Original Article

Duration of periconceptional folic acid supplementation and risk of gestational diabetes mellitus

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Background and Objectives: Increased consumption of folic acid is prevalent, raising concerns about possible adverse effects. This prospective study aimed to explore the associations between the duration of folic acid supplementation and the risk of gestational diabetes mellitus (GDM) in Chinese women. **Methods and Study Design:** A total of 326 pregnant women were prospectively included for detailed information on folic acid supplementation during pre-pregnancy and early pregnancy, lipid profiles at 16-18 weeks, and subsequent GDM diagnosis at 24-28 weeks. Associations among folic acid supplementation, lipid profiles, and risk of GDM were analyzed using linear and logistic regression models, adjusting for potential confounders. **Results:** The incidence of GDM in participants was 10.1%. We observed a U-shape relation between duration of folic acid supplementation and risk of GDM. Women who did not take folic acid and took folic acid for >90 days had a higher incidence of GDM compared to those who took folic acid for ≤60 days. Moreover, lipid profiles were positively correlated with duration of folic acid supplementation and risk of GDM. After adjusting for demographic characters, energy and nutrients intakes and lipid profiles, the adjusted OR of GDM comparing taking folic acid for >90 days with taking folic acid for ≤60 days was 3.45 (95% CI: 1.01, 11.8). **Conclusions:** This prospective study indicate a positive association among prolonged folic acid supplementation, lipid profiles in the second trimester, and risk of GDM. Future studies are warranted to verify the causal relationship and underlying mechanisms.

Key Words: folic acid, duration, gestational diabetes mellitus, lipid profiles, China

INTRODUCTION

Gestational diabetes mellitus (GDM) is a major national and global health concern, and it is defined as glucose intolerance of various degrees that is first detected during pregnancy.[1] Globally, the prevalence of GDM is approximately 17%. However, in the South-East Asia Region, the prevalence of GDM is as high as 25.0%.[2] GDM not only increases the risk of immediate adverse pregnancy and infant outcomes,[3] but also increases the long-term metabolic risk in both mothers and their offspring.[4-6] With the increasing incidence of GDM, it is urgent to explore its risk factors and mechanisms, and to develop effective strategies to reduce the burden of GDM on mothers and offspring.

Folic acid (FA) is an essential vitamin that serves as a source of single carbon units in methionine/homocysteine cycle by supplying 5-methyltetrahydrofolate for the methylation of homocysteine back into methionine.[7] The methionine cycle is responsible for the synthesis of S-adenosylmethionine (SAM). SAM-dependent methylation reactions are critically required for the synthesis of phosphatidylcholine (PC)[8,9] that is an important component of the plasma membrane and very low-density lipoprotein (VLDL).[10] Therefore, there is a link between FA, PC, and lipid metabolism. Recently, it proposed that high FA consumption could result in lipid accumulation in mice by reducing the protein and activity levels of methylenetetrahydrofolate reductase (MTHFR).[11] Moreover, another animal study also reported that excess FA intake might increase lipid storage.[12] Consequently, inappropriate FA supplementation may lead to hyperlipidemia. It is well known that hyperlipidemia is an extremely important risk factor for GDM.[13] However, data linking FA supplementation and blood lipids in pregnant women are limited. In addition, it is unclear whether FA supplementation is related to the risk of GDM.

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FA is well known for its preventive effect on neural tube defects (NTDs). Recommendations for FA supplementation before and during pregnancy have been well established all over the world. The WHO recommends that reproductive women take FA supplements at a dose of 400μg/d for 4-12 weeks before pregnancy and for 8-12 weeks during early pregnancy.[14,15] In fact, most women take FA for a longer duration instead of following the recommendation. For instance, a recent survey from China reported that 48.8% of pregnant women took FA for more than 12 weeks before pregnancy, and 30.7% of women took FA for 12-24 weeks during pregnancy.[16] Moreover, an Irish study indicated that 56.2% of women took FA for more than 12 weeks before pregnancy, and even 17.7% of women took FA for more than one year.[17] To our knowledge, the majority of the available researches were commonly focused on the high dose of FA consumption or the different periods of FA use, but no studies have specifically designed to investigate the effects of duration of FA supplementation on pregnant women. Thus, it remains unclear whether prolonged FA supplementation would cause any adverse effects, including lipid metabolism change and GDM.

Accordingly, the aim of this study was to explore the association between the duration of FA supplementation and the risk of GDM. Furthermore, this study also investigated the association of duration of supplementation with blood lipid profiles to assess the role of lipid profiles in the relationship between FA supplementation and GDM. We hypothesized that prolonged FA supplementation would increase blood lipid profiles and the risk of GDM. Consequently, this study may provide new information regarding the potential adverse effects of prolonged FA supplementation on GDM in Chinese women.

METHODS

Study design and subjects

This prospective study recruited a total of 348 pregnant women with 16-18 weeks of gestation at a hospital in Hefei, Anhui Province between August 2014 to April 2015. Questionnaires were used to collect participant information about sociodemographic, lifestyle characteristics, past medical history, and FA supplementation by face-to-face interviews. At 16-18 weeks of gestation, we collected the information of FA supplementation during pre-pregnancy and early pregnancy and conducted a dietary intake survey. In addition, blood samples were collected to measure triglycerides (TG), high-density lipoprotein-cholesterol (HDL-c), and low-density lipoprotein-cholesterol (LDL-c) concentrations. At 28 weeks of gestation, a 75-g oral glucose tolerance test (OGTT) was performed. According to guidelines from the International Association of Diabetes and Pregnancy Study Groups (IADPSG),[18] diagnosis of GDM was made when any of the following criteria were met: fasting plasma glucose ≥5.1 mmol/L, at 1h ≥10 mmol/L, and at 2h ≥8.5 mmol/L. Among 348 pregnant women, 2 women had missing data regarding FA supplementation, 14 women without blood samples, and 6 women were lost. Finally, 326 participants were involved in the further analysis. Figure 1 shows the

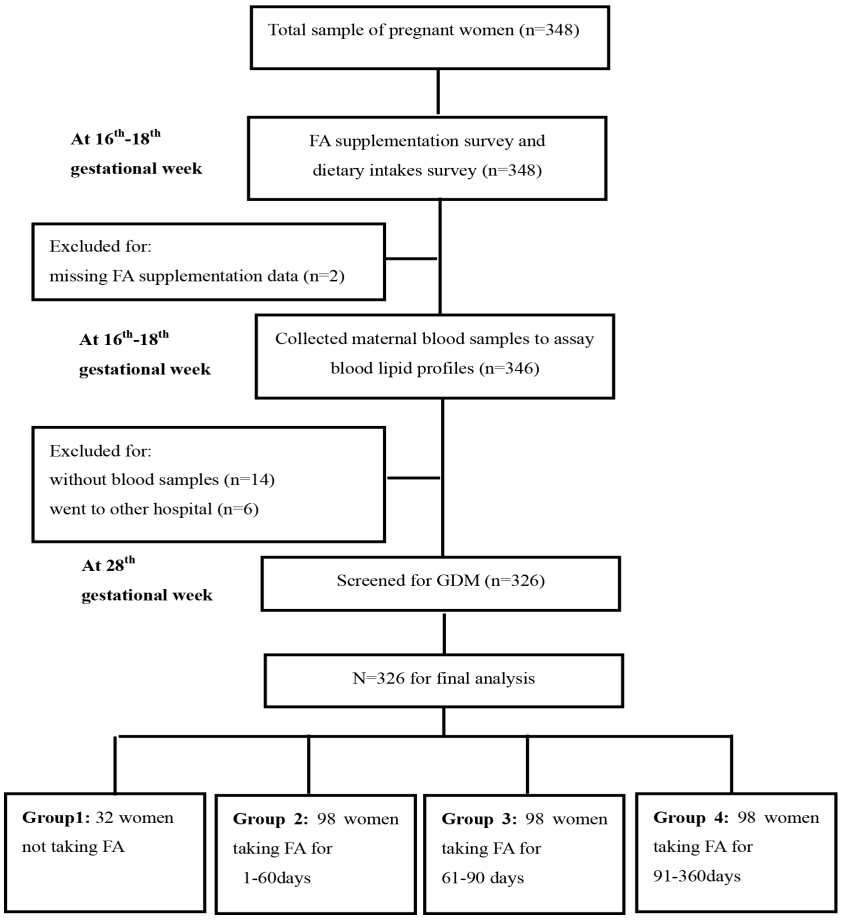

Figure 1. Flow diagram of recruitment and follow-up in this prospective study.

recruitment and follow-up process for this study. This protocol has received ethics approval from the Ethics Committee of Anhui Medical University. All participants provided written informed consent before being entered into the study. All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1995 Declaration of Helsinki as revised in Edinburgh in 2000.

FA Supplementation and dietary intake

In the present study, a self-reported questionnaire was used to collect the information on FA supplementation during pre-pregnancy and early pregnancy, including the supplements brand name, the initiation time of FA supplementation (e.g., Have you taken FA or multivitamins containing FA before pregnancy or during the early pregnancy? When did you start to take FA or multivitamins containing FA?), intake duration, dose, and frequency of use. Additionally, we also recorded the amount of remaining FA supplements to evaluate the duration of FA supplementation. FA supplementation included taking FA supplements, as well as taking FA-containing multivitamins.

Dietary intakes were assessed by means of three consecutive 24-hour dietary recalls, including two weekdays and one weekend day. This dietary survey was administered by personnel trained in the questionnaire development and interview technique. The interview was conducted using a four-step multiple-pass approach proposed by the United States Department of Agriculture (USDA).[19] In the first pass, participants were requested to list all of the foods and drinks consumed in the previous 24 h from midnight to midnight. In the second pass, the interviewer went through the list chronologically, probing for food description details (type, brand, gross or net weight, etc.), preparation methods, and amounts consumed. We provided participants with standard quantitative measures (including standard measuring utensils, life-sized food models and an atlas of food pictures) to help the interviewer accurately measure the weight of foods and liquids consumed. The atlas used in this study was specifically designed for the dietary recall survey by WANG Zhi-Xu from Nanjing Medical University. In the third pass, the interviewer attempted to elicit any forgotten items such as condiments or beverages. In the fourth pass, the interviewer reviewed the reported intake to validate the information and to register omitted foods. Foods and beverages consumed were converted to energy, macronutrients, and micronutrients by using the data from the China Food Composition Tables.[20,21] The daily intake of energy and nutrients was averaged over 3 days to assess usual dietary intake.

Covariates

A general health questionnaire was utilized to collect information, including socio-demographic, lifestyle characteristics and past medical history. Details include maternal age, pre-pregnancy BMI, race, parity, place of residence, educational level, socio-economic status, and passive smoking. Educational level was divided into three categories: ≤9 years, 10–15 years, and >15 years. Monthly income was classified into three categories: <4000 Chinese Yuan (CNY), 4000-8000 CNY, and >8000 CNY. The pre-pregnancy BMI is based on self-reports. The variable "passive smoking" was classified into two categories: yes and no. The main nutrients of interest were carbohydrate, protein, fat, fiber, fatty acids, cholesterol, vitamins, and minerals.

Statistical analysis

Data analysis was performed using the Statistical Package for the Social Sciences software version 21.0. Duration of FA supplementation was divided into tertiles for analysis. Normal distribution test was conducted for continuous variables before data analysis. Blood HDL-c concentration, TG concentration, and dietary fat, protein, dietary fiber, saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids, cholesterol, FA, Fe, Se, vitamin A, vitamin B-1, vitamin B-2 intakes were skewed. Normally distributed variables were presented as mean ± standard deviation (SD), skewed variables were presented median (interquartile range [IQR]), and categorical data were presented as frequencies (n [%]). For comparison of GDM and non-GDM women, the Student's t-test was used for continuous variables and Wilcoxon rank sum test for skewed variables. In addition, ANOVA or Wilcoxon rank sum and Kruskal-Wallis tests were used to test for differences between the four groups. For categorical variables, differences were examined by using chi-square test. Associations were assessed by linear regression for lipid profiles and logistic regression for GDM. HDL-c and TG concentrations were log-transformed (ln) to normality for linear regression analysis.

RESULTS

Participant characteristics

A total of 326 women were enrolled in this study, and the mean age was 28.4 years (SD=3.15, age range: 21–43 years). In this study, the dose of FA supplementation ranged from 140 μg/d to 1200 μg/d. Approximately 93.5% of participants took FA at 400 μg/d. Moreover, 85.0% of participants took FA supplements, and 15.0% of participants took FA-containing multivitamins. For the duration of FA supplementation, our findings showed that only 7.7% of participants took FA for 6 months, 2.1% of participants took FA for more than 6 months, and 9.8% of participants did not take FA.

Duration of FA supplementation was divided into tertiles with the following cutoffs: 1st tertiles (1-60 days), 2nd tertiles (61-90 days), and 3rd tertiles (91-360 days). All pregnant women were divided into four groups for analysis. Women who did not take FA supplements were assigned to one group, and the remaining were distributed into three groups according to the tertiles of the duration. In the four groups, significant differences were found in the educational level, age, and monthly income. Women who took FA for more than 90 days tend to be older, have a higher educational level, and have a higher monthly income. Further details on the study participants are given in Table 1. Table 2 presents the comparison of dietary intakes in participants according to the duration of FA supplementation. No significant difference was observed in energy and nutrient intake among the four groups

Table 1. General characteristics and incidence of GDM in study participants according to the duration of FA supplementation

Variables	All women	FA-users			Non-FA-users	*p*
		Duration of FA supplementation (tertiles)				
		T1 (1-60d)	T2 (61-90d)	T3 (91-360d)		
N	326	98	98	98	32	
Age (y, means±SD)	28.4±3.15	27.7±3.10	28.6±3.10	28.9±3.04	28.5±3.59	0.056
pre-BMI (means±SD)	20.6±2.61	20.4±2.75	20.8±2.42	20.6±2.68	20.4±2.85	0.799
Residence (n (%))						
Urban	273 (83.7)	79 (80.6)	86 (87.8)	86 (87.8)	22 (68.8)	0.127
Suburb	18 (5.5)	8 (8.2)	3 (3.1)	3 (3.1)	4 (12.5)	
Rural	35 (10.7)	11 (11.2)	9 (9.2)	9 (9.2)	6 (18.8)	
Educational (years)						
≤9 (n (%))	37 (11.3)	15 (15.3)	7 (7.1)	6 (6.1)	9 (28.1)	0.012
10–15 (n (%))	128 (39.3)	34 (34.7)	41 (41.8)	40 (40.8)	13 (40.6)	
>15 (n (%))	161 (49.4)	49 (50.0)	50 (51.0)	52 (53.1)	10 (31.3)	
Monthly income (CNY), (n (%))						
<4000	119 (36.5)	38 (38.8)	30 (30.6)	30 (30.6)	21 (65.6)	0.003
4000-8000	151 (46.3)	41 (41.8)	56 (57.1)	46 (46.9)	8 (25.0)	
>8000	56 (17.2)	19 (19.4)	12 (12.2)	22 (22.4)	3 (9.4)	
Passive smoking (n (%))						
No	129 (39.6)	31 (31.6)	45 (45.9)	40 (40.8)	13 (40.6)	0.230
Yes	197 (60.4)	67 (68.4)	53 (54.1)	58 (59.2)	19 (59.4)	
Parity (n (%))						
Primipara	268 (82.2)	75 (76.5)	78 (79.6)	90 (91.8)	25 (78.1)	0.223
Multipara	58 (17.8)	23 (23.5)	20 (20.4)	8 (8.2)	7 (21.9)	
GDM (n (%))						
Yes	33 (10.1)	5 (5.1)	7 (7.1)	18 (18.4)	3 (9.4)	0.012
No	293 (89.9)	93 (94.9)	91 (92.9)	80 (81.6)	29 (90.6)	

T1: 1st tertile; T2: 2nd tertile; T3: 3rd tertile; pre-BMI: pre-pregnancy BMI; GDM: gestational diabetes mellitus; CNY: Chinese Yuan; FA: folic acid.

except for dietary Fe intake. Women who did not take FA supplements consumed less dietary Fe than those who took FA supplements.

Associations between duration of FA supplementation and blood lipid profiles

Table 3 shows the blood lipid profiles in the second trimester according to the duration of the FA supplementation. Women who took FA for more than 90 days had a lower HDL-c concentration and higher TG and LDL-c concentrations compared to those who took FA supplements for less than 90 days. To further investigate the relationship between duration and lipid profiles, we performed a general linear regression analysis among women who took FA supplements. As shown in Table 4, prolonged FA supplementation may be a risk factor of elevated TG and decreased HDL-c concentrations in the second trimester. Interestingly, we also found that women who did not take FA supplements had higher blood lipid concentrations compared to those who took FA supplements for less than 60 days. Meanwhile, women who subsequently developed GDM had a higher concentration of TG in the second trimester. Further details are presented in Table 5. Conclusively, these findings suggested that taking FA for a longer duration during pre-pregnancy and early pregnancy or not taking FA may cause subsequently adverse blood lipid profiles in the second trimester.

Association between duration of FA supplementation and risk of GDM

There was a U-shape relation between duration of FA supplementation and the incidence of GDM. In this study, 10.1% of participants were subsequently developed GDM. However, the incidence of GDM is as high as 18.7% in women who took FA supplements for more than 90 days. In addition, we noticed that women who did not take FA had a higher incidence of GDM (9.4%) compared to women who took FA for less than 90 days. Results assessing the relation between duration of FA supplementation and the risk of GDM are reported in Table 6. From the results of the logistic regression analysis, the crude OR of GDM comparing taking FA supplements for >90 days with taking FA supplements for ≤60 days was 4.18 (95% CI: 1.49, 11.8). After adjusting for pre-pregnancy BMI, age, educational level, parity, and monthly income, the adjusted OR was 3.35 (95% CI: 1.15, 9.81). Especially, the association remained significantly (OR=4.08, 95% CI: 1.22, 13.7) when we adjusted for energy and all nutrients intakes. Finally, after adjusting for demographic characters, energy and nutrients intakes and lipid profiles, the adjusted OR was 3.45 (95%CI: 1.01, 11.8).

DISCUSSION

To the best of our knowledge, no other authors have investigated the association between duration of FA supplementation and risk of GDM in Chinese women. In the present study, the most remarkable result was that taking FA supplements for more than 90 days significantly increased the risk of GDM, and this relation was strong even after adjusting for some relevant confounders. Meanwhile, among women who took FA supplements, a longer duration was associated with higher TG concentration and lower HDL-c concentration in the second trimester. Moreover, it is worth mentioning that women

Table 2. Dietary intakes in study participants according to the duration of FA supplementation

Dietary intakes	FA-users			Non-FA-users	*p*
	Duration of FA supplementation (tertiles)				
	T1 (1-60d)	T2 (61-90d)	T3 (91-360d)		
Total energy (Kcal)†	2440±302	2517±310	2484±331	2435±311	0.315
Protein (g)‡	105 (96.6, 118)	110 (99.7, 124)	107 (97.1, 128)	107 (99.1, 126)	0.589
Fat (g)‡	51.4 (40.1, 68.5)	53.7 (41.9, 67.9)	51.2 (41.0, 70.0)	50.0 (39.4, 80.2)	0.913
Carbohydrates (g)†	389±66.4	409±71.1	397±69.0	385±60.8	0.156
Dietary fiber (g)‡	16.8 (13.8, 22.0)	15.5 (13.2, 20.2)	16.6 (12.4, 19.3)	15.4 (12.5, 17.0)	0.095
Saturated fatty acids (g)‡	4.27 (2.40, 8.76)	4.18 (2.55, 6.09)	5.07 (2.65, 8.93)	5.12 (2.41, 11.7)	0.285
Monounsaturated fatty acids (g)‡	5.56 (2.98, 11.7)	5.26 (2.53, 9.69)	6.11 (3.24, 10.6)	5.81 (2.63, 10.0)	0.630
Polyunsaturated fatty acids (g)‡	3.49 (1.95, 6.52)	3.37 (2.22, 6.44)	3.64 (2.24, 8.46)	3.36 (1.73, 6.16)	0.482
Cholesterol (mg)‡	678 (430, 862)	702 (423, 941)	716 (467, 904)	576 (444, 811)	0.708
FA (μg)‡	315 (248, 393)	369 (279, 519)	329 (250, 497)	343 (255, 518)	0.089
Fe (mg)‡	31.0 (26.4, 34.5)	30.9 (28.3, 34.4)	29.4 (26.8, 32.4)	28.1 (25.5, 31.3)	0.033
Zn (mg)†	16.2±2.76	16.8±2.73	16.7±3.19	16.0±2.86	0.379
Se (μg)‡	76.3 (62.9, 92.9)	80.0 (65.9, 95.4)	83.6 (67.8, 95.1)	81.0 (61.6, 108)	0.605
Vitamin A(μg)‡	966 (746, 1261)	1014 (758, 1348)	978 (780, 1255)	928 (715, 1245)	0.677
Vitamin E (mg)†	16.7±4.40	17.5±4.35	17.5±5.32	17.1±4.96	0.647
Vitamin B-1 (mg)‡	1.40 (1.20, 1.70)	1.50 (1.30, 1.70)	1.40 (1.30, 1.60)	1.40 (1.22, 1.50)	0.052
Vitamin B-2 (mg)‡	1.30 (1.20, 1.50)	1.30 (1.20, 1.60)	1.35 (1.20, 1.50)	1.20 (1.10, 1.40)	0.108
Vitamin C (mg)†	164±61.8	163±50.2	160±55.1	172±49.0	0.772

T1: 1st tertile; T2: 2nd tertile; T3: 3rd tertile.
†Data are presented as mean±SD, *p*-values are for ANOVA of differences between groups.
‡Data are presented as median (IQR), *p*-values are for Kruskal-Wallis tests of differences between groups.

Table 3. Blood lipid profiles in the second trimester according to the duration of FA supplementation

Lipid profiles	FA-users			Non-FA-users	*p*
	Duration of FA supplementation (tertiles)				
	T1 (1-60d)	T2 (61-90d)	T3 (91-360d)		
TG (mmol/L) †	1.61 (1.27, 2.09)	1.87 (1.37, 2.56)	1.97 (1.58, 2.56)	1.88 (1.33, 2.64)	0.008
HDL-c (mmol/L) †	1.80 (1.56, 1.97)	1.77 (1.53, 1.93)	1.64 (1.46, 1.91)	1.59 (1.39, 1.86)	0.026
LDL-c (mmol/L) ‡	3.01±0.707	3.30±0.662	3.35±0.936	3.13±0.441	0.012

T1: 1st tertile; T2: 2nd tertile; T3: 3rd tertile.
†Data are presented as median (IQR), *p*-values are for Kruskal-Wallis tests of differences between groups.
‡Data are presented as mean±SD, *p*-values are for ANOVA of differences between groups.

Table 4. Associations of the duration of FA supplementation with lipid profiles in the second trimester

Lipid parameter	Duration of FA supplementation		
	β	R^2	p
TG†	0.148	0.022	0.013
HDL-c†	-0.136	0.019	0.022
LDL-c	0.065	0.004	0.274

TG: triglyceride; HDL-c: high-density lipoprotein-cholesterol; LDL-c: low-density lipoprotein-cholesterol.
†Log-transformed for linear regression analysis.

Table 5. Blood lipid profiles in the second trimester according to the occurrence of GDM

Lipid parameter	Non GDM	GDM	p
TG (mmol/L)†	1.84 (1.34, 2.42)	2.07 (1.60, 2.60)	0.023
HDL-c (mmol/L)†	1.74 (1.51, 1.91)	1.77 (1.50, 1.96)	0.902
LDL-c (mmol/L)‡	3.20±0.769	3.38±0.685	0.205

TG: triglyceride; HDL-c: high-density lipoprotein-cholesterol; LDL-c: low-density lipoprotein-cholesterol.
†Data are presented as median (IQR), p-values are for Wilcoxon rank sum test of differences between groups.
‡Data are presented as mean ±SD, p-values are for Student's t-test of differences between groups.

Table 6. The correlation between the duration of FA supplementation and the risk of GDM

OR	FA-users			Non-FA-users
	Duration of FA supplementation(tertiles)			
	T1 (1-60d)	T2 (61-90d)	T3 (91-360d)	
Model 1	1	1.43 (0.438, 4.67)	4.18 (1.49, 11.8)**	1.92 (0.433, 8.54)
Model 2	1	1.25 (0.374, 4.17)	3.35 (1.15, 9.81)*	1.66 (0.348, 7.93)
Model 3	1	1.50 (0.395, 5.71)	4.08 (1.22, 13.7)*	1.76 (0.273, 11.4)
Model 4	1	1.25 (0.323, 4.85)	3.45 (1.01, 11.8)*	1.53 (0.237, 9.87)

T1: 1st tertile; T2: 2nd tertile; T3: 3rd tertile.
Model 1 is crude OR.
Model 2 adjusted for pre-pregnancy BMI, age, education level, parity, passive smoking, monthly income.
Model 3 plus energy and overall nutrients intake.
Model 4 plus TG, HDL-c, LDL-c concentrations.
**p<0.01 compared with 1st tertile (reference).
*p<0.05 compared with 1st tertile (reference).

who did not take FA had higher blood lipid concentrations and incidence of subsequent GDM as well. Furthermore, women who subsequently developed GDM had a higher second-trimester TG concentration. Figure 2 illustrates all the findings of this study.

In this study, few women took FA for more than the recommended duration. In China, the National Health and Family Planning Commission (NHFPC) recommends reproductive women to take FA at 400 μg/d regularly from 3 months before pregnancy until the end of the first trimester of pregnancy,[16] that is to say, reproductive women in China are recommended to take FA for 6 months. Our findings showed that only 7.7% of pregnant women adhered to the recommendation, 2.1% of pregnant women took FA for more than 6 months, and 9.8% of pregnant women did not take FA. However, in another Chinese study, 14.4% of the participants took FA for 6 months, 24.3% of the participants took FA for more than 6 months during pregnancy, and 15.3 % of the participants took FA for more than 6 months before pregnancy.[16] Especially, even 17.7% of women took FA for more than one year before pregnancy in one Ireland study.[17] Obviously, women in this study took FA for a shorter duration compared to those women in other studies, which might be related to the relatively backward economy in Anhui Province. Another possible reason for this difference is that we only investigated FA supplementation during pre-pregnancy and early pregnancy. Essentially, these results reflected that the majority of women were less aware of the appropriate duration of FA supplementation.

In our study, we found that longer duration of FA supplementation was significantly associated with the increased risk of GDM. Several investigations have evaluated the correlation between FA supplementation and GDM, while they only focused on the FA concentration or the period (pre-pregnancy, first/second/third trimester) of taking FA. For example, one cohort study from China discovered that daily taking FA in the first trimester was linked to an increased risk of GDM, rather than taking FA before pregnancy alone or in the second trimester alone.[22] Furthermore, there were two studies that focused on the association of FA concentration with the risk of GDM. An early study from southern India had shown that FA concentration was not related to women's adiposity, insulin resistance or the incidence of GDM.[23] However, a recent multi-ethnic study suggested that high FA concentration was significantly associated with the risk of GDM among Indian women, but no substantial associations were observed in Chinese and Malay women.[24] Therefore, the relationship between FA concentration and risk of GDM is inconsistent. Collectively, these results indicate

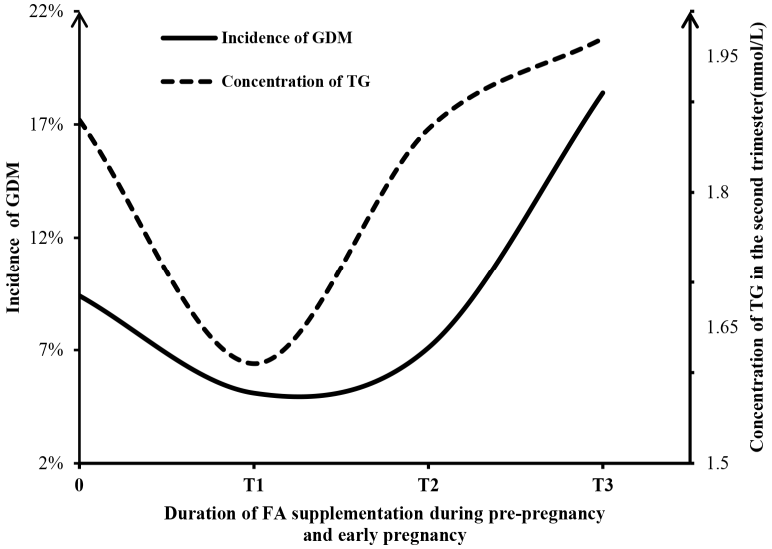

Figure 2. Conceptual diagram to illustrate the findings. 0: not taking FA; T1: 1st tertile, 1-60d; T2: 2nd tertile, 61-90d; T3: 3rd tertile, 91-360d.

that the duration or the different periods of FA supplementation might be associated the incidence of GDM. It is well known that FA supplementation is crucial for fetal growth and maternal health. Especially, it is necessary for pregnant women to routinely take FA to prevent NTDs.[25] Thus, it is critical for women to take FA supplements healthily and safely. From our own perspective, it's probably appropriate for reproductive women to take FA supplements for 90 days, and this duration is in line with the WHO recommendation. WHO recommends that reproductive women take FA supplements at 400 μg/d for 12-24 weeks.[13] Of course, further large-scale cohort studies are warranted to confirm that whether prolonged (>90 days) FA supplementation increase the risk of GDM.

Although there were two studies have reported that FA is involved in the increased risk of GDM,[22,24] the underlying mechanism of this adverse effect has not been investigated. The current study attempted to explore the underlying mechanism. In this study, we measured blood lipid profiles at 16-18 weeks of gestation, and found that a longer duration of FA supplementation was associated with higher TG concentration and lower HDL-c concentration. Previous studies had indicated that high concentrations of TG, LDL-c, VLDL-c and low concentration of HDL-c was associated with the high risk of GDM.[13,26-28] Similarly, we also found that women who subsequently developed GDM had higher concentrations of TG and LDL-c in the second trimester. Taken together, it is plausible that prolonged FA supplementation may cause elevated blood lipid concentrations in the second trimester and subsequently lead to increased risk of GDM in late pregnancy.

As we know, no studies have evaluated the association of FA supplementation with blood lipid profiles in pregnant women. In a Polish study, 122 elderly women were supplemented with FA 400 μg/d for 8 weeks, which resulted in a 15% increase in glucose concentration and a rise in blood TG and LDL concentrations.[29] The mechanism may be lowered MTHFR activity.[29] Moreover, an animal research reported that giving mice FA at 20 mg/kg for 6 months caused pseudo-MTHFR deficiency, altered lipid metabolism, and resulted in liver injury.[11] Thus, these findings indicated that the mechanism by which FA supplementation may cause lipid accumulation is due to lowed MTHFR activity. MTHFR produces methyltetrahydrofolate for remethylation of homocysteine to methionine by methionine synthase. In one-carbon metabolism, lower MTHFR activity was associated with reduced PC synthesis,[7,8] which could consequently cause TG accumulation.[10,24,30] However, little knows about the mechanism of FA supplementation on lowered MTHFR activity. Some researchers speculated that it may be due to the effects of unmetabolized FA, because high plasma concentrations of unmetabolized FA can lead to a build-up of dihydrofolate in the cell, which has been shown to inhibit MTHFR[31] and thereby inhibiting remethylation of homocysteine. Thus, it has been postulated that high plasma concentrations of FA may lead to a "functional" folate deficiency.[32,33] Further investigations are required to examine the effect of FA supplementation on MTHFR activity and further to confirm the effect of lowed MTHFR activity on blood lipids in pregnant women. In conclusion, our results inferred that taking FA for a longer duration (>90 days) during pre-pregnancy and early pregnancy is linked to subsequently adverse blood lipid profiles in the second trimester.

In addition, our results also demonstrated that women who did not take FA supplements had higher blood lipid concentrations compared to women who take FA for less than 90 days. We speculated that not taking any FA supplements might lead to FA deficiency and subsequently increase blood lipid concentrations in the second trimester. It is reported that FA deficiency might be associated with lipid accumulation. Our recent study showed that chronic FA deficiency induced obesity, lipid metabolism disorders, insulin resistance and inhibited insulin signaling.[34] In addition, other studies have confirmed that FA deficiency could result in significantly greater hepatic lipid accumulation.[35,36] Thus, not taking FA or taking FA for a longer duration may not be safe for pregnant women.

The main strength of this study is firstly reported that taking FA supplements for more than 90 days before and

during early pregnancy significantly increased the risk of GDM. Additionally, we also found that FA supplementation may cause lipid metabolism changes in the second trimester. We hypothesize that changes in lipid metabolism may provide a direction for exploring the potential mechanisms by which FA supplementation increases the risk of GDM. Finally, we considered the possible confounding factors, especially dietary intakes.

Likewise, our study has some limitations worth noting. First, our sample size was relatively small, so further large sample studies are needed. Second, this work did not investigate the red blood cell folate concentrations. Third, we did not collect the information of blood glucose value. Fourth, due to the small sample size, we did not conduct a subgroup analysis for different trimester.

Conclusions

In summary, the present study investigated the associations of FA supplementation with GDM risk and lipid metabolism. We observed a U-shape relation between duration of FA supplementation and risk of GDM. Women who did not take FA and took FA for more than 90 days had a higher incidence of GDM compared to those who took FA for ≤90 days. Moreover, blood lipid profiles in the second trimester were positively correlated with duration of FA supplementation and the risk of GDM. Future studies are required to verify the causal relationship and underlying mechanisms. Collectively, our results highlight the importance of appropriate duration of FA supplementation.

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AUTHOR DISCLOSURES

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