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Maternal folic acid supplementation and more prominent birth weight gain in twin birth compared with singleton birth: a cross-sectional study in northwest China

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Abstract

Objective: To investigate the association of folic acid (FA) supplementation with birth weight, the risk of small for gestational age (SGA) and low birth weight (LBW) in singleton and twin pregnancy.

Design: A population-based cross-sectional survey.

Setting: Twenty counties and ten districts in Shaanxi Province of northwestern China, 2013.

Participants: 28 174 pregnant women with their infants, covering 27 818 single live births and 356 twin live births.

Results: The prevalence of FA supplementation in singletons and twins was 63·9 and 66·3 %. The mean birth weight was 3267 (SD 459·1) g, 2525 (SD 534·0) g and 2494 (SD 539·5) g; the prevalence of SGA was 14·3, 51·4 and 53·4 %; the prevalence of LBW was 3·4, 42·4 and 46·6 % among singleton, twin A and twin B, respectively. Compared with non-users, women with FA supplementation were (β 17·3, 95 % CI 6·1, 28·4; β 166·3, 95 % CI 69·1, 263·5) associated with increased birth weight, lower risk of SGA (OR 0·85, 95 % CI 0·80, 0·92; OR 0·45, 95 % CI 0·30, 0·68) and LBW (OR 0·82, 95 % CI 0·71, 0·95; OR 0·50, 95 % CI 0·33, 0·75) in singletons and twins, and more prominent effects in twins. Moreover, there were significant interactions between FA supplementation and plurality on birth weight, SGA and LBW.

Conclusions: The present study suggests the association of periconceptional 0·4 mg/d FA supplementation with increased birth weight and reduced risk of SGA and LBW in both singletons and twins, and this association may be more prominent in twins.

Keywords
Folic acid supplementation
Birth weight
Twins
Interaction effect

Introduction

Birth weight, serving as an important indicator of foetal growth,has been influenced by foetal and maternal factors and affected adult later-life health[1–3]. Small for gestational age (SGA) and low birth weight (LBW) are higher risk factors of morbidity and mortality of infants[4–6]. Maternal nutrition before and during pregnancy plays a critical role in pregnancy outcome[7–9]; the recommendations had been given in many countries, including China, for women of child-bearing age to take folic acid (FA)[10–12]. Since 2009, free FA supplementation at a dose of 0·4 mg/d during the periconceptional period has been given to the women of child-bearing age in China under a policy issued by the Ministry of Health to ensure good maternal and child health. The policy was implemented through the three-level network of health care in China. The health administration department in the rural areas or the districts of urban areas is in charge of purchase and management of FA supplements. In rural areas, the township hospital distributes the supplements to the village clinics where village doctors are responsible for its free distribution to the women in need.

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And the women in urban areas might go to the community health service centre for a free supplement[13]. However, to date, the effects of FA on other pregnancy outcomes, such as birth weight, SGA and LBW, remain inconclusive except neural tube defects[14,15] and congenital diseases[16,17]. Besides, previous studies mainly focused on the effects of FA supplementation on birth weight in singleton births[18–24]; no studies had compared the effects of FA supplementation on birth weight indicators in the same samples of singleton and twin births in the round. The present study aimed to explore whether the associations of FA supplementation with birth weight, SGA and LBW are consistent in singletons and twins, as well as the potential effect modifications concerning plurality in a large population-based cross-sectional survey in China, involving 28 174 pregnant women with their infants, during 2010–2013.

Methods

Study design and participants

A population-based, cross-sectional epidemiological survey aiming to investigate the risk factors of birth outcomes was conducted between August and December 2013 in Shaanxi Province of northwest China. Infants born during 2010–2013 and their mothers were recruited using a stratified multistage random sampling method, which has been described elsewhere[25,26]. Briefly, according to the proportion of rural and urban residents and the fertility level of the population of the whole province of Shaanxi, firstly twenty counties and ten districts were sampled randomly. And then six villages each from six townships were selected randomly in each sampled county; six communities each from three streets were selected randomly in each sampled district. Finally, thirty and sixty participants were selected randomly in each sampled village and community, respectively. 30 027 of 32 400 pregnant women completed the questionnaire in the survey (response rate 92·7 %). In our study, 1853 were excluded for the following reasons: miscarriage (*n* 704); terminations (*n* 42); stillbirth (*n* 15); triplet births (*n* 2); FA supplementation was unknown (*n* 606); missing data for birth weight (*n* 484). The final study sample included 28 174 women with their infants – 27 818 single live births and 356 twin live births. Figure 1 displays the flow diagram with exclusion criteria in this study.

Ascertainment of folic acid supplementation

In China, the policy of free FA supplementation at a dose of 0·4 mg/d during the periconceptional period has been carried

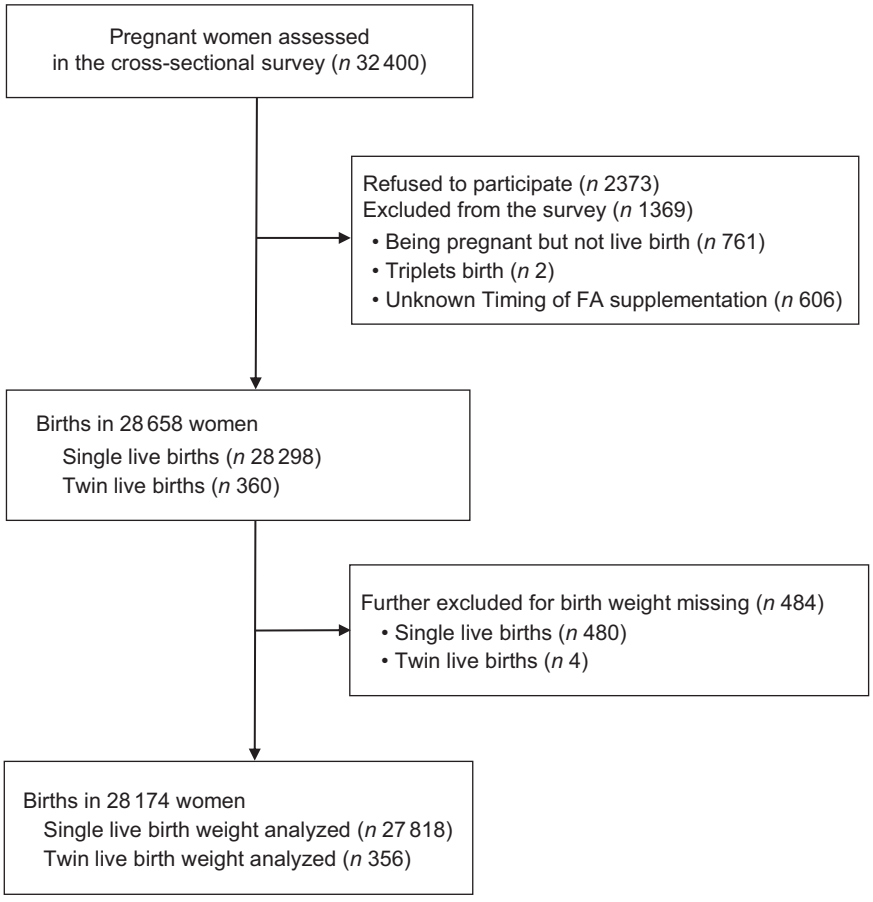

Fig. 1 Study flow diagram with exclusion criteria. FA, folic acid

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out since 2009. The women who plan to be pregnant might get FA supplements freely in village clinics or community health service centres. In this study, FA supplementation was recorded via a retrospective in-person interview. Women were asked to report the brand and duration of FA supplementation use. In this study, women who took FA pills of 0·4 mg/d at any time from preconception (12 weeks before pregnancy) until the end of the first trimester (1–12 weeks during pregnancy) were classified as FA users[14]. FA supplementation was divided into four patterns according to the period of use: (1) preconceptional use (12 weeks before pregnancy); (2) postconceptional use (1–12 weeks during pregnancy); (3) periconceptional use (12 weeks before pregnancy and 1–12 weeks during pregnancy); (4) no use (no supplementation during the above periods). We also collected FA supplementation in the second trimester (13–27 weeks during pregnancy) and third trimester (≥28 weeks during pregnancy). This information was used for sensitivity analysis.

Ascertainment of birth outcome

Birth weight and gestational age (GA) were recorded from a review of the Medical Certificate of Birth. Birth weight was measured with precision to the nearest 10 g. GA was calculated in weeks based on the first day of the last menstrual period (LMP)[22,27,28]. Information about LMP was obtained from the Medical Certificate of Birth. SGA was defined as the infant whose birth weight was <10th centile based on the GA–gender-specific Chinese reference for foetal growth[29]. LBW was defined as birth weight <2500 g. Plurality was defined according to the number at births (singletons or twins)[30].

Assessment of covariates

Questionnaires administered via retrospective in-person interview were used to collect participants' characteristics. Covariates considered were infant gender (female or male), residence (rural or urban), maternal age (<25, 25–29, 30–34, ≥35), maternal education (non-educated, primary, secondary, high school, college or above), household wealth index (poor, middle, rich), antenatal care (ANC) visits (<7 or ≥7), gravidity (1, 2, ≥3), parity (1, ≥2), GA (continuous), alcohol drinking (yes or no), passive smoking (yes or no), tea drinking (yes or no), pregnancy-induced hypertension (PIH; yes or no), cold (yes or no), iron supplementation during pregnancy (yes or no), birth order (twin A, twin B). The household wealth index was built by principal component analysis based on four variables representing the family economic level (monthly income, monthly expenditure, housing condition, vehicle), which was classified according to tertiles of poor, middle and rich[31]. Maternal active smoking was also one of the important predictors of reduced birth weight[32], which should be considered as a confounder. However, the percentage of active women smokers was extremely low in China[33], especially among women of child-bearing age (0·3 % for women with singletons and none for women with twins in our study). Therefore, the covariate of active smoking was not included in the present analysis.

Statistical analysis

The characteristics of participants are reported as mean (SD) for the quantitative variable, and number (percentage) for the categorical variable. Student's *t* test for continuous variables and χ^2 test for categorical variables were used in univariate analyses. Based on the data distribution and type (normal distribution with identity-link function, and binomial distribution with logit-link function), we fitted generalised linear models (GLM) and generalised estimating equation (GEE) models [34–36] to estimate adjusted regression coefficients, or OR and 95 % confidence intervals (CI) for each birth outcome. The consideration of potential confounders was based on previous literature reports[7,20,23,37–40]. Stratified analysis by plurality was built to estimate the association between FA supplementation and birth weight, the risk of SGA and LBW, respectively. In addition, effect modification was examined by adding an interaction term of FA supplementation with plurality in dataset 1 (singleton and twin A) and dataset 2 (singleton and twin B) of the total population, respectively. Furthermore, the association of timing of FA supplementation with birth weight, risk of SGA and LBW was explored. Finally, a sensitivity analysis was conducted to evaluate the robustness of the results by excluding women who sporadically continued to consume FA in the second trimester or third trimester or both (207 cases). And a subgroup analysis was conducted by infant's sex.

Statistical analyses were conducted with SAS software (version 9.4; SAS Institute Inc.). A two-tailed *P* value <0·05 was regarded as statistically significant.

Results

Status of folic acid supplementation

The status of FA supplementation is presented in Table 1. Among all the participants, the prevalence of FA supplementation was 63·9 % in singletons and 66·3 % in the twin sample. Women with FA supplementation accounted for 4·3 % for preconception, 46·9 % for postconception and 12·7 % for periconception, respectively, among the singleton sample; and these figures were 4·5, 45·8, 16·0 %, respectively, among the twin sample, but there was no statistical significance between singleton and twin samples.

Demographic characteristics of participants

A total of 28 174 women aged 15–49 years were included in the survey, covering 27 818 single live births and 356 twin

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Table 1 Maternal and infant characteristics by plurality in northwest China during 2010–2013

	Singleton (*n* 27 818)		Twins (*n* 356)				
			Twin A		Twin B		
Variables	*n*	%	*n*	%	*n*	%	*P* value*
Maternal characteristics							
Residence							
Rural	22 051	79·3	283	79·5			0·917
Urban	5767	20·7	73	20·5			
Age (years)							
Mean	26·6		27·4				<0·001
SD	4·7		4·7				
Age							
<25	10 444	37·5	95	26·7			<0·001
25–29	10 979	39·5	164	46·1			
30–34	4318	15·5	64	18·0			
≥35	2077	7·5	33	9·3			
Education							
Non-educated	504	1·8	5	1·4			0·843
Primary	2788	10·0	37	10·4			
Secondary	13 826	49·7	186	52·2			
High school	5583	20·1	67	18·8			
College or above	5117	18·4	61	17·1			
Household wealth index							
Poor	9269	33·3	120	33·7			0·957
Middle	9272	33·3	116	32·6			
Rich	9277	33·3	120	33·7			
ANC visits							
<7	16 492	59·3	198	55·6			0·162
≥7	11 326	40·7	158	44·4			
Gravidity							
1	14 655	52·7	171	48·0			0·041
2	10 043	36·1	131	36·8			
≥3	3120	11·2	54	15·2			
Parity							
1	16 811	60·4	163	45·8			<0·001
≥2	11 007	39·6	193	54·2			
Passive smoking	6844	24·6	82	23·0			0·494
Alcohol drinking	301	1·1	4	1·1			>0·999
Tea drinking	636	2·3	10	2·8			0·513
PIH	432	1·6	19	5·3			<0·001
Cold	10 539	37·9	118	33·1			0·067
Iron supplementation	1387	5·0	35	9·8			<0·001
FA supplementation	17 780	63·9	236	66·3			0·353
Preconception	1197	4·3	16	4·5			
Postconception	13 054	46·9	183	45·8			
Periconception	3529	12·7	57	16·0			
Infants' characteristics							
Gender							
Female	12 689	45·6	158	44·4	173	48·6	0·643
Male	15 129	54·4	198	55·6	183	51·4	0·262
Birth weight (g)							
Mean	3267		2525		2494		<0·001
SD	459·1		534·0		539·5		
Small for gestational age	3990	14·3	183	51·4	190	53·4	<0·001
Low birth weight	941	3·4	151	42·4	166	46·6	<0·001
Gestational age (weeks)							
Mean	39·6		37·6				<0·001
SD	1·3		2·5				
<32	52	0·2	7	2·0			<0·001
32–36	642	2·3	85	23·9			
≥37	27 124	97·5	264	74·2			

ANC, antenatal care; PIH, pregnancy-induced hypertension, FA, folic acid.
*Comparisons used Student's *t* test for quantitative variables and χ^2 test for categorical variables.

live births. The mean maternal age of the twin sample was 27·4 (SD 4·7) years, and 26·6 (SD 4·7) years for the singleton sample. There was no statistically significant difference in infant's gender (twin A *v.* singleton, and twin B *v.* singleton, $P = 0·643$, 0·262, respectively). Other detailed demographic characteristics of the two samples are presented in Table 1.

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Compared with the mean birth weight of the singleton sample (3267 g; SD 459·1), the mean birth weights of twin A (2525 g; SD 534·0) and twin B (2494 g; SD 539·5) were lower ($P<0·001$). The prevalence rates of SGA in twin A and twin B were higher (51·4 and 53·4 *v.* 14·3 %; $P<0·001$) than that of the singleton sample. The prevalence rates of LBW in twin A and twin B were higher (42·4 and 46·6 *v.* 3·4 %; $P<0·001$) than that of the singleton sample. The twin sample's GA was shorter than that of the singleton sample (37·6 (SD 2·5) *v.* 39·6 (SD 1·3) weeks; $P<0·001$).

Associations between folic acid supplementation and birth weight, small for gestational age, low birth weight by plurality

Figure 2 displays the differences in birth weight, prevalence of SGA and LBW, and GA according to the status of FA supplementation in the singleton and twin samples (twin A, twin B). Accordingly, compared to non-users, infants' birth weight for women with FA supplementation was higher (3283 *v.* 3240 g, $P<0·001$; 2567 *v.* 2444 g, $P=0·040$) in singleton and twin A sample, respectively, but there was no statistically significant difference (2530 *v.* 2426 g, $P=0·085$) in twin B sample. The prevalence of SGA for women with FA supplementation was lower (singleton: 13·0 *v.* 16·8 %, $P<0·001$; twin A: 44·1 *v.* 65·8 %, $P<0·001$; twin B: 47·0 *v.* 65·8 %, $P=0·001$) compared to non-users (Fig. 2(b)). Simultaneously, compared with non-users, the prevalence of LBW for women with FA supplementation was lower (singleton: 2·9 *v.* 4·2 %, $P<0·001$; twin A: 37·7 *v.* 51·7 %, $P=0·012$), but there was no statistically significant difference (44·9 *v.* 50·0 %, $P=0·363$) in twin B (Fig. 2(c)). Compared with non-users, GA for women with FA supplementation was not significantly different in the singleton sample (39·7 *v.* 39·6 weeks, $P=0·306$), but was lower in the twin sample (37·4 *v.* 38·1 weeks, $P=0·007$) (Fig. 2(d)).

Stratified analysis was performed by plurality after an adjustment for major confounding factors (Fig. 3). Compared with non-users, infants' birth weight for women with FA supplementation was higher in the singleton sample (17·3 g, 95 % CI 6·1, 28·4; $P=0·002$), prominently higher in the twin sample (166·3 g, 95 % CI 69·1, 263·5, $P=0·001$; twin A: 179·1 g, 95 % CI 71·7, 286·6, $P=0·001$; twin B: 153·3 g, 95 % CI 43·5, 263·1, $P=0·006$). FA supplementation was associated with a reduced risk of SGA in both singleton and twin samples (singleton: OR 0·85, 95 % CI 0·80, 0·92, $P<0·001$; twins: OR 0·45, 95 % CI 0·30, 0·68, $P<0·001$; twin A: OR 0·43, 95 % CI 0·27, 0·70, $P=0·001$; twin B: OR 0·47, 95 % CI 0·29, 0·76, $P=0·002$) and a reduced risk of LBW (singleton: OR 0·82, 95 % CI

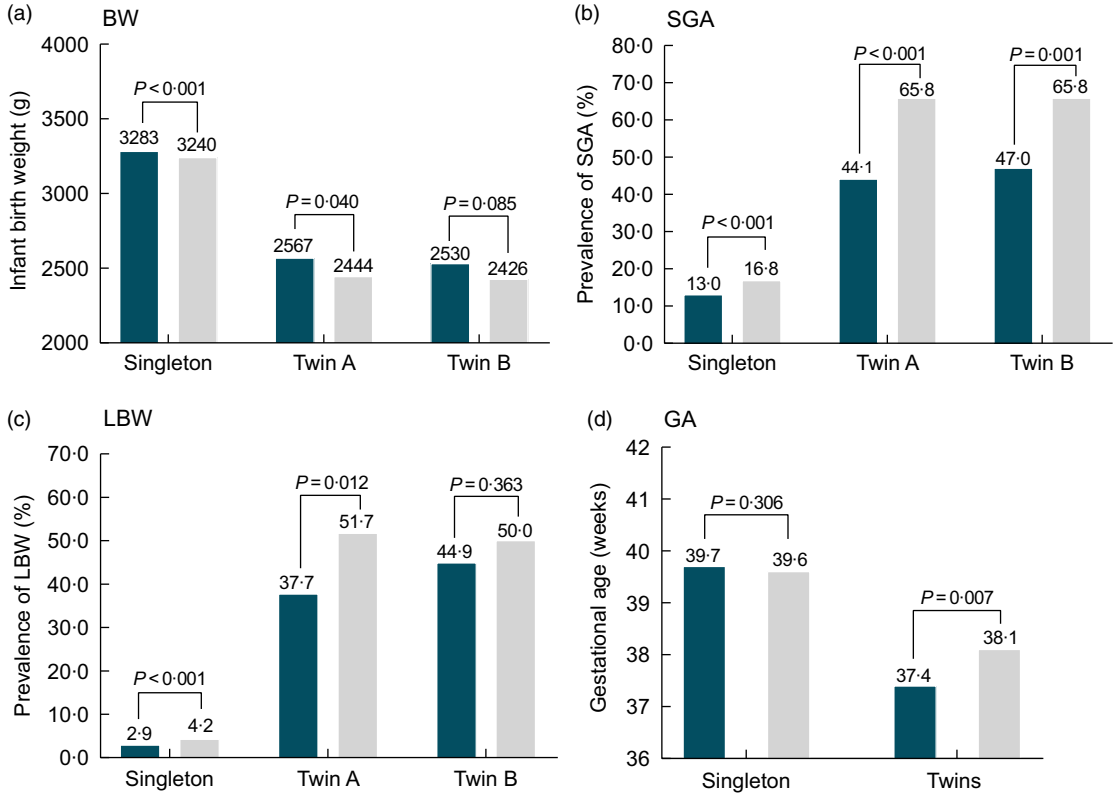

Fig. 2 (colour online) Differences in BW, SGA, LBW and GA by plurality according to the status of FA supplementation in northwest China during 2010–2013. BW, birth weight; FA, folic acid; SGA, small for gestational age; LBW, low birth weight. ■, FA; ■, non-FA.

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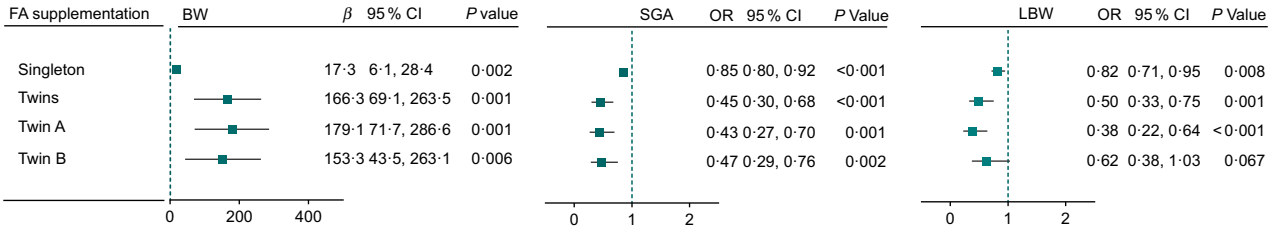

Fig. 3 (colour online) Birth weight associated with FA supplementation in singletons and twins in northwest China during 2010–2013. For BW, β was adjusted for all covariates of infant's gender, residence, maternal age, maternal education, household wealth index, antenatal care visits, gravidity, parity, passive smoking, alcohol drinking, tea drinking, pregnancy-induced hypertension, cold, iron supplementation, FA supplementation, gestational age in generalised linear models (GLM) with normal distribution and identity-link function among singletons, twin A, twin B; and β was adjusted for all covariates above plus birth order in generalised estimating equation (GEE) models with normal distribution and identity-link function in twins. For SGA, OR was adjusted for all covariates above except for GA in GLM with binomial distribution and logit-link function among singletons, twin A, twin B, and OR was adjusted for all covariates above except for GA, plus birth order in GEE with binomial distribution and logit-link function in twins. For LBW, OR was adjusted for all covariates above in GLM with binomial distribution and logit-link function among singletons, twin A, twin B, and OR was adjusted for all covariates above plus birth order in GEE with binomial distribution and logit-link function in twins. BW, birth weight; FA, folic acid; SGA, small for gestational age; LBW, low birth weight

0·71, 0·95, $P = 0·008$; twins: OR 0·50, 95 % CI 0·33, 0·75, $P = 0·001$; twin A: OR 0·38, 95 % CI 0·22, 0·64, $P < 0·001$), but there was no significant reduction of LBW in twin B (OR 0·62, 95 % CI 0·38, 1·03, $P = 0·067$).

Interaction effect analysis of folic acid supplementation with plurality on birth weight, small for gestational age, low birth weight

To identify whether the associations were caused by plurality rather than sampling error, the effect modification was examined by adding an interaction term of FA supplementation with plurality in dataset 1 (singleton and twin A) and dataset 2 (singleton and twin B) of the total population, respectively. Table 2 shows that the main effect terms of FA supplementation (dataset 1: 17·4 g, 95 % CI 6·2, 28·5, $P = 0·002$; dataset 2: 17·2 g, 95 % CI 6·1, 28·4, $P = 0·002$) and the interaction terms of FA supplementation by plurality (dataset 1: 160·0 g, 95 % CI 63·4, 256·6, $P = 0·001$; dataset 2: 139·7 g, 95 % CI 43·1, 236·4, $P = 0·005$) were positively correlated with birth weight. The results indicate that birth weight was approximately 17 g higher in the FA supplementation group compared to non-users for singletons, and was 177·4 g (160·0 + 17·4) and 156·9 g (139·7 + 17·2) higher in twin A and twin B.

As regards SGA, compared with non-users, the main effect terms of FA supplementation (dataset 1 and 2: OR 0·85, 95 % CI 0·79, 0·92, $P < 0·001$) and the interaction terms of FA supplementation by plurality (dataset 1: OR 0·52, 95 % CI 0·33, 0·83, $P = 0·007$; dataset 2: OR 0·59, 95 % CI 0·37, 0·94, $P = 0·027$) were inversely associated with SGA, indicating that FA supplementation was associated with reduced risks of SGA (singleton: OR 0·85; twin A: OR 0·44 (0·85 × 0·52); twin B: OR 0·50 (0·85 × 0·59)).

For LBW, compared with non-users, the main effect terms of FA supplementation (dataset 1 and 2: OR 0·82, 95 % CI 0·71, 0·94, $P = 0·006$) and the interaction terms in dataset 1(OR 0·44, 95 % CI 0·26, 0·75, $P = 0·003$) were inversely associated with LBW, but there was no significant association for the interaction term in dataset 2 (OR 0·73, 95 % CI 0·43, 1·24, $P = 0·248$), indicating that FA supplementation was associated with a reduced risk of LBW for singletons (OR 0·82) and twin A (OR 0·36 (0·82 × 0·44)), but there was no statistically significant correlation in twin B (OR 0·60 (0·82 × 0·73)).

Timing of folic acid supplementation and birth weight by plurality

Figure 4 shows the association between timing of FA supplementation and birth weight, the risk of SGA and LBW in singletons and twins, respectively. Preconceptional, postconceptional and periconceptional FA supplementation was associated with increased birth weight and reduced SGA in both singletons and twins. Postconceptional and periconceptional FA supplementation was associated with a significant reduction of LBW in singletons and twins, but there was no significant reduction of LBW in preconceptional FA use in singletons and twins.

Sensitivity analysis

An additional sensitivity analysis indicated a robust association of FA supplementation with birth weight, SGA and LBW when excluding women who sporadically continued to consume FA in the second trimester or third trimester or both (online Supplemental Table S1), and no statistically significant interaction effect was found between FA supplementation and infant's sex (online Supplemental Table S2).

Discussion

FA supplementation from preconception (12 weeks before pregnancy) to the first trimester was associated with increased infant birth weight and reduced risk of SGA and LBW at birth in both singletons and twins, and this

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Table 2 Interaction effect of FA supplementation with plurality on BW, SGA, LBW in the total population of northwest China during 2010–2013

	BW				SGA				LBW			
	Dataset 1*		Dataset 2†		Dataset 1*		Dataset 2†		Dataset 1*		Dataset 2†	
Risk factors	β‡	95 % CI	β‡	95 % CI	OR§	95 % CI	OR§	95 % CI	OR‖	95 % CI	OR‖	95 % CI
FA	17·4	6·2, 28·5	17·2	6·1, 28·4	0·85	0·79, 0·92	0·85	0·79, 0·92	0·82	0·71, 0·94	0·82	0·71, 0·94
Plurality	–673·6	–752·4, –594·8	–685·9	–764·8, –607·0	10·68	7·26, 15·72	10·70	7·27, 15·75	18·60	12·21, 28·32	16·59	10·88, 25·31
Interaction	160·0	63·4, 256·6	139·7	43·1, 236·4	0·52	0·33, 0·83	0·59	0·37, 0·94	0·44	0·26, 0·75	0·73	0·43, 1·24

BW, birth weight; FA, folic acid; SGA, small for gestational age; LBW, low birth weight.
*Singleton and twin A samples included in analysis.
†Singleton and twin B samples included in analysis.
‡β represents an estimate of generalised linear models (GLM) with normal distribution and identity-link function, including all covariates of infant's gender, residence, maternal age, maternal education, household wealth index, antenatal care visits, gravidity, parity, passive smoking, alcohol drinking, tea drinking, pregnancy-induced hypertension, cold, iron supplementation, FA supplementation, gestational age.
§OR represents results of GLM with binomial distribution and logit-link function, including covariates above except for GA.
‖OR represents results of GLM with binomial distribution and logit-link function, including all covariates above.

association might be more prominent in twins. Our findings are in accordance with previous studies showing that FA supplementation is associated with increased birth weight and reduced risk of LBW and SGA[19–22]. In contrast, a majority of studies principally focused on singleton birth population, which excluded twin births. And few studies had compared twin and singleton births in the same sample. The birth weight of twins could increase 166·3 g if their mothers took FA, whereas this figure was only 17·3 g for singletons, and this association became more prominent when mothers took FA during 1–12 weeks before pregnancy. Further, the risk of SGA and LBW might be reduced by 55 and 50 % for twins if mothers took FA, but only 15 and 18 % for singletons. Therefore, twins might benefit much more from earlier-pregnancy FA supplementation. As we know, twins are the high-risk group of small size at birth and neonatal mortality[41,42]; they require more attention. Considering the growth and development of the foetus and infant survival, FA supplementation might be beneficial for pregnant women with a family history of twins.

The association of timing of FA supplementation and birth weight has been explored in singleton birth population. The Generation R study in the Netherlands[19] suggested that preconceptional FA supplementation is associated with higher birth weight and lower risk of LBW and SGA. A prospective cohort study in Jiaxing indicated[20] an association between preconceptional FA supplementation and lower risk of SGA, but a large prospective cohort study in Jiangsu and Zhejiang provinces[22] showed that periconceptional or postconceptional FA supplementation is associated with a reduced risk of SGA and LBW. Nevertheless, our study found that FA supplementation is associated with increased birth weight, reduced risk of SGA for women who took FA during preconceptional, postconceptional and periconceptional periods in both singletons and twins. Similarly, postconceptional and periconceptional FA supplementation was associated with a reduced risk of LBW in singletons and twins, but there was no significant reduction of LBW in preconception FA use in both singletons and twins.

Previous studies have suggested that nutrient supplementation during pregnancy increased infant birth weight through prolonging gestational weeks[7,43]. However, in our study, there was no difference in gestational weeks between the FA group and non-FA group in singletons, but the gestational week of the FA group was a little shorter than that of the non-FA group in twins. This result implies that increased infant birth weight with FA supplementation during pregnancy might not be through prolonging gestational weeks. Moreover, a higher sex ratio (about 119) at birth was observed in our sample, which was close to 118·06 from the Sixth Censuses of China in 2010[44]. Moreover, our sample was in line with the characteristics of birth population in China. Meanwhile, a subgroup analysis by infant's sex suggested that there was no significant interaction effect between FA use and infant's sex on birth weight, risk of SGA and LBW.

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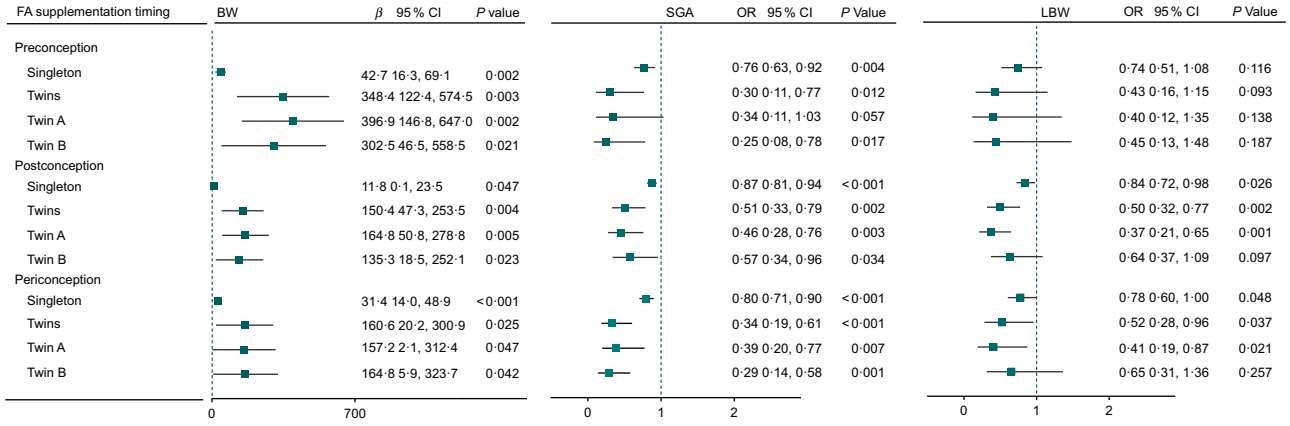

Fig. 4 (colour online) Birth weight associated with timing of FA supplementation in singletons and twins in northwest China during 2010–2013. For BW, β was adjusted for covariates of infant's gender, residence, maternal age, maternal education, household wealth index, antenatal care visits, gravidity, parity, passive smoking, alcohol drinking, tea drinking, pregnancy-induced hypertension, cold, iron supplementation, FA supplementation, gestational age (GA) in generalised linear models (GLM) with normal distribution and identity-link function among singletons, twin A, twin B, and β was adjusted for all covariates above plus birth order in generalised estimating equation (GEE) models with normal distribution and identity-link function in twins. For SGA, OR was adjusted for all covariates above except for GA in GLM with binomial distribution and logit-link function among singletons, twin A, twin B, and OR was adjusted for all covariates above except for GA, plus birth order in GEE with binomial distribution and logit-link function in twins. For LBW, OR was adjusted for all covariates above in GLM with binomial distribution and logit-link function among singletons, twin A, twin B, and OR was adjusted for all covariates above plus birth order in GEE with binomial distribution and logit-link function in twins.

The exact mechanisms behind the association of FA deficiency with adverse pregnancy outcomes were not fully understood[45]. FA is an essential water-soluble B vitamin, acting as a substrate in the biological pathways of cellular processes[12,46], which might affect foetal growth indirectly by the optimisation of the FA-dependent homocysteine pathway because of its critical role in DNA synthesis and repair, as well as methylation. Higher serum homocysteine accumulation is associated with decreased foetal growth, while reduced FA status is associated with elevated homocysteine. Furthermore, the improvement of birth weight might be due to epigenetic modification for the effect of periconceptional women with FA supplementation[47].

A systematic review has reported[48] a relationship between periconceptional FA supplements and increased twinning. But a population-based cohort study has suggested[49] that FA supplementation during pregnancy is not associated with an increased occurrence of multiple births. Our data also found no significant association (crude OR: 1·26, 95 % CI 0·97, 1·63) between preconceptional FA supplementation and the occurrence of twins. This problem requires more evidence. However, our study confirms a positive association between periconceptional 0·4 mg/d FA supplementation and increased birth weight in singletons and twins, which implies that periconceptional supplementation of 0·4 mg/d would play an active role in improving birth weight. It is noteworthy that high doses of FA supplements (≥1 mg/d) might have a detrimental effect on child's birth weight and neuropsychological development[23,50]. Thus, further researches are required to examine the mechanism behind the association of FA supplementation with foetal development.

Our study has several strengths. First, it was a large population-based, cross-sectional study with a high response rate in northwest China using a stratified multistage random sampling method, which is generalisable to some extent. Second, birth weight and GA were recorded from a review of Medical Certificate of Birth, which might be relatively accurate. Third, a multi-parameter (birth weight, SGA, LBW) and multi-statistical (stratified analysis, interaction effect analysis, sensitivity analysis) approach was employed, and the results were robust and reliable. Besides, the associations of FA supplementation with birth weight, SGA and LBW in twins were explored, which had not been reported previously.

However, some limitations should be addressed. First, since this study was observational research, information on FA supplementation and other covariates was retrospectively self-reported by mothers after delivery. FA supplementation was not determined and supervised by the researcher. Therefore, there might be a misclassification of FA supplementation types due to the retrospective investigation. To minimise recall bias, efforts were made to help participants recall as accurately as possible. For one thing, standard and detailed classification questionnaires were used to control the recall bias. For another thing, before the formal investigation, interviewers were trained rigorously and a pilot study was performed. During the analysis, we just included live births in order to analyse the association of birth weight with FA supplementation. We had also controlled for possible confounders when data-analysing as far as possible. However, the potential for bias in our findings as a result of only including live births might still exist. Accordingly, systematic reviews and meta-analyses

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of high-quality prospective longitudinal data should be done to assist in clarifying this association. In addition, causal modelling and mediation analysis of direct and indirect effects could be also helpful. Second, the sample size was relatively small in the twin sample, and the power of analysis was limited. The association might be more prominent if the sample size was larger. In addition, data on the availability of maternal or infant folate biomarkers were lacking. Finally, observational studies are subject to unobserved confounding factors even though the observed confounding factors are controlled in the multivariable regression analysis, but residual confounding might still exist. Maternal height and weight gain during pregnancy could be an important predictor of the size of the baby at birth. Unfortunately, maternal height and weight gain was not recorded in this cross-sectional survey, especially because mothers could not effectively recall such information during pregnancy.

Conclusion

The present study suggests an association of periconceptional 0·4 mg/d FA supplementation with increased birth weight and reduced risk of SGA and LBW in both singletons and twins, and this association may be more prominent in twins. Further researches with high-quality prospective longitudinal data are required to explore their associations and interpret underlying mechanisms.

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Supplementary material

For supplementary material accompanying this article visit https://doi.org/10.1017/S1368980019004580

References

1. Horikoshi M, Beaumont RN, Day FR *et al.* (2016) Genome-wide associations for birth weight and correlations with adult disease. *Nature* **538**, 248–252.
2. Victora CG, Adair L, Fall C *et al.* (2008) Maternal and child undernutrition: consequences for adult health and human capital. *Lancet* **371**, 340–357.
3. Whincup PH, Kaye SJ, Owen CG *et al.* (2008) Birth weight and risk of type 2 diabetes: a systematic review. *JAMA* **300**, 2886–2897.
4. Katz J, Lee AC, Kozuki N *et al.* (2013) Mortality risk in preterm and small-for-gestational-age infants in low-income and middle-income countries: a pooled country analysis. *Lancet* **382**, 417–425.
5. Iliodromiti S, Mackay DF, Smith GC *et al.* (2017) Customised and noncustomised birth weight centiles and prediction of stillbirth and infant mortality and morbidity: a cohort study of 979,912 term singleton pregnancies in Scotland. *PLoS Med* **14**, e1002228.
6. McIntire DD, Bloom SL, Casey BM *et al.* (1999) Birth weight in relation to morbidity and mortality among newborn infants. *N Engl J Med* **340**, 1234–1238.
7. Zeng LX, Cheng Y, Dang SN *et al.* (2008) Impact of micronutrient supplementation during pregnancy on birth weight, duration of gestation, and perinatal mortality in rural western China: double blind cluster randomised controlled trial. *Br Med J* **337**, 1–11.
8. Liu JM, Mei Z, Ye R *et al.* (2013) Micronutrient supplementation and pregnancy outcomes: double-blind randomized controlled trial in China. *JAMA Intern Med* **173**, 276–282.
9. West KP Jr, Shamim AA, Mehra S *et al.* (2014) Effect of maternal multiple micronutrient vs iron-folic acid supplementation on infant mortality and adverse birth outcomes in rural Bangladesh: the JiVitA-3 randomized trial. *JAMA* **312**, 2649–2658.
10. De Wals P, Tairou F, Van Allen MI *et al.* (2007) Reduction in neural-tube defects after folic acid fortification in Canada. *N Engl J Med* **357**, 135–142.
11. Thaler CJ (2014) Folate metabolism and human reproduction. *Geburtsh Frauenheilk* (Obstetrics gynecology) **74**, 845–851.
12. Tamura T & Picciano MF (2006) Folate and human reproduction. *Am J Clin Nutr* **83**, 993–1016.
13. Maternal and Child Health Division (2010) Project management plan on folic acid supplementation prevented neural tube defects. Notification of the Ministry of Health. http://www.gov.cn/zwgk/2010-06/28/content_1639533.htm (accessed June 2010).
14. Berry RJ, Li Z, Erickson JD *et al.* (1999) Prevention of neural-tube defects with folic acid in China. *N Engl J Med* **341**, 1485–1490.
15. Czeizel AE & Dudas I. (1992) Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. *N Engl J Med* **327**, 1832–1835.

16. Shaw GM, Lammer EJ, Wasserman CR *et al.* (1995) Risks of orofacial clefts in children born to women using multivitamins containing folic acid periconceptionally. *Lancet* **346**, 393–396.
17. Liu S, Joseph KS, Luo W *et al.* (2016) Effect of folic acid food fortification in Canada on congenital heart disease subtypes. *Circulation* **134**, 647–655.
18. Van Dijk AE, Van Eijsden M, Stronks K *et al.* (2010) Maternal depressive symptoms, serum folate status, and pregnancy outcome: results of the Amsterdam Born Children and their Development study. *Am J Obstet Gynecol* **203**, e561–e567.
19. Timmermans S, Jaddoe VWV, Hofman A *et al.* (2009) Periconception folic acid supplementation, fetal growth and the risks of low birth weight and preterm birth: the Generation R Study. *Br J Nutr* **102**, 777–785.
20. Zheng JS, Guan YH, Zhao YM *et al.* (2016) 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. *Br J Nutr* **115**, 509–516.
21. Hodgetts VA, Morris RK, Francis A *et al.* (2015) Effectiveness of folic acid supplementation in pregnancy on reducing the risk of small-for-gestational age neonates: a population study, systematic review and meta-analysis. *BJOG* **122**, 478–490.
22. Li N, Li ZW, Ye RW *et al.* (2017) Impact of periconceptional folic acid supplementation on low birth weight and small-for-gestational-age infants in China: a large prospective cohort study. *J Pediatr* **187**, 105–110.
23. Pastor-Valero M, Navarrete-Munoz EM, Rebagliato M *et al.* (2011) Periconceptional folic acid supplementation and anthropometric measures at birth in a cohort of pregnant women in Valencia, Spain. *Br J Nutr* **105**, 1352–1360.
24. Papadopoulou E, Stratakis N, Roumeliotaki T *et al.* (2013) The effect of high doses of folic acid and iron supplementation in early-to-mid pregnancy on prematurity and fetal growth retardation: the mother-child cohort study in Crete, Greece (Rhea study). *Eur J Nutr* **52**, 327–336.
25. Yang JM, Cheng Y, Pei LL *et al.* (2017) Maternal iron intake during pregnancy and birth outcomes: a cross-sectional study in Northwest China. *Br J Nutr* **117**, 862–871.
26. Yang J, Dang S, Cheng Y *et al.* (2017) Dietary intakes and dietary patterns among pregnant women in Northwest China. *Public Health Nutr* **20**, 282–293.
27. Li Z, Ye R, Zhang L, *et al.* (2014) Periconceptional folic acid supplementation and the risk of preterm births in China: a large prospective cohort study. *Int J Epidemiol* **43**, 1132–1139.
28. Conde-Agudelo A, Belizan JM, Norton MH *et al.* (2005) Effect of the interpregnancy interval on perinatal outcomes in Latin America. *Obstet Gynecol* **106**, 359–366.
29. Zhu L, Zhang R, Zhang S *et al.* (2015) Chinese neonatal birth weight curve for different gestational age. *Chin J Pediatr* **2**, 97–103.
30. Schieve LA, Meikle SF, Ferre C *et al.* (2002) Low and very low birth weight in infants conceived with use of assisted reproductive technology. *N Engl J Med* **346**, 731–737.
31. Filmer D & Pritchett LH (2001) Estimating wealth effects without expenditure data – or tears: an application to educational enrollments in states of India. *Demography* **38**, 115–132.
32. Larsen S, Haavaldsen C, Bjelland EK *et al.* (2018) Placental weight and birthweight: the relations with number of daily cigarettes and smoking cessation in pregnancy. A population study. *Int J Epidemiol* **47**, 1141–1150.
33. Liu S, Zhang M, Yang L *et al.* (2017) Prevalence and patterns of tobacco smoking among Chinese adult men and women: findings of the 2010 national smoking survey. *J Epidemiol Community Health* **71**, 154–161.
34. Pollack H, Lantz PM & Frohna JG (2000) Maternal smoking and adverse birth outcomes among singletons and twins. *Am J Public Health* **90**, 395–400.
35. Kibel M, Kahn M, Sherman C *et al.* (2017) Placental abnormalities differ between small for gestational age fetuses in dichorionic twin and singleton pregnancies. *Placenta* **60**, 28–35.
36. Zeger SL & Liang KY (1986) Longitudinal data analysis for discrete and continuous outcomes. *Biometrics* **42**, 121–130.
37. Zeng L, Yan H, Cheng Y *et al.* (2011) Modifying effects of wealth on the response to nutrient supplementation in pregnancy on birth weight, duration of gestation and perinatal mortality in rural western China: double-blind cluster randomized controlled trial. *Int J Epidemiol* **40**, 350–362.
38. Sengpiel V, Elind E, Bacelis J *et al.* (2013) Maternal caffeine intake during pregnancy is associated with birth weight but not with gestational length: results from a large prospective observational cohort study. *BMC Med* **11**, 42.
39. Banhidy F, Acs N, Puho E *et al.* (2006) Pregnancy complications and delivery outcomes of pregnant women with common cold. *Cent Eur J Public Health* **14**, 10–14.
40. Acharya D, Singh JK, Kadel R *et al.* (2018) Maternal factors and utilization of the antenatal care services during pregnancy associated with low birth weight in rural Nepal: analyses of the antenatal care and birth weight records of the MATRI-SUMAN trial. *Int J Environ Res Public Health* **15**, e2450.
41. Monden CWS & Smits J (2017) Mortality among twins and singletons in sub-Saharan Africa between 1995 and 2014: a pooled analysis of data from 90 demographic and health surveys in 30 countries. *Lancet Glob Health* **5**, e673–e679.
42. Hanson C, Munjanja S, Binagwaho A *et al* (2019) National policies and care provision in pregnancy and childbirth for twins in Eastern and Southern Africa: a mixed-methods multi-country study. *PLoS Med* **16**, e1002749.
43. Kang YJ, Dang SN, Zeng LX *et al.* (2017) Multi-micronutrient supplementation during pregnancy for prevention of maternal anaemia and adverse birth outcomes in a high-altitude area: a prospective cohort study in rural Tibet of China. *Br J Nutr* **118**, 431–440.
44. Shi R (2013) Changing patterns of sex ratio at birth in China: a comparative analysis of data from the fifth and sixth censuses of China. *Population Research* **37**, 66–72.
45. Van der Molen EF, Verbruggen B, Novakova I *et al.* (2000) Hyperhomocysteinemia and other thrombotic risk factors in women with placental vasculopathy. *BJOG* **107**, 785–791.
46. Bailey LB & Gregory JF. (1999) Folate metabolism and requirements. *J Nutr* **129**, 779–782.
47. Sinclair KD, Allegrucci C, Singh R *et al.* (2007) DNA methylation, insulin resistance, and blood pressure in offspring determined by maternal periconceptional B vitamin and methionine status. *Proc Natl Acad Sci U S A* **104**, 19351–19356.
48. Muggli EE & Halliday JL (2007) Folic acid and risk of twinning: a systematic review of the recent literature, July 1994 to July 2006. *Med J Aust* **186**, 243–248.
49. Li Z, Gindler J, Wang H *et al.* (2003) Folic acid supplements during early pregnancy and likelihood of multiple births: a population-based cohort study. *Lancet* **361**, 380–384.
50. Valera-Gran D, Garcia de la Hera M, Navarrete-Munoz EM *et al.* (2014) Folic acid supplements during pregnancy and child psychomotor development after the first year of life. *JAMA Pediatr* **168**, e142611.

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