adjusted; [b] aOR: logistic regression, adjusted odds ratio. Adjusted for maternal age, pre-pregnancy BMI, occupation, education, parity and smoking status. Cases with missing data for confounding variables were defined as a new category. [c] aOR: logistic regression, adjusted odds ratio. Adjusted for maternal age, pre-pregnancy BMI, occupation, education, parity, smoking status, and consumption of eggs, milk, vegetables and fruits. Cases with	Abbreviations: GDM, gestational diabetes mellitus; FA, folic acid. [a] cOR: logistic regression, crude odds ratio. No confounders were adjusted; [b] aOR: logistic regression, adjusted odds ratio. Adjusted for maternal age, pre-pregnancy BMI, occupation, education, parity and smoking status. Cases with missing data for confounding variables were defined as a new category. [c] aOR: logistic regression, adjusted odds ratio. Adjusted for maternal age, pre-pregnancy BMI, occupation, education, parity, smoking status, and consumption of eggs, milk, vegetables and fruits. Cases with	Abbreviations: GDM, gestational diabetes mellitus; FA, folic acid. [a] cOR: logistic regression, crude odds ratio. No confounders were adjusted; [b] aOR: logistic regression, adjusted odds ratio. Adjusted for maternal age, pre-pregnancy BMI, occupation, education, parity and smoking status. Cases with missing data for confounding variables were defined as a new category. [c] aOR: logistic regression, adjusted odds ratio. Adjusted for maternal age, pre-pregnancy BMI, occupation, education, parity, smoking status, and consumption of eggs, milk, vegetables and fruits. Cases with

FA supplementation	Total	Non-GDM	GDM	cOR (95%CI) [a]	aOR(95%CI) [b]	aOR(95%CI) [c]

Table 3. Interaction between the category of folic acid supplementation and Hb levels for GDM

Characters
FA only and Hb [?] 130 g/L
MM-FA and Hb [?] 130 g/L
FA only and Hb > 130 g/L
MM-FA and Hb > 130 g/L

Abbreviations: GDM, gestational diabetes mellitus; FA, folic acid; Hb, hemoglobin. [a] aOR: logistic regression, adjusted odd

Figure legends

Figure 1 . aORs[a] and 95% CI of GDM according to periconceptional (A) and pre-conception (B) folic acid supplementation stratified by pre-pregnancy BMI.

[a] aOR: logistic regression, adjusted for maternal age, occupation, education, parity, smoking status, and consumption of eggs, milk, vegetables and fruits. Cases with missing data for confounding variables were defined as a new category.