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Pre-conceptional intake of folic acid supplements is inversely associated with risk of preterm birth and small-for-gestational-age birth: a prospective cohort study

Ju-Sheng Zheng[1,2]†, Yuhong Guan[3]†, Yimin Zhao[1], Wei Zhao[3], Xuejuan Tang[3], Hua Chen[3], Meilong Xu[3], Lingping Wu[3], Shanlin Zhu[3], Huijuan Liu[3], Tao Huang[4] and Duo Li[1]

[1]*Department of Food Science and Nutrition, Zhejiang University, Hangzhou, 310058, People's Republic of China*
[2]*MRC Epidemiology Unit, University of Cambridge School of Clinical Medicine, Institute of Metabolic Science, Cambridge Biomedical Campus, Cambridge CB2 0QQ, UK*
[3]*Jiaxing Maternity and Child Health Care Hospital, Jiaxing, 314051, People's Republic of China*
[4]*Department of Nutrition, Harvard School of Public Health, Boston 02115, MA, USA*

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Abstract
Associations of folic acid supplementation with risk of preterm birth (PTB) and small-for-gestational-age (SGA) birth were unclear for the Chinese populations. The aim of the present study was to investigate the associations in a large Chinese prospective cohort study: the Jiaxing Birth Cohort. In the Jiaxing Birth Cohort, 240 954 pregnant women visited local clinics or hospitals within their first trimester in Southeast China during 1999–2012. Information on anthropometric parameters, folic acid supplementation and other maternal characteristics were collected by in-person interviews during their first visit. Pregnancy outcomes were recorded during the follow-up of these participants. Multinomial logistic regression was used to examine the association of folic acid supplementation with pregnancy outcomes. The prevalence of folic acid supplementation was 24·9 % in the cohort. The prevalence of PTB and SGA birth was 3·48 and 9·2 %, respectively. Pre-conceptional folic acid supplementation was associated with 8 % lower risk of PTB (relative risk (RR) 0·92; 95 % CI 0·85, 1·00; $P = 0·04$) and 19 % lower risk of SGA birth (RR 0·81; 95 % CI 0·70, 0·95; $P = 0·008$), compared with non-users. Higher frequency of pre-conceptional folic acid use was associated with lower risk of PTB ($P_{trend} = 0·032$) and SGA birth ($P_{trend} = 0·046$). No significant association between post-conceptional initiation of folic acid supplementation and either outcome was observed. In conclusion, the present study suggests an association between pre-conceptional, but not post-conceptional, folic acid supplementation and lower risk of PTB and SGA birth in the Jiaxing Birth Cohort. Further research in other cohorts of large sample size is needed to replicate these findings.

Key words: Prospective cohorts: Folic acid: Pregnancy: Preterm birth: Small-for-gestational-age birth

Folate is a water-soluble B vitamin, playing a critical role in the one-carbon metabolism process, which is essential for DNA synthesis, repair and methylation[1]. It is also important for periods of rapid cell division and growth, which occur during pregnancy and infancy[1–3]. Pregnant women may be at higher risk of folate deficiency compared with non-pregnant women, as the folate requirement is 5- to 10-fold higher during pregnancy[3]. It has been well accepted that maternal folate status is protectively associated with neural tube defects[4,5] and other congenital disorders[6,7]. However, the associations of folate supplementation with other pregnancy outcomes such as preterm birth (PTB) or small-for-gestational-age (SGA) birth are still inconclusive[8–16]. Some observational studies suggest a protective association of folate supplementation with the risk of PTB or SGA birth[9,10,12,14], whereas others have not found such a protective association[15,16]. Results from randomised controlled trials also show conflicting results[13].

In 2009, free folic acid supplements (400 mg/d) were given to rural women of child-bearing age across China as a policy issued by the Ministry of Health in China. Although numerous observational studies or randomised trials have reported the association between folic acid supplementation and PTB or SGA, studies based on Chinese populations are still limited[17,18]. A prospective cohort study conducted in central China, involving 4448 participants, suggested that folic acid supplementation is associated with lower risk of SGA birth[17]. Another large Chinese cohort study indicated that peri-conceptional folic acid supplementation was inversely associated with risk of PTB[18]. However, all the participants included in this large cohort were recruited 20 years ago (in mid 1990s). In addition, only the association of

Abbreviations: LBW, low birth weight; PTB, preterm birth; RR, relative risk; SGA, small-for-gestational-age.

* **Corresponding author**: Professor D. Li, fax +86 571 88982024, email duoli@zju.edu.cn

† These authors contributed equally to the present work.

folic acid supplementation with PTB was investigated, and the potential dose–response relationship was not examined[18]. More updated and larger prospective cohort studies are warranted to comprehensively explore these associations in Chinese populations. The aim of the present study was to examine the association between folic acid supplementation and risk of PTB and SGA birth, as well as potential dose–response relationships in a large Chinese cohort, involving 240 954 participants, during 1999–2012.

Methods

Study population

The present population-based prospective cohort study was based on a birth defect surveillance system in China, initiated in 1993[19]. During 1999–2012, 240 954 pregnant women visited local clinics and hospitals within their first trimester in Southeast China, Jiaxing area of Zhejiang Province, and were enrolled in the 'Jiaxing Birth Cohort'. Information on anthropometric parameters, folic acid supplementation and other maternal characteristics was collected by in-person interviews during their first visit to the clinics or hospitals. Subjects were excluded if they gave multiple births or non-live births (n 2086), reported any missing information on folic acid supplementation (n 5531), had missing data on the child's sex (n 119), had extreme values of maternal age (<16 or >50 years) (n 36), extreme gestational age (<28 or >42 weeks) (n 1566) or gave birth to children with extreme birth weight (<1000 or >5000 g) (n 382); subjects with extreme menarcheal age (<10 or >20 year) were also excluded (n 55). Finally, 231 179 participants were eligible for the statistical analysis.

The study was conducted according to principles laid down in the Declaration of Helsinki. The study protocol was approved by the Ethics Committee of the College of Biosystem Engineering & Food Science at Zhejiang University, China. All the participants gave their oral informed consent.

Folic acid supplementation and pregnancy outcomes

Folic acid supplementation status was assessed by three questions during an interview. (1) Have they ever taken folic acid supplements during this pregnancy (yes or no); (2) did they start taking the folic acid supplements before or after conception; (3) if they took folic acid supplements, how often did they take the pills: occasionally (<15 capsules/month), sometimes (15–25 capsules/month) or often (≥25 capsules/month). Pre- and post-conceptional folic acid supplementation was defined when participants started taking folic acid before and after conception, respectively. The mean gestational age for the time of the interview was 9·5 (SD 2·2) weeks.

PTB was defined as delivery before gestational week 37. Gestational age was calculated based on the self-reported 1st day of the woman's last normal menstrual period. SGA birth was determined as the birth weight below the 10th percentile for the gestational age, based on a global reference for fetal weight and birth weight percentiles by Mikolajczyk *et al.*[20]. The local weight percentiles across different gestational ages were obtained when the mean birth weight at 40 weeks and standard deviation of birth weight for the present population were entered into the excel-based calculator derived from the study by Mikolajczyk *et al.*[20]. Birth weight was measured to the nearest 0·01 kg by trained nurses right after the infant was born.

Covariates

Maternal educational status was categorised as > high school, high school and < high school, and occupation was categorised into four groups: farm work, routine job, temporary job and unemployed. Maternal smoking status was defined as current smoker *v.* non-current smoker, and alcohol drinking status was defined as current drinker *v.* non-current drinker. Maternal parity status was defined as primiparous or multiparous. Maternal age, BMI and menarcheal age were used as continuous variables in the statistical models, except when they were used to describe the population characteristics. Recruiting before or after 2010 (<2010 *v.* ≥2010) was also considered as a confounder, as folic acid supplements became freely available in China for rural women of pregnancy age in late 2009. Since then, the prevalence of folic acid supplementation increased significantly in the population. This new policy dramatically changed the pattern of folic acid supplementation among women, which may be associated with some unknown confounding factors.

Statistical analysis

Multinomial logistic regression was used to examine the relationship between folic acid supplementation and risk of PTB and SGA birth. Within these statistical models, potential covariates included maternal age (continuous), BMI (continuous), menarcheal age (continuous), parity status (primiparous or multiparous), educational status (<high school, high school, >high school), occupational status (farm work, routine job, temporary job, unemployed), maternal residence (rural *v.* urban), child' sex and recruiting year (<2010, ≥2010). Maternal smoking status, alcohol drinking status, history of diabetes, hypertension and heart disease were not included in the statistical model as their prevalence was extremely low (0·71 % for the current alcohol drinker, <0·2 % for the other four covariates) in this population and had negligible influence on the associations.

Among the users of folic acid, initiation of supplement use before or after conception was analysed separately to examine their potentially differential associations with pregnancy outcomes. In addition, the associations between frequency of folic acid use and pregnancy outcomes were explored. For each outcome, effect modification was examined by adding an interaction term of folic acid with a potential covariate, such as maternal age, BMI, menarcheal age, parity status, educational status, occupational status, maternal residence, child's sex and recruiting year.

A two-tailed P value < 0·05 was regarded as statistically significant. All the statistical analyses were performed using STATA software version 12 (StataCorp LP).

Results

Population characteristics and folic acid supplementation

Among all the participants, the prevalence of folic acid supplementation was 24·9 %. Post-conceptional folic acid use showed higher prevalence (14 %) compared with pre-conceptional folic acid use (10 %). When stratified by the recruiting year, the prevalence of folic acid supplementation ranged from 7·6 to 24·2 % during the 11 years from 1999 to 2009, and it increased to 48·7 % in 2010 and to 67·2 % and 64·6 % in 2011 and 2012, respectively (Fig. 1).

Folic acid users, compared with non-users, were more likely to be within the age range of 26–30 years, primiparous, had a menarcheal age <14, had higher educational level and were living in urban areas (Table 1). The prevalence of PTB and SGA birth was 3·48 and 9·20 %, respectively. The prevalence of PTB was similar among non-users (3·49 %), pre-conceptional folic acid users (3·34 %) and post-conceptional folic acid users (3·51 %). Among non-users, the prevalence of SGA birth (9·39 %) was higher than that in pre-conceptional (8·57 %) and post-conceptional folic acid users (8·68 %).

Folic acid supplementation and preterm birth

Folic acid supplementation was associated with 5 % lower risk of PTB (adjusted relative risk (RR) = 0·95; 95 % CI 0·90, 1·01) (Table 2). When separated by the timing of folic acid introduction, pre-conceptional folic acid supplementation was associated with 8 % decreased risk of PTB (adjusted RR 0·92; 95 % CI 0·85, 1·00). In addition, a significant dose–response relationship between folic acid use and risk of PTB was found in the pre-conceptional folic acid users ($P_{trend} = 0·032$), but not in post-conceptional users (Table 3). Folic acid use showed no significant interaction with any covariate on PTB risk.

Folic acid supplementation and small-for-gestational-age birth

A significant inverse association between folic acid supplementation and risk of SGA birth was observed (RR 0·91; 95 % CI 0·88, 0·94) in the crude model. The inverse association did not change remarkably in the multivariable model, and 12 % lower risk of SGA birth (adjusted RR 0·89; 95 % CI 0·80, 1·00) was observed. The protective association of folic acid supplementation against SGA birth was observed only when folic acid was taken pre-conceptionally (adjusted RR 0·81; 95 % CI 0·70, 0·95), and no significant association (adjusted RR 0·95; 95 % CI 0·83, 1·09) was observed among post-conceptional folic acid users (Table 2). Consistently, a significant dose–response association was observed only among the pre-conceptional folic acid users ($P_{trend} = 0·046$) (Table 3).

A significant interaction between folic acid use and recruiting year for the risk of SGA birth was observed ($P_{interaction} = 0·014$) (Table 4). The inverse association between folic acid supplementation and risk of SGA birth was only observed in participants recruited before 2010 (adjusted RR 0·81; 95 % CI 0·71, 0·93). Furthermore, in participants recruited before 2010, the protective association of folic acid use against SGA birth was only observed in participants who took the supplementation pre-conceptionally (adjusted RR 0·71; 95 % CI 0·58, 0·88).

Discussion

The present study suggested that folic acid supplementation was associated with lower risk of PTB and SGA birth in women who started taking folic acid supplements before conception, but not in those who started taking the supplements after conception. The present study has provided, so far, the largest sample size to examine the relationship between folic acid supplementation and PTB and SGA birth in a Chinese setting. The present results provide important and valuable information for the prevention of PTB and SGA in the Chinese populations.

Pre-conceptional folic acid use was suggested to be inversely associated with the risk of PTB or SGA birth in a number of prospective cohort studies[9,14,17,18,21,22]. In a cohort of 34 480 US women who pre-conceptionally started folic acid supplementation, there was a 50–70 % reduction in the incidence of early spontaneous PTB[9]. In another cohort of 6353 women in the Generation R Study from the Netherlands, women who

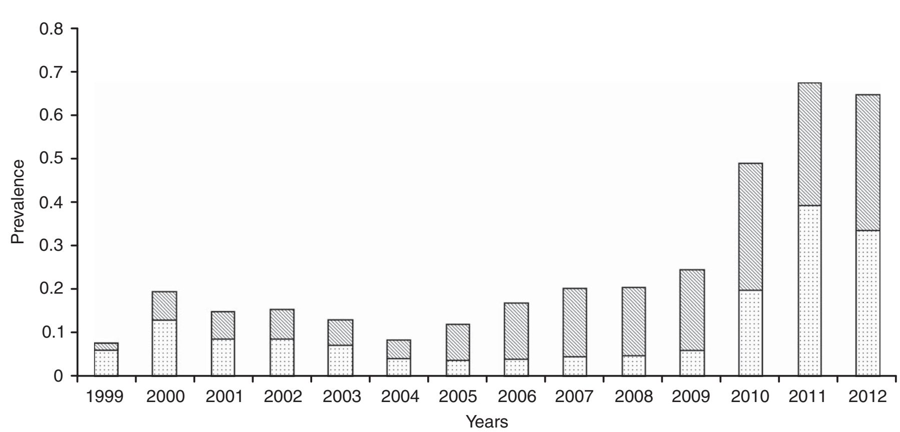

Fig. 1. Prevalence of folic acid supplementation in the Jiaxing Birth Cohort during 1999–2012. The number of participants included from 1999 to 2012 was 2755, 13 897, 19 353, 18 370, 18 005, 19 807, 17 300, 15 693, 17 372, 19 265, 21 339, 22 520, 19 741 and 5762, respectively. ▧, Post-conceptional use; ⬚, pre-conceptional use.

Table 1. Population characteristics by status of folic acid supplementation in the Jiaxing Birth Cohort*
(Number of subjects and percentages)

				Users		
	n	%	Non-users (*n* 173 545)	Pre-conceptional users (*n* 25 260)	Post-conceptional users (*n* 32 374)	*P*
Maternal age (years)						<0·001
≤25	133 568	57·8	58·7	54·9	55·4	
26–30	69 580	30·1	28·5	35·5	34·5	
31–35	24 398	10·6	11·2	8·33	8·76	
≥36	3595	1·56	1·63	1·26	1·39	
Maternal BMI (kg/m^2)						<0·001
<18·5	44 995	19·6	19·1	21·2	21·2	
18·5–24·9	169 755	74·0	74·4	73·1	72·7	
25–29·9	13 080	5·70	5·83	5·08	5·51	
≥30	1462	0·64	0·64	0·65	0·63	
Parity						<0·001
Primiparous	185 731	80·3	78·7	85·9	84·9	
Multiparous	45 448	19·7	21·3	14·1	15·1	
Menarcheal age (years)						<0·001
<14	39 462	17·1	16·1	20	20·4	
14–15	139 944	60·7	60·8	60·9	60·1	
>15	51 196	22·2	23·2	19·2	19·5	
Maternal educational status						<0·001
<High school	145 302	62·9	68·9	45·5	44·8	
High school	42 003	18·2	17·8	17·7	20·8	
>High school	43 550	18·9	13·3	36·9	34·4	
Maternal occupation						<0·001
Farm work	142 499	61·8	65·6	53·6	47·5	
Routine job	45 575	19·8	18·3	24·2	19·8	
Temporary job	15 040	6·52	6·12	7·3	6·52	
Unemployed	27 612	12·0	10	14·9	12	
Maternal residence						<0·001
Rural	168 101	72·7	76·5	65·5	58·1	
Urban	63 078	27·3	23·5	34·5	41·9	
Preterm birth	8044	3·48	3·49	3·34	3·51	0·49
Small-for-gestational-age birth	21 265	9·20	9·39	8·57	8·68	<0·001

* *P* value was calculated by the χ^2 test comparing the maternal characteristics between folic acid users (pre- plus post-conceptional) and non-users.

Table 2. Association of folic acid supplementation with risk of preterm birth and small-for-gestational-age birth*
(Relative risk (RR) and 95 % confidence interval)

	Non-user		Users			Pre-conceptional use			Post-conceptional use		
	RR	95 % CI	RR	95 % CI	*P*	RR	95 % CI	*P*	RR	95 % CI	*P*
Risk of preterm birth											
Case/study subjects	6065/173 545		1979/57 634			843/25 260			1136/32 374		
Model 1	1	Ref.	0·98	0·93, 1·03	0·49	0·95	0·89, 1·03	0·20	1·00	0·94, 1·07	0·90
Model 2	1	Ref.	0·95	0·90, 1·01	0·08	0·92	0·85, 1·00	0·040	0·97	0·91, 1·04	0·40
Risk of small-for-gestational-age birth											
Case/study subjects	16 290/173 545		4975/57 634			2165/25 260			2810/32 374		
Model 1	1	Ref.	0·91	0·88, 0·94	<0·001	0·90	0·86, 0·95	<0·001	0·92	0·88, 0·96	<0·001
Model 2	1	Ref.	0·89	0·80, 1·00	0·044	0·81	0·70, 0·95	0·008	0·95	0·83, 1·09	0·47

Ref. referent values.
* Model 1 is crude RR. Model 2 is adjusted for maternal age (continuous), BMI (continuous), menarcheal age (continuous), parity status, educational status (<high school, high school, >high school), occupational status (farm work, routine job, temporary job, unemployed), maternal residence (rural *v.* urban), child' sex and recruiting year (<2010 *v.* ≥2010).

pre-conceptionally started taking folic acid supplements showed a 60 % reduction of SGA birth risk compared with non-users[14]. In a recent cohort study in China, peri-conceptional use of folic acid supplementation was associated with 30 % decreased risk of spontaneous PTB. These studies are in line with the present study, showing that the protective association of folic acid supplements against PTB or SGA birth only existed among those who started taking the supplements pre-conceptionally, but not in those who started taking the supplements after conception. It appeared that the protective association of pre-conceptional folic acid supplementation in the present study was much weaker compared with the aforementioned studies. It may be because of the residual confounding factors in the present study, as it involved participants from 1999 to 2012. During this time period, diet, lifestyles and certain socio-economic statuses may have changed remarkably

Table 3. Association between the frequency of folic acid supplementation and risk of preterm birth and small-for-gestational-age birth*
(Relative risk (RR) and 95 % confidence interval)

	Pre-conceptional use									Post-conceptional use						
	Non-users		<15 cap/months		15–25 cap/months		≥25 cap/months			<15 cap/months		15–25 cap/months		≥25 cap/months		
	RR	95 % CI	RR	95 % CI	RR	95 % CI	RR	95 % CI	P_{trend}	RR	95 % CI	RR	95 % CI	RR	95 % CI	P_{trend}
Risk of preterm birth																
Case/study subjects	6065/173 545		12/284		39/1343		792/23 633			31/604		143/4018		962/27 752		
Model 1	1	Ref.	1·22	0·68, 2·17	0·83	0·60, 1·14	0·96	0·89, 1·03	0·21	1·49	1·04, 2·15	1·02	0·86, 1·21	0·99	0·93, 1·06	0·90
Model 2	1	Ref.	1·24	0·70, 2·22	0·82	0·60, 1·13	0·92	0·84, 1·00	0·032	1·53	1·06, 2·20	0·96	0·81, 1·14	0·96	0·89, 1·04	0·33
Risk of small-for-gestational-age birth																
Case/study subjects	16 290/173 545		18/284		101/1343		2046/23 633			64/604		339/4018		2407/27 752		
Model 1	1	Ref.	0·65	0·41, 1·05	0·79	0·64, 0·96	0·91	0·87, 0·96	<0·001	1·14	0·88, 1·48	0·89	0·79, 1·00	0·92	0·88, 0·96	<0·001
Model 2	1	Ref.	0·38	0·12, 1·21	0·54	0·30, 0·96	0·87	0·74, 1·03	0·046	0·94	0·38, 2·29	1·12	0·79, 1·59	0·95	0·82, 1·10	0·58

Ref. referent values.
* Model 1 is crude RR. Model 2 is adjusted for maternal age (continuous), BMI (continuous), menarcheal age (continuous), parity status, educational status (<high school, high school, >high school), occupational status (farm work, routine job, temporary job, unemployed), maternal residence (rural *v.* urban), child' sex and recruiting year (<2010 *v.* ≥2010).

due to the rapid economic growth across the country. Among pre-conceptional folic acid users, it appeared that less frequent folic acid use was much stronger related to SGA than more frequent supplement use. Misclassification of folic acid supplement use may contribute to such results. In addition, among the category '<15 cap/month', the number of study participants was rather small, which may not provide sufficient statistical power to examine the associations.

The mechanisms behind the relationship between folic acid supplementation and PTB and SGA birth are not fully understood. Folate is a critical B vitamin, which acts as a one-carbon donor that is implicated in biological pathways of many cellular processes such as cell multiplication, apoptosis and intracellular signalling, as well as fetal and placental growth and development[1]. Furthermore, folic acid supplementation may affect fetal growth by regulating the folate-dependent homocysteine pathway. Hyperhomocysteinaemia was associated with decreased fetal growth and placental vasculopathy[23], whereas high folate status was associated with lower homocysteine level[24]. Consistent with previous reports by Timmermans *et al.*[14], the present study found a significant inverse association between folic acid use and risk of common adverse pregnancy outcomes when taken pre-conceptionally, but not post-conceptionally. This may be because pre-conceptional exposure to folic acid may exert some additional benefits to fetal growth via epigenetic modification[25,26].

Since late 2009, rural Chinese women of child-bearing age were given free folic acid supplements (400 mg/d) from the Government as part of a public health project initiated by the Ministry of Health in China. As a result of this policy, the prevalence of folic acid supplementation in the present study population increased from 24·2 % in 2009 to 48·7 % in 2010. The prevalence continued to increase to 67·2 and 64·6 % in 2011 and 2012, respectively. Interestingly, a significant interaction between folic acid use and recruiting year for SGA birth was observed. This may reflect some residual confounders in the present study. Indeed, the initiation of the new policy may have increased the health consciousness of women of child-bearing age and reduced other risk factors of adverse pregnancy outcomes, thereby attenuating or even eliminating the inverse association of folic acid supplements with SGA birth. On the other hand, women who used folic acid supplements before they were free may be more health-conscious and associated with healthier lifestyles or better economic status, thereby strengthening the inverse association of folic acid supplements with SGA birth. Nevertheless, no interaction between folic acid supplementation and recruiting year for PTB was observed. Therefore, the influence of the free folic acid distribution policy on the association between folic acid supplementation and adverse pregnancy outcomes warrants further investigation.

The prevalence of folic acid supplementation found in this study is in agreement with some other studies in China[27–31]. Between 2002 and 2004, 483 rural women were recruited to investigate the blood folate concentrations and the status of folic acid supplementation in Shanxi Province in China. This survey found that 47·6 % of the women had erythrocyte folate deficiency, and <10 % reported having ever taken folic acid supplements during their current pregnancy[27]. In another

Table 4. Stratified analysis of the association between folic acid supplementation and risk of small-for-gestational-age birth*
(Relative risk (RR) and 95 % confidence interval)

	Non-users		Users			
	RR	95 % CI	RR	95 % CI	P	$P_{interaction}$
Recruiting year						0·014
Before 2010						
Case/study subjects	14 434/153 491		2593/29 665			
Adjusted RR	1	Ref.	0·81	0·71, 0·93	0·003	
After 2010						
Case/study subjects	1856/20 054		2382/27 969			
Adjusted RR	1	Ref.	1·10	0·90, 1·35	0·36	
Maternal age (years)						0·16
≤25						
Case/study subjects	10 669/101 787		3105/31 781			
Adjusted RR	1	Ref.	0·86	0·75, 0·99	0·034	
26–30						
Case/study subjects	4003/49 442		1536/20 138			
Adjusted RR	1	Ref.	0·92	0·74, 1·15	0·47	
31–35						
Case/study subjects	1419/19 458		291/4940			
Adjusted RR	1	Ref.	1·10	0·71, 1·71	0·66	
≥36						
Case/study subjects	199/2826		43/769			
Adjusted RR	1	Ref.	1·60	0·51, 4·98	0·42	
Maternal BMI (kg/m^2)						0·23
<18·5						
Case/study subjects	4593/32 858		1567/12 137			
Adjusted RR	1	Ref.	0·80	0·64, 1·01	0·06	
18·5–24·9						
Case/study subjects	10 975/127 956		3217/41 799			
Adjusted RR	1	Ref.	0·91	0·79, 1·04	0·16	
25–29·9						
Case/study subjects	528/10 029		148/3051			
Adjusted RR	1	Ref.	1·15	0·63, 2·12	0·65	
≥30						
Case/study subjects	48/1094		18/368			
Adjusted RR	1	Ref.	0·35	0·06, 1·94	0·23	
Parity						0·62
Primiparous						
Case/study subjects	13 640/136 536		4502/49 195			
Adjusted RR	1	Ref.	0·86	0·77, 0·98	0·018	
Multiparous						
Case/study subjects	2650/37 009		473/8439			
Adjusted RR	1	Ref.	1·12	0·82, 1·53	0·47	
Menarcheal age (years)						0·65
<14						
Case/study subjects	2386/27 830		928/11 632			
Adjusted RR	1	Ref.	0·95	0·72, 1·25	0·71	
14–15						
Case/study subjects	9651/105 151		2998/34 793			
Adjusted RR	1	Ref.	0·88	0·76, 1·01	0·08	
>15						
Case/study subjects	4187/40 072		1043/11 124			
Adjusted RR	1	Ref.	0·89	0·70, 1·12	0·32	
Maternal educational status						0·10
<High school						
Case/study subjects	11 984/119 343		2444/25 959			
Adjusted RR	1	Ref.	0·86	0·74, 0·99	0·036	
High school						
Case/study subjects	2579/30 817		929/11 186			
Adjusted RR	1	Ref.	0·84	0·64, 1·11	0·23	
>High school						
Case/study subjects	1706/23 102		1598/20 448			
Adjusted RR	1	Ref.	1·01	0·79, 1·29	0·96	
Maternal occupation						0·80
Farm work						
Case/study subjects	11 281/113 650		2658/28 849			
Adjusted RR	1	Ref.	0·86	0·75, 0·99	0·042	
Routine job						
Case/study subjects	2724/31 748		1165/13 827			
Adjusted RR	1	Ref.	0·95	0·74, 1·23	0·71	

Table 4. *Continued*

	Non-users		Users			
	RR	95 % CI	RR	95 % CI	*P*	$P_{interaction}$
Temporary job						
Case/study subjects	895/10 603		365/4437			
Adjusted RR	1	Ref.	0·80	0·50, 1·29	0·37	
Unemployed						
Case/study subjects	1362/17 236		774/10 376			
Adjusted RR	1	Ref.	0·97	0·71, 1·34	0·88	
Maternal residence						0·95
Rural						
Case/study subjects	13 168/132 732		3268/35 369			
Adjusted RR	1	Ref.	0·88	0·77, 1·01	0·06	
Urban						
Case/study subjects	3122/40 813		1707/22 265			
Adjusted RR	1	Ref.	0·92	0·74, 1·15	0·47	

Ref. referent values.

* All the statistical models were adjusted for maternal age (continuous), BMI (continuous), menarcheal age (continuous), parity status, educational status (<high school, high school, >high school), occupational status (farm work, routine job, temporary job, unemployed), maternal residence (rural *v.* urban), child's sex and recruiting year (<2010 *v.* ≥2010), except for the covariate that was stratified for the subgroup analysis.

cross-sectional study of 1338 women of child-bearing age who were recruited in 2008 in Shanghai, only 14·9 % of the subjects took folic acid supplements at least once per day, whereas 74·3 % of them had never taken it[28]. In addition, within this Shanghai population, nearly half of them did not know that folic acid should be taken before pregnancy.

We noticed that the prevalence of PTB in the present study was much lower than that reported by a recent WHO report (7·1 %)[32]. However, accordingly to a recent global report on PTB and still birth[33], the prevalence of PTB in China has decreased from 7·5 % in 1981–1982 to 3·5 % in 1998[34]. Another large-scale study[35], based on 542 923 women in ten counties from South and North China, suggested that the PTB rate declined steadily from 1993 to 2005, with an overall PTB rate of 4·49 % during 1993 and 2005 and 3·62 % in 2005. A more recent large study, involving 26 611 pregnancies in Southeast China's Jiangsu Province, also found that the PTB prevalence ranged from 2·6 to 2·9 % in urban and rural areas[36]. Therefore, our report about the prevalence of PTB was consistent with these studies. The reason for the inconsistency between the WHO report and present study and other aforementioned studies is not clear and needs further clarification.

There are several strengths to the present study. First, to the best of our knowledge, this study was the largest prospective cohort study to comprehensively examine the association between folic acid supplementation and risk of PTB and SGA in Chinese populations. The large sample size gave us sufficient power to examine the topics of concern. Second, the present study was a population-based prospective cohort, which would have minimised the influence of selection or recall bias. In addition, almost all the participants were of Han ethnicity and shared similar lifestyle and dietary patterns, which would have reduced the residual confounding factors.

Several limitations are present for this study. First, we did not collect information on multivitamin use, which is a major limitation of the present study. Indeed, use of folic acid and multivitamin might be highly correlated and influence the associations of folic acid use with birth outcomes. Second, our participants were recruited from Southeast China, and the generalisability of the present results to other regions of China may be inappropriate. Nonetheless, the prevalence of folic acid supplementation and other maternal characteristics in the present study were consistent with populations of other regions in China[27–30]. This suggested that our population could, to some extent, represent women from other regions. Third, the observational nature of the study design made it subject to some residual confounding factors. Fourth, there may be misclassification of folic acid supplements, especially for the frequency of folic acid use, and potential sporadic folic acid use. In addition, we did not collect information on the actual dose of folic acid supplements. In this study, we assumed that 400 mg tablets of folic acid supplements were used by the present population, as after 2009 folic acid supplements were freely available in the form of 400 mg tablets. This dose was also widely recommended for women of child-bearing age or during pregnancy in China before 2009. Fifth, we did not measure the duration of folic acid supplementation of the folic acid users, and therefore could not examine the influence of folic acid use duration on birth outcomes. Finally, recall and outcome misclassification may exist – for example, gestational age was based on self-reported last normal menstrual period, which is known to be error prone.

In conclusion, the present study suggests an inverse association of pre-conceptional folic acid supplementation with lower risk of PTB and SGA birth. Further studies in this field are warranted to replicate these findings.

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The authors declare that there are no conflicts of interest.

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