Preconception homocysteine and B vitamin status and birth outcomes in Chinese women

Alayne G Ronnenberg, Marlene B Goldman, Dafang Chen, Iain W Aitken, Walter C Willett, Jacob Selhub, and Xiping Xu

ABSTRACT

Background: The associations between homocysteine, B vitamin status, and pregnancy outcomes have not been examined prospectively.

Objective: We assessed the associations of preconception homocysteine and B vitamin status with preterm birth and birth of low-birth-weight (LBW) and small-for-gestational-age (SGA) infants in Chinese women.

Design: This was a case-control study of women aged 21–34 y. Preterm cases (n = 29) delivered living infants at <37 wk gestation; term controls (n = 405) delivered infants at ≥37 wk. LBW cases (n = 33) had infants weighing <2500 g; normal-birth-weight controls (n = 390) had infants weighing ≥2500 g. SGA cases (n = 65) had infants below the 10th percentile of weight-for-gestational-age; appropriate-for-gestational-age controls (n = 358) had infants above this cutoff. Nonfasting plasma concentrations of homocysteine, folate, and vitamins B-6 and B-12 were measured before conception.

Results: Elevated homocysteine (≥12.4 μmol/L) was associated with a nearly 4-fold higher risk of preterm birth (OR: 3.6; 95% CI: 1.3, 10.0; P < 0.05). The risk of preterm birth was 60% lower among women with vitamin B-12 ≥258 pmol/L than among vitamin B-12-deficient women (OR: 0.4; 95% CI: 0.2, 0.9; P < 0.05) and was 50% lower among women with vitamin B-6 ≥30 nmol/L than among vitamin B-6-deficient women (OR: 0.5; 95% CI: 0.2, 1.2; NS). Folate status was not associated with preterm birth, and homocysteine and B vitamin status were not associated with LBW or SGA status.

Conclusions: Elevated homocysteine and suboptimal vitamin B-12 and B-6 status may increase the risk of preterm birth. These results need to be confirmed in larger prospective studies.

KEY WORDS Homocysteine, vitamin B-12, vitamin B-6, preterm birth, low birth weight, pregnancy, pregnancy outcome, China

INTRODUCTION

Low birth weight (LBW; <2500 g) affects nearly 8% of births in the United States (1) and as many as 20% of births worldwide (2). Although less is known about the incidence of preterm birth (birth before 37 completed weeks of gestation), evidence suggests that up to 10% of births in some developed countries may be preterm (3), and the incidence is likely to be even higher in underdeveloped areas. Both preterm birth and LBW remain significant risk factors for virtually all causes of neonatal and postneonatal death (4–6). In China, where the proportion of LBW infants was estimated to be between 5.0% and 9.9% in 1990 (2), the risk of perinatal death was 15–80 times that among LBW and preterm infants than among infants of normal weight and gestational age (7). Preterm birth and LBW also increase the risk of infant morbidity (4), including childhood asthma (8) and neurodevelopmental handicaps (6), and may have detrimental effects that reach well into adulthood (9).

Several previous studies suggested that maternal B vitamin status may influence the risk of both LBW and preterm birth. Hibbard (10) reported an association between maternal red blood cell folate concentrations at or before 16 wk gestation and the proportions of preterm and small-for-gestational-age (SGA) infants. More recently, Scholl et al (11) found that lower dietary intake of folate and lower serum folate at week 28 were associated with a 3-fold increase in the risk of preterm birth and LBW. Although vitamin B-12 status has not been linked to these pregnancy outcomes, Kubler (12) and Reinken and Dapunt (13) reported positive relations between maternal vitamin B-6 status during pregnancy and infant birth weight, and Brophy and Siiteri (14) found an inverse association between the vitamin B-6 concentration in cord blood and the risk of preeclampsia, which is itself a risk factor for preterm birth (15). Recent attention has focused on the potential role of the amino acid homocysteine in pregnancy outcomes. Elevated homocysteine, which can result from genetic abnormalities and suboptimal folate, vitamin B-12, or vitamin B-6 status (16), was associated with preeclampsia (17–20) and both LBW and preterm birth (21).

Little is known about the relation between B vitamin status and birth outcomes in Chinese women. However, a folate supplementation trial in China found a marked reduction in the risk of neural tube defects after supplementation, suggesting that folate deficiency was widespread among women in these regions (22). We
recently reported a high prevalence of B vitamin deficiencies in a
group of young women who were attempting to become pregnant
and were living in Anqing, China (23). We also observed that rou-
tine prenatal vitamin supplementation is uncommon in this area
of China (23), and we suspected that the high prevalence of vita-
m deficiencies, coupled with the additional nutrient demands of
pregnancy, could place these women and their infants at increased
risk of adverse pregnancy outcomes. The purpose of this case-control
study was to determine whether there are associations between
preconception homocysteine and B vitamin status and the risk of
preterm birth or the birth of LBW or SGA infants in young Chi-
nese women.

SUBJECTS AND METHODS

Subjects

The current case-control study of preterm birth and of the
birth of LBW or SGA infants was conducted in conjunction
with an ongoing prospective study of the effects of rotating shift
work on reproductive outcomes among female textile workers in
Anqing, China. Eligible subjects were selected from among
married women enrolled in the shift-work study between
August 1996 and December 1998. All women employees of the
textile mills receive prenatal, delivery, and postnatal care in
the nearby hospital. All the subjects were between 21 and 34 y
of age, had never smoked, and had obtained governmental per-
mission to have a child. Women were excluded if they were
pregnant at the initial interview, had tried unsuccessfully to
become pregnant for ≥1 y, had previously experienced a clini-
cally recognized spontaneous abortion, or planned to quit their
job, change jobs, or move out of the city in the coming year.
Women were monitored during any ensuing pregnancy or for
up to 1 y after they started trying to conceive. All suspected
pregnancies were confirmed by a positive urinary human chorionic
gonadotropin test, and all pregnancy outcomes were
recorded.

The gestational age of the infants (in weeks) was determined
by calculating the number of days between the first day of the last
menstrual period and the birth of the infant. A woman was classi-
fied as a preterm case (n = 29) if her infant was born before 37 wk
of completed gestation and as a term control (n = 405) if the infant
was born at or beyond 37 wk gestation. Infant birth weight data
were available for 423 women. A woman was defined as a LBW
control if the infant weighed ≥ 2500 g (n = 390). Only 5
infants had birth weights <2000 g. SGA cases (n = 65) were
women whose infants had weights below the 10th percentile for
their gestational age according to intrauterine growth standards
for ethnic Chinese (24), and appropriate-for-gestational-age con-
trols were women whose infants were above the 10th percentile
of weight-for-age (n = 358).

Measurements

At enrollment in the prospective study, the women’s body
weight in light clothing and height were measured to the nearest
0.1 kg and 0.1 cm, respectively, with a beam weighing scale
and measuring system. Body mass index (BMI) was calculated in kg/
m². Also at the time of enrollment, interviewers administered
a previously validated questionnaire to the women and their hus-
bands to collect baseline information on sociodemographic,
environmental, and personal characteristics that might be related
to reproductive outcomes. Included among these variables were
education (in number of years completed), type of shift work
(rotating or nonrotating), passive smoking in the home, use of
vitamin or mineral supplements, and consumption of tea and alco-
hol. Infant weight (to the nearest 0.01 kg) was recorded immedi-
ately after delivery.

Blood samples were obtained from all the women before the
initial interview when they were not fasting. The samples were
drawn via venipuncture and were collected into 10-mL, metal-
free EDTA-treated tubes. Blood was kept on ice until cen-
trifuged at 4000 × g for 10 min at 4 °C. The plasma was stored
at −20 °C until shipped on dry ice to the Harvard School of
Public Health, where it was stored at −70 °C before nutritional
analyses. Frozen samples were transported to the US Depart-
ment of Agriculture Human Nutrition Research Center on
Aging, Tufts University, Boston, where plasma concentrations
of homocysteine, folate, and vitamins B-6 and B-12 were
measured. The Human Subjects Committees at the Harvard
School of Public Health and the local institute approved all
study procedures, and informed consent was obtained from
each participant.

Total homocysteine concentration in plasma was determined
with a method derived from the principles described by Araki
and Sako (25). Although the analyses were performed on plasma
samples obtained from nonfasting subjects, fasting status was
shown to have no appreciable effect on homocysteine concen-
trations (26). Moreover, a single measurement of homocysteine
was shown to reliably reflect an individual’s average long-term
homocysteine concentration (27). Plasma folate and vitamin B-
12 concentrations were determined by using a radioimmunoas-
say method with a commercially available kit from BioRad
Diagnostics Group (Hercules, CA). Plasma vitamin B-6 (as pyri-
doxal 5′-phosphate) was measured with the tyrosine decarboxy-
lase apoenzyme method (28). Homocysteine and vitamin meas-
urements were completed in 4 batches over an 11-mo period,
with from 63 to 282 samples in each batch. Typical CVs for
in-house control plasma samples were <8% for homocys-
teine, folate, and vitamin B-12 and <9% for vitamin B-6.

Statistical analyses

Statistical analyses were performed with SAS for WINDOWS,
release 8.0, version 4.10 (SAS Institute Inc, Cary, NC). Data were
analyzed in a manner consistent with a case-control study design
rather than a prospective cohort study, because nutritional anal-
yses were not performed on samples from the entire cohort. Instead,
samples were selected for biochemical analysis on the basis of the
recorded pregnancy outcome. Because of their skewed distribu-
tions, nutrition variables and covariates are presented as medians
with the range of values or as geometric means with their 95%
CIs. Differences between cases and controls were assessed with
the Wilcoxon rank sum test (for the medians) or a t test on loga-
rithmically transformed variables. The correlations between nutri-
tion variables (homocysteine, folate, vitamins B-6 and B-12, and
BMI) were estimated after logarithmic transformation and are
shown as Pearson’s product-moment correlation coefficients.

Plasma vitamin concentrations were compared with published
reference values to determine the proportions of case and control
subjects with biochemical evidence of vitamin deficiencies. Vita-
m deficiency was defined as plasma concentrations < 6.8 nmol/L
(3 ng/mL) for folate (29), <30 nmol/L of pyridoxal 5′-phosphate
TABLE 1
Maternal and infant characteristics of cases and controls according to birth status

<table>
<thead>
<tr>
<th>Variable</th>
<th>Preterm birth</th>
<th>Low birth weight</th>
<th>Small for gestational age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases (n = 29)</td>
<td>Controls (n = 405)</td>
<td>Cases (n = 33)</td>
</tr>
<tr>
<td>Maternal age (y)</td>
<td>24.8 (23.0–34.2)</td>
<td>24.7 (21.5–32.4)</td>
<td>24.8 (23.0–34.2)</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.6 (1.5–1.7)</td>
<td>1.6 (1.4–1.7)</td>
<td>1.6 (1.4–1.6)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>48.5 (41.0–57.5)</td>
<td>48.5 (37.5–78.0)</td>
<td>48.0 (35.0–66.0)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>19.4 (17.1–22.1)</td>
<td>19.5 (15.8–31.4)</td>
<td>19.0 (16.1–31.4)</td>
</tr>
<tr>
<td>Gestational age (wk)</td>
<td>35.7 (30.1–36.9)</td>
<td>39.9 (37.0–45.3)</td>
<td>38.6 (30.1–42.6)</td>
</tr>
<tr>
<td>Infant birth weight (g)</td>
<td>2625 (1400–3975)</td>
<td>3100 (1800–4700)</td>
<td>2300 (1400–2450)</td>
</tr>
</tbody>
</table>

1 Preterm birth defined as a live birth with a gestational age < 37 wk gestation, low birth weight (LBW) as an infant birth weight < 2500 g, and small for gestational age (SGA) as an infant weight below the 10th percentile of weight for gestational age. The numbers of case and control subjects vary slightly because of missing values for some variables.

2 Median; range in parentheses.

3 Significantly different from corresponding controls, P < 0.005 (Wilcoxon rank-sum test).

4 Significantly different from corresponding controls, P < 0.001 (Wilcoxon rank-sum test).

5 Significantly different from corresponding controls, P < 0.001 (Wilcoxon rank-sum test).

6 The proportions of cases and controls with these characteristics did not differ significantly (Pearson’s χ² P > 0.05).

7 n in brackets.

8 Data were available for only 275 women in the preterm analysis and 270 women in the LBW and SGA analyses.

for vitamin B-6 (30), and < 258 pmol/L (350 pg/mL) for vitamin B-12 (31, 32). There is no standard definition of elevated homocysteine. For these analyses, we defined elevated homocysteine as a plasma concentration of homocysteine ≥ 12.4 μmol/L. This value was above the 90th percentile among women (n = 311) with plasma concentrations of both folate and vitamin B-12 above the cutoffs used to define deficiency (i.e., folate ≥ 6.8 nmol/L and vitamin B-12 ≥ 258 pmol/L).

Significant differences between the proportions of case and control women with vitamin deficiencies or elevated homocysteine were evaluated by using Pearson’s chi-square analyses and Fisher’s exact test (homocysteine). The associations of preterm birth and the birth of LBW and SGA infants with elevated homocysteine were estimated by using logistic regression and were expressed as odds ratios with 95% CIs, with plasma homocysteine < 12.4 μmol/L as the referent. Odds ratios for preterm birth and the birth of LBW or SGA infants were similarly calculated for B vitamin status by using plasma concentrations below those used to define deficiency as the referents. All logistic models were adjusted for the batch in which the plasma samples were analyzed. Additional adjustments in preterm models were made for mother’s age, BMI, and hemoglobin concentration, all as continuous variables. LBW and SGA models were also adjusted for mother’s age, BMI, and hemoglobin concentration; in addition, LBW models were adjusted for infant sex and gestational age. Variables for the B vitamins were not included in the final logistic models containing elevated homocysteine. However, models exploring the potential associations between deficiency of one B vitamin and preterm birth or the birth of LBW or SGA infants were adjusted for plasma concentrations of the other 2 vitamins (as continuous variables). Because infant sex was not known for 6 subjects, the number of controls was reduced to 384 in adjusted LBW models. Statistical significance was defined as P ≤ 0.05.

RESULTS

The gestational age of live-born infants was available for 434 women. There were 29 births before 37 wk gestation; of these preterm births, 4 (14%) had an estimated gestational age < 34 wk. Infant birth weight was available for 423 women with available gestational age data (33 LBW cases and 390 controls). Five of these LBW infants weighed < 2000 g and one weighed < 1500 g. Nine LBW infants (27%) were born preterm, and 32% of preterm infants weighed < 2500 g. Of the 423 infants with recorded birth weights, 65 were classified as SGA. Of the SGA infants, 26 (40%) were also LBW, although only 4 (6%) were also born preterm. There were no significant differences in terms of maternal age, BMI, education, work shift, passive smoking in the home, or use of vitamins, tea, or alcohol between preterm or LBW cases and their respective controls (Table 1). However, the median weight and BMI of women with SGA infants were significantly less than values of their respective controls. In general, women in the study tended to be young and lean; most had a middle-school education, and nearly all worked rotating shifts. Only 11 women (<3%) reported using vitamin supplements of any kind. As expected, median infant birth weight was significantly lower for preterm, LBW, and SGA cases than for controls.

We assessed the degree of correlation between logarithmically transformed plasma concentrations of homocysteine and the B vitamins in the combined group of 434 case and control women (Table 2). Total homocysteine concentration was inversely correlated with both folate and vitamin B-12 concentrations and was positively correlated with vitamin B-6 concentration. Significant
positive correlations between the 3 vitamins were also observed. A small, significant correlation was observed between BMI and plasma folate (r = 0.11), but BMI was not significantly related to homocysteine or vitamins B-6 and B-12 (data not shown).

In general, B vitamin deficiencies were common in both case and control women, with plasma folate and vitamin B-6 concentrations indicative of deficiency detected in > 20% of women overall and vitamin B-12 deficiency detected in 19%. However, combined vitamin deficiencies were uncommon: only 2.5% of women were deficient in folate and vitamin B-12, 9% were deficient in folate and vitamin B-6, 4.4% were deficient in vitamins B-6 and B-12, and 1.4% were deficient in all 3 vitamins. Although mean concentrations of homocysteine, folate, and vitamins B-6 and B-12 were not significantly different between preterm case subjects and control subjects (Table 3), the prevalence of elevated homocysteine was more than twice as high in women who delivered preterm as in control women (Fishers exact test, chi square; P = 0.06). In addition, vitamin B-12 deficiency tended to be more common in women with preterm deliveries than in control subjects (31.0% and 17.8%, respectively; P = 0.08). Vitamin B-6 deficiency was =50% more common in preterm cases than in control subjects (34.5% and 22.5%, respectively), although this difference was not statistically significant. Mean homocysteine and vitamin concentrations and the proportions of women with elevated homocysteine concentrations or B vitamin deficiencies were not significantly different between LBW case and control subjects or between SGA case and control subjects (Table 3).

In logistic regression analyses, a strong association was observed between preterm birth and elevated homocysteine (Table 4). In models adjusted only for analytic batch (unadjusted OR), the odds of delivering preterm were nearly 4-fold higher among women with elevated homocysteine than among those with lower plasma homocysteine. This association was not appreciably altered by adjustment for age, BMI, or hemoglobin concentration (adjusted OR). In addition, higher plasma vitamin B-12 concentrations were associated with reduced risk of preterm birth. The adjusted OR (adjusted for covariates and concentrations of the other 2 vitamins) was 0.4 (95% CI: 0.2, 0.9) for women with plasma vitamin B-12 ≥ 258 pmol/L compared with those with B-12 deficiency (< 258 pmol/L). The risk of preterm birth also tended to be 50% lower among women with adequate vitamin B-6 status (pyridoxal 5'-phosphate ≥ 30 mmol/L) compared with women with vitamin B-6 deficiency, although this association was not statistically significant (P = 0.09). Folate status was not associated with preterm birth.

To determine whether the association between preterm birth and elevated homocysteine was independent of vitamin B-12 status and vitamin B-6 status, we added the dichotomized variable for elevated homocysteine to the adjusted logistic regression models for vitamin B-12 and vitamin B-6 that are shown in Table 4. Addition of elevated homocysteine to the model comparing vitamin B-12 sufficiency with deficiency had virtually no effect on the estimated associations between preterm birth and elevated homocysteine or vitamin B-12 (OR for homocysteine: 3.7, 95% CI: 1.3, 10.4; OR for vitamin B-12: 0.40, 95% CI: 0.2, 0.9). However, when elevated homocysteine was added to the model com-

### Table 2

<table>
<thead>
<tr>
<th>Homocysteine</th>
<th>Folate</th>
<th>Vitamin B-12</th>
<th>Vitamin B-6</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>r</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.00</td>
<td>–0.18</td>
<td>0.27</td>
</tr>
<tr>
<td><strong>P</strong></td>
<td>&lt;0.01</td>
<td>0.04</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

**TABLE 2**

Correlation matrix for plasma concentrations of homocysteine and the B vitamins.

1Pearson product-moment correlation coefficient on logarithmically transformed variables; n = 434 for all correlations.

### Table 3

Preconception homocysteine and B vitamin concentrations in cases and controls by birth status.

<table>
<thead>
<tr>
<th>Homocysteine</th>
<th>Preterm birth</th>
<th>Low birth weight</th>
<th>Small for gestational age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases (n = 29)</td>
<td>Controls (n = 405)</td>
<td>Cases (n = 33)</td>
</tr>
<tr>
<td>Concentration (µmol/L)</td>
<td>8.6 (7.4, 10.0)</td>
<td>8.1 (7.8, 8.4)</td>
<td>8.1 (7.4, 8.9)</td>
</tr>
<tr>
<td>Elevated (%)</td>
<td>24.1</td>
<td>10.8</td>
<td>12.1</td>
</tr>
<tr>
<td>Vitamin B-12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentration (pmol/L)</td>
<td>332 (290, 379)</td>
<td>344 (333, 356)</td>
<td>333 (296, 374)</td>
</tr>
<tr>
<td>Deficient (%)</td>
<td>31.0</td>
<td>17.8</td>
<td>17.9</td>
</tr>
<tr>
<td>Vitamin B-6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentration (nmol/L)</td>
<td>37.4 (31.5, 44.2)</td>
<td>38.4 (37.1, 39.7)</td>
<td>40.2 (35.7, 45.3)</td>
</tr>
<tr>
<td>Deficient (%)</td>
<td>34.5</td>
<td>22.5</td>
<td>18.2</td>
</tr>
<tr>
<td>Folate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentration (nmol/L)</td>
<td>9.4 (7.8, 11.2)</td>
<td>9.0 (8.7, 9.4)</td>
<td>9.3 (8.1, 10.8)</td>
</tr>
<tr>
<td>Deficient (%)</td>
<td>20.7</td>
<td>21.0</td>
<td>24.2</td>
</tr>
</tbody>
</table>

1There were no significant differences between the means of the cases and controls by birth status (t test on logarithmically transformed variables). Elevated homocysteine was defined as a plasma concentration ≥12.4 µmol/L; vitamin deficiency was defined as <258 pmol/L for vitamin B-12, <30 nmol pyridoxal 5'-phosphate/L for vitamin B-6, and <6.8 mmol/L for folate. Preterm birth defined as a live birth with a gestational age <37 wk gestation, low birth weight as an infant birth weight <2500 g, and small for gestational age as an infant weight below the 10th percentile of weight-for-gestational-age.

2Values are geometric means; 95% CIs in parentheses.

3Pearson’s χ², P > 0.05 for all case-control comparisons.

4Pearson’s χ², P = 0.06 for difference between cases and controls.

5Pearson’s χ², P = 0.08 for the preterm case-control comparison.
DISCUSSION

We found a strong association between preconception plasma homocysteine concentration and subsequent risk of preterm birth in a relatively homogeneous group of Chinese women. The risk of preterm birth was nearly 4-fold higher among women with preconception homocysteine concentrations ≥ 12.4 μmol/L compared with women who had lower homocysteine concentrations. To our knowledge, this is the first prospective study conducted among women planning to conceive that has shown a link between elevated homocysteine and preterm birth. Several previous studies reported an association between homocysteine and preeclampsia (18, 19, 21), which increases the risk of preterm birth (15), and it is possible that some of our findings are related to preeclampsia or other pregnancy-associated changes in blood pressure. One important shortcoming of most earlier studies, however, is that homocysteine was measured either near the time of delivery (18, 19) or up to several years before or after the index pregnancy (21), thus obscuring the possible temporal relation between elevated homocysteine and pregnancy outcomes. Only one previous study reporting an association between homocysteine and preeclampsia assessed homocysteine status in the second trimester of pregnancy, before the onset of preeclampsia (20). By measuring homocysteine in plasma obtained before pregnancy, our study establishes that elevated homocysteine precedes the occurrence of preterm birth.

We also found that preterm birth was related to B vitamin status. The risk of preterm birth was 60% lower among women with vitamin B-12 ≥ 258 pmol/L than among women with lower vitamin B-12 concentrations. Lindenbaum et al (31) have argued that vitamin B-12 deficiency. This may be particularly true for women who have increased vitamin requirements (33).
The risk of preterm birth also appeared to be \( \approx 50\% \) lower among women with adequate preconception vitamin B-6 status compared with those with deficiency. Although previous studies have related vitamin B-6 status to various pregnancy outcomes, including placental abruption or infarction (34), preeclampsia (14), and infant birth weight (12, 13), to our knowledge this is the first report suggesting a possible association between preconception vitamin B-6 status and preterm birth. Because our observations have not been reported previously, additional prospective studies are needed to confirm these possible protective effects.

We assessed the relations between the pregnancy outcomes and vitamin nutritional status by measuring plasma concentrations of homocysteine and the B vitamins and dividing the values into 2 categories (ie, high versus normal homocysteine and vitamin sufficiency versus deficiency). We chose this approach instead of analyzing the nutrients as continuous variables because we reasoned that we were unlikely to observe a linear relation between vitamin nutritional status and the birth outcomes. Instead, it seemed more biologically plausible to expect a threshold effect, with greater risk of adverse outcomes occurring when vitamin concentrations were indicative of biochemical deficiency. Moreover, because the numbers of cases of adverse birth outcomes were rather small, we were unable to explore potential dose-response relations between vitamin concentrations and pregnancy outcomes. Larger prospective studies are needed to clarify these relations.

The associations of preterm birth with elevated homocysteine and preterm birth with B vitamin concentrations appear to be independent effects. Although we found correlations between plasma concentrations of homocysteine and both vitamin B-12 and vitamin B-6, adjustment for these vitamins did not weaken the associations between preterm birth and either homocysteine or vitamin status. In fact, the detrimental effects of elevated homocysteine and the protective effects of vitamin B-6 sufficiency were enhanced in adjusted logistic models containing both variables. It is important to note that we observed a significant positive association between homocysteine and vitamin B-6 concentrations, which is contrary to the observations of others (16) and which we are unable to explain. It is possible that diet-gene interactions are involved, and we hope that obtaining dietary and genotype data for other Chinese populations will shed light on this unexpected correlation. Regardless of whether the associations between preterm birth, homocysteine, and B vitamin status are independent, numerous intervention trials have shown that appropriate vitamin supplementation both improves B vitamin status and lowers homocysteine concentrations (35–38). Vitamin supplementation trials among pregnant women are needed in this population to determine whether improving vitamin nutritional status also reduces the risk of preterm birth.

The mechanism or mechanisms by which homocysteine and vitamins B-12 and B-6 influence preterm delivery are not known. Some investigators have speculated that, in addition to a possible role in preeclampsia (18, 20), elevated homocysteine may also compromise pregnancy outcomes by interfering with connective tissue integrity, thereby increasing the risk of preterm premature rupture of membranes (39). Elevated homocysteine also has been linked to reduced nitric oxide concentration and glutathione peroxidase activity (40), and it is possible that such disruptions could affect the length of gestation. Vitamin B-12 is critical for nucleotide synthesis and amino acid metabolism, and vitamin B-6 serves as a coenzyme in > 100 reactions, including many involved in amino acid and neurotransmitter synthesis and those necessary to form collagen cross-links (41). Therefore, it appears plausible that deficiencies of either vitamin could contribute to chorionic, hormonal, or other abnormalities involved in preterm birth.

In contrast with the findings of others, we found no relation between folate status and either length of gestation (11, 42) or infant birth weight (11, 43). We reported previously that folate status in the cohort of Chinese women from which subjects in the current study were drawn varied significantly depending on the season in which blood was sampled, with significantly lower plasma folate concentrations measured in summer (23). We believe these differences probably reflect seasonal variations in the availability of folate-rich foods. Because plasma folate concentrations are sensitive to short-term dietary changes, it is possible that maternal plasma folate measured before pregnancy does not reliably reflect the amount of folate ultimately available to maternal and fetal tissues during the last trimester of pregnancy, when fetal growth is greatest.

One strength of the present study was that B vitamin and homocysteine status were measured prospectively. Other important strengths include that the subjects were of similar age and educational background and worked similar shifts in the same industry. None of the subjects smoked, and use of alcohol and vitamin supplements was extremely rare. Moreover, all women were primiparous, because China has a fairly stringent one-child policy, and women who reported a previous pregnancy or clinical spontaneous abortion were excluded from the study. The most serious shortcoming of the present study is the small number of pregnancy outcomes. In addition, our analyses were limited 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 preterm birth and both elevated plasma homocysteine and low plasma vitamin B-12, but not folate, measured before conception in young Chinese women. We did not observe an association between birth of either LBW or SGA infants and homocysteine or B vitamin status. Although recent public health campaigns to prevent neural tube defects generally have focused on increasing periconceptional and prenatal intake of folate alone (44), the potential involvement of related vitamins in other, more prevalent pregnancy outcomes, including preterm birth, should not be overlooked. Appropriate vitamin supplementation not only improves vitamin status and lowers homocysteine concentrations (38) but it is also relatively inexpensive and easy to administer. Well-controlled clinical trials in this or similar populations are needed to determine whether improving maternal B vitamin status through supplementation reduces the risk of preterm birth and other common complications of pregnancy.

REFERENCES