

# Periconceptional folic acid supplementation and vitamin B<sub>12</sub> status in a cohort of Chinese early pregnancy women with the risk of adverse pregnancy outcomes

Ting Yang,<sup>1</sup> Yan Gu,<sup>1</sup> Xiaoping Wei,<sup>1</sup> Xiaohua Liang,<sup>1</sup> Jie Chen,<sup>1</sup> Youxue Liu,<sup>1</sup> Ting Zhang<sup>2,\*</sup> and Tingyu Li<sup>1,\*</sup>

<sup>1</sup>Children Nutrition Research Centre, Children's Hospital of Chongqing Medical University, Ministry of Education Key Laboratory of Child Development and Disorders, China International Science and Technology Cooperation Base of Child Development and Critical Disorders, Chongqing Key Laboratory of Pediatrics, No. 136 Zhongshan Er Rd, Yuzhong District, Chongqing 400014, China

<sup>2</sup>Capital Institute of Pediatrics, No. 2 Yabao Er Rd, Chaoyang District, Beijing 100020, China

(Received 2 May, 2016; Accepted 9 August, 2016; Published online 8 February, 2017)

**Maternal folate and vitamin B<sub>12</sub> deficiency predict poor pregnancy outcome. To improve pregnancy outcomes in rural area of China, we investigate rural women's folic acid supplementation (FAS) status and the associations between maternal vitamin B status during the first trimester and subsequent adverse pregnancy outcomes. We collected the questionnaire information and drew 5 ml of blood from 309 early pregnant rural women. The birth outcomes were retrieved from medical records after delivery. Out of the total, 257 had taken FAS, including 50 before conception (group A) and 207 during the first trimester (group B). The concentration of plasma folate and the RBC folate supplementation groups were obviously higher than that of no-supplementation group (group N,  $p < 0.01$ ). The mean vitamin B<sub>12</sub> levels in FAS group were significantly higher than those in groups N and B ( $p < 0.05$ ). Women who delivered SGA or premature infants had reduced plasma folate levels ( $p < 0.05$ ) compared with controls. The multiple linear regression models revealed that RBC folate levels affected the infant birth weight ( $p < 0.01$ ) and birth length ( $p < 0.05$ ). In conclusion, FAS can significantly improve plasma folate and RBC folate levels in childbearing-age women and reduce the risk of subsequent adverse pregnancy outcomes.**

**Key Words:** folate, vitamin B<sub>12</sub>, adverse pregnancy outcomes, early pregnancy, newborn

Folate and vitamin B<sub>12</sub> play a vital role in one carbon metabolism which is crucial for the general wellbeing of pregnancy and particularly for fetal growth.<sup>(1)</sup> It is widely recognised that peri-conceptional FAS reduces the risk of neural tube defects (NTDs) and other congenital malformations. Some observational studies have suggested that reduced circulating folate in pregnancy is associated with increased risk for prematurity, SGA (<10th centile) and other adverse pregnancy outcomes.<sup>(2-5)</sup>

The prevalence of NTDs in China is approximately 2.7 per 1,000 pregnancies, which is significantly higher than that of approximately 1 per 1,000 pregnancies in Caucasian Americans.<sup>(6,7)</sup> Daily consumption of a supplement containing 400 µg folic acid before and during the first trimester of pregnancy has been shown to be effective in preventing both the occurrence of NTDs and numerous adverse pregnancy outcomes.<sup>(6,8,9)</sup> However, only 3–5% of Chinese childbearing-age women take folic acid, although 20–30% of them know the importance of folic acid in preventing NTDs and some adverse pregnancy outcomes.<sup>(10-12)</sup> A recent publication stated that further research to measure the effect of

folate intake during pregnancy on reducing low birth weight and adverse pregnancy outcomes is an 'urgent priority'.<sup>(5)</sup> As there are strategies to reduce the occurrence of these defects by folic acid fortification of the diet are under way in several parts of the world. In 2009, the Chinese provincial government began distributing free peri-conceptional FAS (400 µg per day) to rural women through the Family Planning System and Maternal and Child Health Care System.

Unpublished data from a population-based surveillance system demonstrated that the prevalence of NTDs in China is in rural counties, where women tend to have more children than urban women do,<sup>(12)</sup> this is of significance because farmers comprise 70% of the total population in China. Tongliang County is a rural district of Chongqing, China and a typical mountainous area with low socioeconomic development. However, most pregnant rural women visit the prenatal clinic not until after the second month of pregnancy; therefore, few receive the supplements when their use is most crucial.

Red blood cell (RBC) and plasma folate concentration levels help in the assessment of folate status in humans. Plasma folate concentrations change immediately after ingestion of folic acid and are therefore commonly measured during the fasting state for bioavailability studies, whereas RBC folate concentrations change slowly, providing a better indication of long-term folate status.<sup>(5,13)</sup> By contrast, the effects of low vitamin B<sub>12</sub> status in reproduction are not well defined. Vitamin B<sub>12</sub> acts as a cofactor for enzyme reactions in one-carbon metabolism and plays a vital role in folate circulation. A study in Bangalore, South India, low maternal vitamin B<sub>12</sub> concentrations predicted fetal growth retardation.<sup>(14,15)</sup> One study from China found that inadequate prepregnancy serum vitamin B<sub>12</sub> status was associated with a 60% increased risk of preterm birth.<sup>(1)</sup> Folate and vitamin B<sub>12</sub> status are important determinants of plasma homocysteine and raised total homocysteine levels in maternal blood and amniotic fluid are associated with an increased risk of adverse pregnancy outcomes. This raises the question whether supplementation with folic acid periconceptional supplementation will further improve the pregnancy outcomes and have any potential correlation with Vitamin B<sub>12</sub> status. Over all, the implications of starting pregnancy and lactation with low vitamin B<sub>12</sub> status need further investigation.

\*To whom correspondence should be addressed.

E-mail: tyli@vip.sina.com (Tingyu Li); zhangtingcv@aliyun.com (Ting Zhang)

To our knowledge, no previous study has been conducted in County (i.e., Tongliang County) on self-reported folic acid supplementation and optimal folic acid use (i.e., regularly from 12 weeks before pregnancy through 12 weeks after delivery), especially the concentrations of plasma folate, RBC folate and vitamin B<sub>12</sub> among women of child-bearing age during the periconceptional period, at the population level. In the present study, our aim was to understand the rates of periconceptional folic acid supplementation and to identify their correlations with blood folate concentrations and adverse pregnancy outcomes of the rural women.

## Materials and Methods

**Study participants.** This study was an on-going population-based prospective cohort study. It was conducted in Tongliang Maternal and Children Health Care Hospital of Chongqing, China, which was the only one maternal and children health care hospital in the county. And most of the pregnancy would come to do antenatal examinations. Study participants were local (Tongliang County) women in early pregnancy (gestational age <12 weeks) and 18–35 years old. (Tongliang County, Chongqing, China is a rural district with low socioeconomic development.) Only healthy mothers were admitted into the protocol; the exclusion criteria were pregnancy toxemia, bronchial asthma, active hepatitis, malignant tumours, chronic renal failure and heart failure. In addition, we had excluded those pregnant women using other dietary supplements (i.e., multivitamins, Fe) during pregnancy ( $n = 4$ ). In the present study, A total of 309 eligible consenting women were recruited and completed the interview, 299 of them contributed a blood sample in early pregnancy. There were 253 mother-infant pairs who remained in the study after delivery, for whom we had complete data on all measurements and variables required for the analysis.

The study protocol was reviewed and approved by the institutional ethical committee of the Capital Institute of Paediatrics, Beijing, China. Informed consent was obtained from all participants before their entry into the study.

**Questionnaire interview.** Information on maternal age, educational level, income, demographic information, height, weight, active and passive smoking, parity and folic acid supplement use were obtained from the questionnaire at enrolment in the study. FAS use was categorised into three groups: (i) started before conception, (ii) started during the first trimester and (iii) no-supplementation.<sup>(16)</sup> FAS use was defined as each woman having taken 400 µg folic acid per day and greater than or equal to 5 days each week. Pregnancy outcome data included date of delivery, gestational age, neonatal gender, birth weight, birth length, head circumference, malformations, maternal height, pregnancy weight and complications of pregnancy or in delivery; details of medications used were collected after delivery by examination of the full clinical records and confirmed after review by senior clinical medical staff.

**Biological sample collection and analysis.** In early pregnancy (about 6–12 weeks of gestation), blood samples (about 5 ml) of each subject were drawn via venipuncture and were collected into 6 ml, metal-free EDTA-treated tubes at the Tongliang Maternal and Children Health Care Hospital. The blood was centrifuged at 3,500 rotations per minute for 15 min at -4°C, and then the plasma and packed RBC were separated and stored at -80°C. To analyse plasma folate, RBC folate and vitamin B<sub>12</sub> concentrations, plasma samples (about 1 ml) and RBC samples (about 1 ml) were selected and transported to the Department of Clinical Chemistry at the Capital Institute of Paediatrics, Beijing, China. After thawing, the plasma folate, RBC folate and vitamin B<sub>12</sub> were measured with a competitive receptor binding immunoassay (Chemiluminescent Immunoenzyme Assay Access Immunoassay System; Beckman Coulter, Krefeld, Geyrman).<sup>(17)</sup> The intra assay CV for serum folate and vitamin B<sub>12</sub> were 3.8–6.5% and 5–7%,

respectively. RBC folate was determined by a microbiological assay.<sup>(18)</sup>

**Birth outcomes.** Information concerning gestational age at birth (weeks), offspring gender, placental weight (grams) and birthweight (grams) were obtained from hospital clinical records. The definition of SGA was a gestational age-adjusted and sex-adjusted birthweight below the tenth centile in this study cohort (<-1.79 SD),<sup>(19)</sup> and prematurity was defined as a spontaneous vaginal birth of an infant before 37.0 weeks of gestation by the WHO.<sup>(20)</sup>

**Statistical analyses.** Data were presented as the mean ± SD for the normal variables and median (P25, P75) for non-normal variables. Effects with  $p < 0.05$  were considered statistically significant. Two independent samples *t* tests were used to observe the differences of the plasma vitamins concentrations in both SGA and prematurity group compared with non-SGA and non-prematurity group separately. The differences in change among the three groups were estimated with ANOVA, and the percentage rates were assessed with Fisher's probability test. Multiple linear regression models were used to estimate the effects of independent variables on head circumference, birth length and birth weight. Data were entered and analysed with the EpiData 3.0 statistical package (Centers for Disease Control and Prevention, Atlanta, GA) and the SPSS software package ver. 19.0 (SPSS Inc., Chicago, IL), respectively.

## Results

**The characteristics of the participants.** We recruited 309 women, and the subjects' characteristics are shown in Table 1. Of the total, 299 contributed a blood sample in early pregnancy (Ten were excluded for a failure in drawing blood). Among the 299 pregnant women, information on birth outcomes was retrieved after delivery only from 253 mother-infant pairs. The mean age of the women in the entire cohort was 26.8 years, and BMI was 21.1. The mean gestational age was 39.0 weeks. Of the participants, 64.4% were nulliparous, 56.9% had finished higher education and 83.2% had taken FAS, with 16.2% of the women having started FAS before conception. Among the 253 newborns, the mean birthweight was 3,310.0 g, 6.3% were preterm and 6.8% were SGA.

**Awareness and use of folic acid among pregnancy women.** As is shown in Table 2, most of the pregnancy women (83.2%) received free folic acid during the pregnancy and almost all the pregnant woman (98.7%) didn't know the role of folic acid in other congenital defect prevention. Only 16.2% pregnant women began taking folic acid 3 months before pregnancy and 1.3% knew the role of folic acid in other complications. Further, obstetricians/gynaecologists and relatives/friends were the main source of learning about folic acid.

As is shown in Table 3, there is no statistically significant association with the use of folic acid among pregnant with respect to food expenditure, employment, gravidity and multivariate-adjusted analyses revealed. With regard to the relationship between folic acid intake and selected factors, pregnant that had higher education levels, income, multiparous and age were more likely to take folic acid during preconception. In addition, pregnant who were aged 25–30 years old, high-income and multiparous were more likely to start folic acid supplementation before conception.

**Folic acid supplement intake and B vitamins levels in women.** From the total study cohort, 249 (83.3%) subjects reported FAS intake and contributed a blood sample. Of that number, 49 (16.4%) started supplementation before conception, and 200 (66.9%) started supplementation during the first trimester. Maternal plasma folate and RBC folate levels were 8.60 ± 3.27 ng/ml and 618.37 ± 274.03 ng/ml in group N; 16.78 ± 5.04 ng/ml and 827.57 ± 271.92 ng/ml in group A; 13.97 ± 5.86 ng/ml and 776.14 ± 307.68 ng/ml in group B. Plasma folate and RBC folate

**Table 1.** Demographics and clinical characteristics of the subjects

Characteristic	Value n (%)
<b>Pregnancy characteristics (n = 309)</b>	
Maternal age (years)	
<25	134 (43.4)
25–30	118 (38.2)
>30	57 (18.4)
BMI index before pregnancy (kg/m <sup>2</sup> )	
<18.5	58 (18.8)
18.5–24.9	218 (70.6)
≥25	33 (10.6)
Monthly income of the family (RMB)	
<3,500	75 (24.3)
3,500–4,500	177 (57.3)
>4,500	57 (18.4)
Gravidity	
Primigravida	101 (32.7)
Multigravida	208 (67.3)
Parity	
Nulliparous	199 (64.4)
Multiparous	110 (35.6)
Education	
Primary school or lower	15 (4.9)
Secondary school	214 (69.3)
University or above	80 (25.8)
Employment	
No	217 (70.2)
Yes	92 (29.8)
Passive smoking exposure	
No	108 (35.0)
Yes	201 (65.0)
<b>Neonate characteristics (n = 253)</b>	
Birth length	50.3 ± 1.4*
Head circumference	33.9 ± 0.9*
Birth weight (g)	3,310.0 ± 413.0*
LBW	7 (2.8)
SGA	
<10th percentile	17 (6.8)
LGA	
>90th percentile	15 (6.0)
Preterm	
<37 weeks	16 (6.3)

\*Data were shown as the mean ± SD.

supplementation groups were obviously higher than that of group N ( $p < 0.01$ ; Table 4). There were statistically significant differences between the three groups. The mean vitamin B<sub>12</sub> levels was  $903.62 \pm 374.93$  pg/ml in group A, significantly higher than that of  $787.84 \pm 302.28$  pg/ml in group N and of  $762.33 \pm 325.60$  pg/ml in group B ( $p < 0.05$ ). However, there was no significant difference between groups N and B (Table 4).

**Daily early pregnancy folic acid implementation and pregnancy outcomes.** The associations between daily folic acid implementation and pregnancy outcomes are shown in Table 5. The SGA and premature incidence rates (17.5% and 15.0%) of the no-use group were higher than those in the implementation group (4.7% and 4.7%). Fisher's probability test indicated that there were statistical differences between the two groups in both SGA and prematurity ( $p < 0.05$ ). The mean birth weight of group N was much lower than group A and B combined ( $3,181.3 \pm 423.2$  vs  $3,315.4 \pm 411.9$ ). However, there was no significant difference between the two groups ( $p = 0.631$ ). This may also suggest that folic acid plays an important role in birth-weight. We speculated that it might be limited by small samples.

**Table 2.** Awareness and use of folic acid among pregnancy women in the cohort study

Items	Value n (%)
Enrolled subjects	309
Received free folic acid	
No	52 (16.8)
Yes	257 (83.2)
Know about the role of folic acid in birth defect prevention	
No	68 (22.0)
Yes	241 (78.0)
Know about the role of folic acid in other complications during pregnancy	
No	305 (98.7)
Yes	4 (1.3)
Means of learning about folic acid	
Media	36 (11.7)
Obstetricians and gynaecologists	158 (51.1)
Relatives and friends	98 (31.7)
Others	17 (5.5)
Previous thought about correct time of folic acid supplement	
Before pregnancy	171 (55.3)
Early in pregnancy	33 (10.7)
In late pregnancy	3 (1.0)
Do not know	102 (33.0)
Began taking folic acid	
Never	52 (16.8)
Early in pregnancy	207 (67.0)
3 months before pregnancy	50 (16.2)
Times of folic acid supplement each week	
1–3	4 (1.3)
3–4	8 (2.6)
≥5	257 (83.2)

### Concentrations of folate and vitamin B<sub>12</sub> biomarkers in cases and controls according to birth status.

The associations of folate and vitamin B<sub>12</sub> Concentrations in cases and controls are shown in Table 6. The mean plasma folate levels were found to be  $10.08 \pm 4.54$  ng/ml in the SGA group and  $13.64 \pm 5.44$  ng/ml in the control group ( $p < 0.05$ ); we also found to be  $10.28 \pm 5.47$  ng/ml in the prematurity group and  $13.59 \pm 5.40$  ng/ml in the control group ( $p < 0.05$ ). However, we did not find that in RBC folate and vitamin B<sub>12</sub> between cases and controls.

We investigated the factors that related to physical development at birth by a multiple linear regression model in which the dependent variables were plasma folate, RBC folate and vitamin B<sub>12</sub> (Table 7). The model revealed that RBC folate levels affected the infant birth weight ( $B = 0.197$ ,  $p < 0.01$ ) and birth length ( $B = 0.149$ ,  $p < 0.05$ ).

### Discussion

It is well-known that plasma folate, RBC folate and vitamin B<sub>12</sub> have received a great deal of attention in that lower levels of them have been linked to SGA, prematurity and the risk of vascular-related pregnancy complications.<sup>(14,21)</sup> Chinese government began distributing free peri-conceptual FAS (400 µg per day) to rural women, but there are few studies evaluated vitamin B<sub>12</sub> and effect of the FAS program on concentrations of folate, ultimately improving and pregnancy outcomes in rural area. In the present study, we have demonstrated the positive effects on Chinese government FAS program on folic acid supplementation rate and improving pregnancy outcomes in rural area.

As is shown in Table 2, most of the pregnancy women received

**Table 3.** Descriptive, univariate, and multivariate analyses of all enrolled pregnant concerning the use of folic acid

	Supplement of folic acid			Took folic acid during the first trimestery			Took folic acid before pregnancy		
	<i>n</i> (%)	Unadjusted OR <sup>a</sup> (95% CI)	Adjusted OR <sup>a</sup> (95% CI)	<i>n</i> (%)	Unadjusted OR <sup>a</sup> (95% CI)	Adjusted OR <sup>a</sup> (95% CI)	<i>n</i> (%)	Unadjusted OR <sup>a</sup> (95% CI)	Adjusted OR <sup>a</sup> (95% CI)
All enrolled pregnancy women	257 (83.2)			207 (70.0)			50 (16.2)		
Age (years)									
<25	105 (40.8)	1	1	92 (44.4)	1	1	13 (26.0)	1	1
25–30	101 (39.3)	1.7 (0.78–3.71)	1.66 (0.58–4.69)	77 (37.2)	2.18 (1.16–4.14)*	7.83 (2.11–28.99)*	24 (48.0)	1.48 (1.21–4.10)*	5.54 (1.71–17.94)*
>30	51 (19.9)	1.32 (0.62–2.81)	1.07 (0.46–2.52)	38 (18.4)	1.82 (1.66–2.25)*	4.113 (1.31–12.91)*	13 (26.0)	0.98 (0.46–2.08)	1.74 (0.77–3.92)
Education									
primary school or lower	12 (4.7)	1	1	10 (4.8)	1	1	2 (4.0)	1	1
Secondary school	171 (66.5)	3.09 (0.68–14.08)	0.28 (0.05–1.52)	140 (67.6)	0.81 (0.25–2.62)	1.13 (0.17–7.29)	31 (62.0)	0.57 (0.12–2.78)	1.287 (0.22–7.69)
University or above	74 (28.8)	1.7 (1.27–7.58)*	4.25 (1.51–12.99)*	57 (27.6)	0.76 (0.44–1.34)	0.87 (0.37–2.07)	17 (34)	0.63 (0.33–1.21)	1.07 (0.21–5.49)
Employment									
No	180 (70.0)	1	1	150 (72.5)	1	1	30 (60.0)	1	1
Yes	77 (30.0)	0.95 (0.49–1.83)	1.24 (0.60–2.60)	57 (27.5)	1.38 (0.83–2.29)	1.27 (0.55–2.63)	20 (40.0)	0.58 (0.31–1.08)	1.2 (0.57–2.54)
Monthly income (RMB)									
<3,500	62 (24.1)	1	1	53 (25.6)	1	1	9 (18.0)	1	1
3,500–4,500	144 (56.0)	1.67 (0.20–13.98)	4.1 (0.35–47.81)	117 (56.5)	3.89 (0.70–21.75)	4.97 (0.63–39.22)	2,754.0	0.17 (0.02–1.87)	0.429 (0.03–5.83)
>4,500	51 (19.9)	1.08 (0.23–5.15)	2.59 (0.44–15.04)	37 (17.9)	1.658 (0.49–5.57)	2.05 (0.39–10.74)	14 (28.0)	1.52 (1.13–5.04)*	1.814 (1.17–3.863)*
Food expenditure (RMB)**									
<1,500	34 (13.2)	1	1	26 (12.6)	1	1	8 (16.0)	1	1
1,500–2,000	195 (75.9)	0.43 (0.03–5.53)	0.12 (0.00–3.68)	157 (75.8)	0.833 (0.07–10.27)	0.4 (0.09–1.74)	38 (76.0)	0.4 (0.78–3.71)	2.5 (0.60–10.44)
>2,000	28 (10.9)	1.08 (0.42–2.76)	1.04 (0.38–2.82)	24 (11.6)	0.829 (0.38–1.81)	0.8 (0.24–2.66)	4 (8.0)	1.53 (0.51–4.54)	1.392 (0.43–4.49)
Gravidity [ <i>n</i> (%)]									
Primigravida	170 (66.1)	1	1	134 (64.7)	1	1	36 (72.0)	1	1
Multigravida	87 (33.9)	1.55 (0.85–2.84)	1.3 (0.52–3.26)	73 (35.3)	1.05 (0.6–14.71)	1.66 (0.73–3.76)	14 (28.0)	1.51 (0.78–2.95)	3.96 (1.48–10.60)
Parity [ <i>n</i> (%)]									
Nulliparous	84 (32.7)	1	1	72 (34.8)	1	1	12 (24.0)	1	1
Multiparous	173 (67.3)	1.08 (0.53–1.89)	1.42 (0.56–3.57)	135 (65.2)	1.34 (1.20–2.25)*	4.69 (1.51–14.51)*	38 (76.0)	1.6 (1.30–2.21)*	4.08 (1.11–11.57)*

OR, odds ratio; CI, confidence interval; 1, reference layer. <sup>a</sup>Adjusted ORs were derived from multivariate logistic regression; unadjusted ORs were derived from univariate logistic regression. \**p*<0.05. \*\*Monthly food expenditure of the family (RMB).

**Table 4.** Concentrations of folate and vitamin B<sub>12</sub> biomarkers in those who did and did not supplement with folic acid (*n* = 299)<sup>a</sup>

Biomarker	Daily folic acid implementation			<i>p</i> value
	No-use (group N)	Start before conception (group A)	During the first trimester (group B)	
<i>N</i>	50	49	200	
Plasma folate (ng/ml)	8.60 ± 3.27	16.78 ± 5.04 <sup>c</sup>	13.97 ± 5.86 <sup>d,e</sup>	0.000
RBC folate (ng/ml)	618.37 ± 274.03	827.57 ± 271.92 <sup>c</sup>	776.14 ± 307.68 <sup>d</sup>	0.001
Vitamin B <sub>12</sub> (pg/ml)	787.84 ± 302.28	903.62 ± 374.93	762.33 ± 325.60 <sup>e</sup>	0.028

<sup>a</sup>All values are mean ± SD. *N* represents the number of subjects with data available. <sup>b</sup>The differences in change between three groups were estimated with ANOVA. <sup>c</sup>*p*<0.01 when group A compared with group N. <sup>d</sup>*p*<0.01 when group B compared with group N. <sup>e</sup>*p*<0.01 when group A compared with group B.

**Table 5.** Associations between daily folic acid implementation and pregnancy outcomes

	Daily folic acid implementation		<i>p</i> value <sup>a</sup>
	No-use ( <i>n</i> = 40)	folic acid implementation ( <i>n</i> = 213)	
SGA <sup>b</sup>	7 (17.5)	10 (4.7)	0.009
Prematurity <sup>b</sup>	6 (15.0)	10 (4.7)	0.025
Birth weight <sup>c</sup> (g)	3,181.3 ± 423.2	3,315.4 ± 411.9	0.631

<sup>a</sup>The percentage rates were assessed with fisher probabilities test or independent samples *t* test between the two groups. <sup>b</sup>Values were *n* (%). <sup>c</sup>Data were shown mean ± SD.

free folic acid during the pregnancy and knew the role of folic acid in birth defect prevention. It indicated that Chinese government FAS program had improved the pregnancy awareness of folic acid supplement, especially about the important role in birth defect prevention. However, few pregnant women began taking folic acid 3 months before pregnancy (16.2%) and know the role of folic acid in other complications during pregnancy (1.3%). Further only 11.7% of the pregnant women were learning about folic acid

by media. The rates were much lower than those in developed countries.<sup>(22–24)</sup> It indicated that health education should be strengthened, specially making more efforts on prepregnancy education and cooperating with public media. Agreement with other studies,<sup>(23,25)</sup> the ones with higher education levels, income, multiparous and age were more likely to take folic acid before pregnancy. Therefore, preconceptional health education should take more concern on the pregnant women with lower education

**Table 6.** Concentrations of folate and vitamin B<sub>12</sub> biomarkers in cases and controls according to birth status<sup>a</sup>

Biomarker	SGA birth		p <sup>b</sup>	Prematurity		p <sup>c</sup>
	Cases (n = 16)	Controls (n = 229)		Cases (n = 15)	Controls (n = 231)	
Plasma folate (ng/ml)	10.08 ± 4.54	13.64 ± 5.44	0.011	10.28 ± 5.47	13.59 ± 5.40	0.022
RBC folate (ng/ml)	765.58 ± 270.51	768.02 ± 311.92	0.976	740.97 ± 311.78	767.36 ± 307.45	0.749
Vitamin B <sub>12</sub> (pg/ml)	808.67 ± 306.38	775.72 ± 341.17	0.707	732.50 ± 311.44	783.78 ± 339.79	0.570

<sup>a</sup>All values are mean ± SD. *N* represents the number of subjects with data available. SGA defined as, 10th customized birth weight percentile. Prematurity defined as a live birth with a gestational age <37 weeks gestation. The numbers of case and control subjects vary slightly because of missing values for some variables. <sup>b</sup>*p* values between SGA and non-SGA births, by independent samples *t* test between the two groups. <sup>c</sup>*p* values between prematurity and non-prematurity births, by independent samples *t* test between the two groups.

**Table 7.** Associations between early pregnancy biomarker concentrations and head circumference, birth length and birth weight<sup>a</sup>

Blood nutrients	Head circumference		Birth length		Birthweight	
	95% CI <sup>b</sup>	<i>p</i> value	95% CI <sup>b</sup>	<i>p</i> value	95% CI <sup>b</sup>	<i>p</i> value
Serum folate (ng/ml)	0.060 (-0.012, 0.031)	0.373	-0.032 (-0.040, 0.024)	0.625	-0.090 (-16.773, 3.192)	0.178
RBC folate (ng/ml)	-0.016 (0.000, 0.000)	0.82	0.197 (0.000, 0.001)	0.003	0.149 (0.017, 0.381)	0.032
Vitamin B <sub>12</sub> (pg/ml)	0.464 (0.000, 0.000)	0.464	-0.086 (-0.001, 0.000)	0.169	-0.041 (-0.205, 0.104)	0.523

<sup>a</sup>Multivariate linear regression analysis with head circumference, birth length and birthweight as dependent variables and Serum folate, RBC folate and vitamin B<sub>12</sub> concentrations as independent variables. <sup>b</sup>All values in this column are regression coefficients (95% CI) and their corresponding *B* value.

levels, income and nulliparous.

Our study was conducted in a rural mountain area, which had distributed free periconceptual folic acid supplements (400 µg per day); this enabled us to accurately assess the consumption of folate supplements and the association with subsequent adverse pregnancy outcomes, including SGA and prematurity. In contrast, previous studies were conducted in the general population to find out the association between vitamin B levels with some adverse pregnancy outcomes.<sup>(21,26)</sup> The consumption of folate supplements among study subjects and the correlations with maternal vitamin B levels could not be completely ruled out.<sup>(27–29)</sup>

In this cohort study of pregnant women who resided in Tongliang, Chongqing, 83.2% reported taking folic acid supplementation during the periconceptual period; 67.0% started folic acid use during the first trimester, and 16.2% of the women began taking it before conception. Recently, another survey conducted in China also revealed that more than 80% of women take folic acid supplementation during the periconceptual period.<sup>(25)</sup> The rates are similar with developed countries, such as Canada (91.3%),<sup>(23)</sup> the USA (73.9%)<sup>(24)</sup> and the Netherlands (86%).<sup>(22)</sup> This indicates that the FAS program in China had greatly helped to periconceptual folic acid supplementation and the supplemental rate among the Chinese population increased over time. Meanwhile, our findings confirmed that the women taking folic acid supplementation during the periconceptual period tended to have timely initiation of prenatal care, higher incomes, higher education, and planned pregnancies.

As is shown in Table 4–6 that the lower plasma folate, RBC folate and vitamin B<sub>12</sub> concentrations in early pregnancy in the no-supplementation group were associated with lower birth weight and a higher risk of having an SGA infant and of prematurity. Furthermore, women who delivered SGA or premature infants had reduced plasma folate compared with the control group. To our knowledge, this is one of the few prospective studies that conducted among women planning to conceive that has shown the correlations between the practice of folic acid supplementation and the maternal blood folate concentrations in early pregnancy. Moreover, we did establish a significant association between folic acid supplementation and lower risk of having an SGA infant or premature birth (Table 5). It is consistent with previous studies.<sup>(16,30,31)</sup>

Vitamin B<sub>12</sub> acts as a cofactor for enzyme reactions in one-carbon metabolism and plays a vital role in folate circulation. This is not surprising given the critical role vitamin B<sub>12</sub> plays in folate metabolism. Data on the impact of maternal blood concentration of vitamin B<sub>12</sub> are conflicting. As Sutton *et al.*<sup>(32)</sup> reported, vitamin B<sub>12</sub> levels were not lower in women whose conceptuses had NTDs or other types of defects. However, Molloy *et al.*<sup>(33)</sup> have also found NTD pregnancies to be associated with low maternal levels of vitamin B<sub>12</sub> independent of folate status. In the present study, we did not find any association between maternal vitamin B<sub>12</sub> concentration in early pregnancy and SGA, as well as prematurity (Table 6). This might be explained by the fact that folate is a substrate and vitamin B<sub>12</sub> serves as a cofactor in the homocysteine metabolism, and so is not often a limiting factor.<sup>(34)</sup> Furthermore, it is also possible that diet-gene interactions are involved and small number of pregnancy outcomes. We hope that obtaining dietary and genotype data for other Chinese populations will shed light on this unexpected correlation.

In a multiple linear regression model (Table 7), we found that lower concentrations of folate may result in a relative lighter tendency of birth weight and shorter tendency birth length. However, we had not observed the differences of the levels of RBC folate between cases and controls of SGA or prematurity (Table 6). But it's noteworthy "a relative lighter birth weight" is not equal to "small for gestational age at birth (SGA)". So although there was not observed the differences of the levels of RBC folate on the SGA between the cases and controls, we still consider it's a positive result. Previous research had shown high concentrations of maternal folate levels have an effect on birth weight but not on birth length.<sup>(26)</sup> Another study found that iron-folic acid supplements were significantly associated with increased birth length compared with folic acid alone and that multiple vitamins administered together result in a significant improvement in physical development.<sup>(35)</sup> Therefore, our results may be explained by RBC folate concentrations changing slowly; it provides a better indication of long-term folate status, as women with high RBC folate concentrations have a better nutritional status.

Nonetheless, there are some limitations to our study. The study's subjects were not randomly selected: those women who came to seek early prenatal care may be more conscientious about

their health and that of their unborn babies, or they may enjoy a higher socioeconomic status. This would result in an overestimation of folic acid use and blood folate levels. Furthermore, although the subjects had typical lifestyles representative of the farmers in Chongqing municipality directly under the central government, it may be difficult to extrapolate the conclusion to other rural areas in China. The most serious limitation of this study is the small number of pregnancy outcomes. In addition, our analyses were restricted by the lack of reliable dietary data and measures of maternal nutritional and health status, including maternal weight gain and blood pressure, later in pregnancy.

In summary, we found significant associations between FAS in rural women of childbearing age and maternal folate and vitamin B<sub>12</sub> levels, the higher folate and vitamin B<sub>12</sub> concentration and lower risk of SGA infant and prematurity in early pregnancy in women with FAS intake compared with no-use women in early pregnancy. Furthermore, the mean of maternal plasma folate concentrations in early pregnancy in women who delivered SGA infant or premature infants were lower compared to the control group. In addition, health education should be strengthened, specially making more efforts on prepregnancy education and cooperating with public media.

## References

- Molloy AM, Kirke PN, Brody LC, Scott JM, Mills JL. Effects of folate and vitamin B12 deficiencies during pregnancy on fetal, infant, and child development. *Food Nutr Bull* 2008; **29** (2 Suppl): S101–S111.
- Hibbard ED. The Figlu-excretion test and defective folic-acid metabolism in pregnancy. *Lancet* 1964; **2**: 1146–1149.
- Ray JG, Laskin CA. Folic acid and homocyst(e)ine metabolic defects and the risk of placental abruption, pre-eclampsia and spontaneous pregnancy loss: a systematic review. *Placenta* 1999; **20**: 519–529.
- Lindblad B, Zaman S, Malik A, et al. Folate, vitamin B12, and homocysteine levels in South Asian women with growth-retarded fetuses. *Acta Obstet Gynecol Scand* 2005; **84**: 1055–1061.
- Czeizel AE, Puhó EH, Langmar Z, Acs N, Bánhidy F. Possible association of folic acid supplementation during pregnancy with reduction of preterm birth: a population-based study. *Eur J Obstet Gynecol Reprod Biol* 2010; **148**: 135–140.
- Berry RJ, Li Z, Erickson JD, et al. Prevention of neural-tube defects with folic acid in China. China-U.S. Collaborative Project for Neural Tube Defect Prevention. *N Engl J Med* 1999; **341**: 1485–1490.
- Li Z, Ren A, Zhang L, et al. Extremely high prevalence of neural tube defects in a 4-county area in Shanxi Province, China. *Birth Defects Res A Clin Mol Teratol* 2006; **76**: 237–240.
- MRC Vitamin Study Research Group. Prevention of neural tube defects: results of the Medical Research Council Vitamin Study. MRC Vitamin Study Research Group. *Lancet* 1991; **338**: 131–137.
- Czeizel AE, Dudás I. Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. *N Engl J Med* 1992; **327**: 1832–1835.
- Ren A, Zhang L, Li Z, Hao L, Tian Y, Li Z. Awareness and use of folic acid, and blood folate concentrations among pregnant women in northern China--an area with a high prevalence of neural tube defects. *Reprod Toxicol* 2006; **22**: 431–436.
- Li Z, Ren A, Zhang L, Liu J, Li Z. Periconceptional use of folic acid in Shanxi Province of northern China. *Public Health Nutr* 2007; **10**: 471–476.
- Zhang L, Ren A, Li Z, Hao L, Tian Y, Li Z. Folate concentrations and folic acid supplementation among women in their first trimester of pregnancy in a rural area with a high prevalence of neural tube defects in Shanxi, China. *Birth Defects Res A Clin Mol Teratol* 2006; **76**: 461–466.
- Pietrzik K, Bailey L, Shane B. Folic acid and L-5-methyltetrahydrofolate: comparison of clinical pharmacokinetics and pharmacodynamics. *Clin Pharmacokinet* 2010; **49**: 535–548.
- Yajnik CS, Deshpande SS, Jackson AA, et al. Vitamin B12 and folate concentrations during pregnancy and insulin resistance in the offspring: the Pune Maternal Nutrition Study. *Diabetologia* 2008; **51**: 29–38.
- Muthayya S, Kurpad AV, Duggan CP, et al. Low maternal vitamin B12 status is associated with intrauterine growth retardation in urban South

## Acknowledgments

We thank all the mothers and their children for their participation in the survey as well as the leaders and doctors of the health care workers in Tongliang County, Chongqing, China, especially Tiangeng Liu Shengying Liu and Ying Pu, for their diligent assistance. We also thank the entire field team for their dedication and hard work in successfully conducting the study. We are grateful to children's Nutrition Research Center Laboratory of Children's Hospital of Chongqing Medical University and the Department of Clinical Chemistry at the Capital Institute of Paediatrics, Beijing, China. All authors contributed to the final version of the manuscript.

## Financial Support

The People's Republic of China of Ministry of Health, Maternal and Child Health and Community Health Division commissioned.

## Conflict of Interest

No potential conflicts of interest were disclosed.

- Indians. *Eur J Clin Nutr* 2006; **60**: 791–801.
- Timmermans S, Jaddoe VW, Hofman A, Steegers-Theunissen RP, Steegers EA. Periconception folic acid supplementation, fetal growth and the risks of low birth weight and preterm birth: the Generation R Study. *Br J Nutr* 2009; **102**: 777–785.
- Minet JC, Bissé E, Aebischer CP, Beil A, Wieland H, Lütsch J. Assessment of vitamin B-12, folate, and vitamin B-6 status and relation to sulfur amino acid metabolism in neonates. *Am J Clin Nutr* 2000; **72**: 751–757.
- Hao L, Ma J, Stampfer MJ, et al. Geographical, seasonal and gender differences in folate status among Chinese adults. *J Nutr* 2003; **133**: 3630–3635.
- Jin H, Huang D, Guang X. *The Utility Neonatology* (3rd ed). People's Medical Publishing House, 2003; 4.
- WHO. *International Statistical Classification of Diseases and Related Health Problems. 10th revision, (2008 ed)*. Geneva: WHO, 2009; 95.
- Scholl TO, Hediger ML, Schall JI, Khoo CS, Fischer RL. Dietary and serum folate: their influence on the outcome of pregnancy. *Am J Clin Nutr* 1996; **63**: 520–525.
- van Eijsden M, van der Wal MF, Bonsel GJ. Folic acid knowledge and use in a multi-ethnic pregnancy cohort: the role of language proficiency. *BJOG* 2006; **113**: 1446–1451.
- Manniën J, de Jonge A, Cornel MC, Spelten E, Hutton EK. Factors associated with not using folic acid supplements preconceptionally. *Public Health Nutr* 2014; **17**: 2344–2350.
- Branum AM, Bailey R, Singer BJ. Dietary supplement use and folate status during pregnancy in the United States. *J Nutr* 2013; **143**: 486–492.
- Liu J, Jin L, Meng Q, et al. Changes in folic acid supplementation behaviour among women of reproductive age after the implementation of a massive supplementation programme in China. *Public Health Nutr* 2015; **18**: 582–588.
- Bergen NE, Jaddoe VW, Timmermans S, et al. Homocysteine and folate concentrations in early pregnancy and the risk of adverse pregnancy outcomes: the Generation R Study. *BJOG* 2012; **119**: 739–751.
- Hangler M, Burmølle M, Schneider I, Allermann K, Jensen B. The serine protease Esperase HPF inhibits the formation of multispecies biofilm. *Biofouling* 2009; **25**: 667–674.
- Wald NJ, Hackshaw AD, Stone R, Sourial NA. Blood folate and vitamin B12 in relation to neural tube defects. *Br J Obstet Gynaecol* 1996; **103**: 319–324.
- Blom HJ, Shaw GM, den Hejjer M, Finnell RH. Neural tube defects and folate: case far from closed. *Nat Rev Neurosci* 2006; **7**: 724–731.
- Kim HS, Jaffé PR. Degradation of toluene by a mixed population of archetypal aerobes, microaerophiles, and denitrifiers: laboratory sand column experiment and multispecies biofilm model formulation. *Biotechnol Bioeng* 2008; **99**: 290–301.
- Goldenberg RL, Tamura T, Cliver SP, Cutter GR, Hoffman HJ, Copper RL. Serum folate and fetal growth retardation: a matter of compliance? *Obstet*

*Gynecol* 1992; **79**: 719–722.

- 32 Sutton M, Mills JL, Molloy AM, *et al.* Maternal folate, vitamin B12 and homocysteine levels in pregnancies affected by congenital malformations other than neural tube defects. *Birth Defects Res A Clin Mol Teratol* 2011; **91**: 610–615.
- 33 Molloy AM, Kirke PN, Troendle JF, *et al.* Maternal vitamin B12 status and risk of neural tube defects in a population with high neural tube defect prevalence and no folic Acid fortification. *Pediatrics* 2009; **123**: 917–923.
- 34 Finkelstein JD. Methionine metabolism in mammals. *J Nutr Biochem* 1990; **1**: 228–237.
- 35 Zeng L, Dibley MJ, Cheng Y, *et al.* 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. *BMJ* 2008; **337**: a2001.