Maternal Folate Status, but Not That Of Vitamins B-12 or B-6, Is Associated with Gestational Age and Preterm Birth Risk in a Multiethnic Asian Population1,2

Ling-Wei Chen,3 Ai Lin Lim,4 Marjorelee Colega,4 Mya-Thway Tint,5 Izzuddin M Aris,6 Chuen Seng Tan,3 Yap-Seng Chong,4,5 Peter D Gluckman,6,7 Keith M Godfrey,8 Kenneth Kwek,9 Seang-Mei Saw,7 Fabian Yap,10,11 Yung Seng Lee,4,6 Mary Foong-Fong Chong,4,6,12,14* and Rob M van Dam3,13,14*

3Saw Swee Hock School of Public Health, Departments of 5Obstetrics and Gynecology, 6Pediatrics, and 13Medicine, Yong Loo Lin School of Medicine, National University of Singapore and National University Health System, Singapore; 4Singapore Institute for Clinical Sciences, A*STAR, Singapore; 7Liggins Institute, University of Auckland, Auckland, New Zealand; 8Medical Research Council Lifecourse Epidemiology Unit and National Institute for Health Research Southampton Biomedical Research Centre, University of Southampton and University Hospital Southampton National Health Service (NHS) Foundation Trust, Southampton, United Kingdom; Departments of 7Maternal Fetal Medicine and 9Pediatric Endocrinology, KK Women’s and Children’s Hospital, Singapore; and 13Duke-NUS Graduate Medical School, Lee Kong Chian School of Medicine, Singapore

Abstract

Background: Maternal folate, vitamin B-12, and vitamin B-6 concentrations during pregnancy have been shown to influence birth outcomes, but the evidence is inconclusive.

Objective: We aimed to examine the associations of maternal B-vitamin status with gestational age, birth weight, and length in a birth cohort study in Singapore.

Methods: Maternal blood samples (n = 999) collected during weeks 26–28 of gestation were assayed for plasma folate, vitamin B-12, and vitamin B-6 concentrations. Birth weight and gestational age data were obtained from hospital records, and other anthropometric variables were measured within 72 h after birth. Relations between B-vitamin status and birth outcomes were assessed by linear or logistic regression with adjustment for potential confounders.

Results: Median (IQR) plasma concentrations were 34.4 (24.5–44.6) nmol/L for folate, 209 (167–258) pmol/L for vitamin B-12, and 61.8 (25.9–113) nmol/L for vitamin B-6. We found that higher plasma folate concentrations were associated with a longer gestational age (0.12 wk per SD increase in folate; 95% CI: 0.02, 0.21) and tended to be associated with lower risk of all preterm birth (delivery at <37 wk of gestation; OR: 0.79; 95% CI: 0.63, 1.00) and spontaneous preterm birth (OR: 0.76; 95% CI: 0.56, 1.04). Overall, concentrations of maternal folate, vitamin B-12, and vitamin B-6 were not independently associated with birth weight or being born small for gestational age (SGA; birth weight <10th percentile for gestational age).

Conclusions: Higher maternal folate concentrations during late pregnancy were associated with longer gestational age and tended to be associated with a lower risk of preterm birth in this multiethnic Asian population. In contrast, the results of our study suggested little or no benefit of higher folate concentrations for reducing the risk of SGA or of higher vitamin B-6 and vitamin B-12 concentrations for reducing the risk of preterm birth or SGA. J Nutr 2015;145:113–20.

Keywords: pregnancy, folate, vitamin B-12, vitamin B-6, gestational age, preterm birth, birth weight, small for gestational age, birth length, birth outcomes

Introduction

Globally, it has been estimated that ~23.8% of all infants are born small for gestational age (SGA)15; birth weight <10th percentile for gestational age) every year, whereas 9.6% are born preterm (before 37 completed weeks) (1–3). SGA and preterm birth are not only major determinants of neonatal mortality and morbidity (1, 4–6) but are also associated with long-term

15 Abbreviations used: GDM, gestational diabetes mellitus; GUSTO, Growing Up in Singapore Towards Healthy Outcomes; KKH, KK Women’s and Children’s Hospital; NUH, National University Hospital; SGA, small for gestational age.

*To whom correspondence should be addressed. E-mail: rob_martinus_van_dam@nuhs.edu.sg (RM van Dam), mary_chong@sics.a-star.edu.sg (MF-F Chong).
adverse health and social consequences (1, 7). For example, SGA is associated with a higher risk of chronic diseases such as type 2 diabetes, whereas preterm birth is associated with a higher risk of disabilities such as cerebral palsy and intellectual disability later in life (8, 9).

Vitamin B-6, folate (vitamin B-9), and vitamin B-12 are water-soluble vitamins involved in the one-carbon metabolism that is important in cellular biosynthesis reactions (10). Folate is essential in the synthesis of purine and thymidine nucleotides, the building blocks of DNA (10, 11). In addition, folate and vitamin B-12 are required for the conversion of homocysteine to methionine (11), whereas vitamin B-6 is required for the conversion of homocysteine to cysteine (12, 13). Methionine is the precursor of S-adenosylmethionine, a cofactor that can alter gene expression through epigenetic mechanisms involving DNA methylation (10, 11). Insufficient concentrations of vitamin B-6, folate, and vitamin B-12 can thus impair cell division and methylation activity, which could subsequently interfere with fetal growth and development (11).

The relations of folate, vitamin B-12, and vitamin B-6 concentrations with gestational age and birth weight have been evaluated in several studies, but the results were equivocal. Higher maternal folate concentrations during pregnancy were associated with longer gestational age or lower preterm birth risk in some studies (14–19) but not in others (20–22). Higher preconceptional maternal vitamin B-12 status was associated with lower preterm birth risk in a Chinese study (22) but not in other studies (14, 18). In a Korean study, no association was observed between vitamin B-6 and duration of gestation (20).

For birth weight outcomes, higher maternal folate status was associated with higher birth weight or lower risk of SGA in some (14, 23–25) but not all (16, 17, 21, 22, 26–28) studies. In general, vitamin B-12 (14, 18, 27, 28) and vitamin B-6 (22, 29) were not associated with birth weight. However, lower vitamin B-12 status was associated with higher risk of SGA in a study in urban South Indians (30).

Most of the previous studies were conducted in Western settings with limited data from Asian populations (20, 22–24, 30). The associations between these vitamins and birth outcomes may differ in an Asian setting, as seen in the Chinese (22) and Indian (30) studies. In this study, we aimed to further elucidate the associations of maternal folate, vitamin B-12, and vitamin B-6 concentrations in relation to gestational age, birth weight, and birth length in a multietnic birth cohort study in Singapore including participants of Chinese, Malay, and Indian ethnicity.

Methods

Study design. We used data from the Growing Up in Singapore Towards Healthy Outcomes (GUSTO) study, a birth cohort study in Singapore involving detailed assessment of the characteristics of pregnant women and their offspring from birth onward. The study was described in detail elsewhere (31). Briefly, the GUSTO study is designed to investigate the effect of early-life events on the risk of developing metabolic diseases later in life. The study was granted ethical approval by the Institutional Review Board of the KK Women’s and Children’s Hospital (KKH) and National University Hospital (NUH). GUSTO participants were recruited from pregnant women who were attending their antenatal care (<14 wk of gestation) from June 2009 to September 2010 at the KKH and the NUH, which house the major public maternity units in Singapore. Written informed consent was collected from all participants upon recruitment. The inclusion criteria included age range between 18 and 50 yr, intention to reside in Singapore for the next 5 yr, intention to finally deliver in KKH and NUH, and willingness to donate cord, cord blood, and placenta. Only Chinese, Malay, and Indian women whose parents and whose husbands’ parents were of the same ethnicity were included in the study. Women with serious health conditions such as psychosis and type 1 diabetes were excluded.

Assessment of maternal folate, vitamin B-12, and vitamin B-6 concentrations. Maternal fasting blood samples were collected at the 26th–28th week of pregnancy visit, processed within 4 h, and stored at −80°C thereafter. Plasma folate and vitamin B-12 concentrations were analyzed at the NUH Referral Laboratory by using the competitive electrochemiluminescence immunoassay on the ADVIA Centaur Immunoassay System (SIEMENS). Between-assay CVs for plasma measurements were 6.0–10.5% for folate samples between 4.1 and 22.2 nmol/L and 4.3–8.8% for vitamin B-12 samples between 160 and 490 pmol/L. Plasma vitamin B-6 was analyzed by using the reverse-phase HPLC method with post–column derivatization and fluorimetric detection (MRC Human Nutrition Research, Elsie Widdowson Laboratory). The between-assay CV was <5%.

Infant characteristics at birth. Information on birth weight, gestational age, infant sex, and birth order was retrieved from birth delivery reports. Gestational age was determined on the basis of a dating ultrasound scan in the first trimester. SGA was defined as birth weight <10th percentile for gestational age by using a birth weight reference developed from 19,634 infants born at the NUH, Singapore (32). Preterm birth was defined as the delivery of a live birth before 37 wk of gestation (33, 34). In addition, birth lengths were measured within 72 h after birth by using a mobile measuring mat (SECA model 210), recorded to the nearest 5 mm.

Maternal characteristics. Data on ethnicity, maternal age, educational level, and self-reported prepregnancy weights were collected from the participants during recruitment; and information on physical activity, dietary supplement use, cigarette smoking, and alcohol consumption habits during pregnancy was gathered at the clinic visit during the 26th–28th week of pregnancy. A 24-h food recall was also administered to ascertain participants’ pregnancy diets, and total energy intake was calculated on the basis of the recall data. At the same clinic visit, maternal weight and height were also measured. Standing heights were measured in duplicate by a stadiometer (SECA model 213) and weights measured by a digital scale (SECA model 803). Weight gains up until 26–28 wk of gestation were calculated by subtracting self-reported prepregnancy weights from weights measured at 26–28 wk of gestation. The participants also underwent oral-glucose-tolerance testing at the 26th–28th week of pregnancy visit and subsequently had their gestational diabetes mellitus (GDM) status diagnosed on the basis of WHO criteria (35).

Statistical analyses. Of the 1247 recruited GUSTO participants, 70 dropped out during pregnancy mainly due to personal reasons (n = 36), family disapproval (n = 10), and loss to follow-up (n = 15). We further excluded participants who underwent in vitro fertilization (n = 85) in this study. Of the remaining participants, 999 had an aliquot of plasma available and had their folate, vitamin B-12, and vitamin B-6 concentrations measured. A subset of these participants had information on birth outcomes including birth weight and gestational age (n = 986) and birth length (n = 940). Because birth weight and gestational age are the main outcomes in this study, the study population for all analyses was restricted to the 986 participants with complete information for vitamin B-6, folate, vitamin B-12, birth weight, and gestational age.

Maternal characteristics were summarized according to quintiles of folate, vitamin B-12, and vitamin B-6 concentrations. The P-trend values for the associations between folate, vitamin B-12, and vitamin B-6 concentrations.
concentrations and maternal characteristics were assessed by modeling the median values of the quintiles in linear regression analysis. For categorical maternal characteristics, the P-trend values were assessed by using Cochran-Mantel-Haenszel tests.

The associations of maternal plasma folate, vitamin B-12, and vitamin B-6 concentrations with gestational age, birth weight, and birth length were analyzed by using linear regression analysis. For binary birth outcomes (SGA and preterm birth), logistic regression analysis was used. The values for folate, vitamin B-12, and vitamin B-6 were log-transformed because they were not normally distributed. The log-transformed values were truncated to the nearest possible value if they were >4 SDs from the mean. We also modeled folate, vitamin B-12, and vitamin B-6 as quintiles. We adjusted for several maternal characteristics that might act as confounding factors based on results from previous studies, namely ethnicity, maternal age, birth order, maternal height, prepregnancy BMI, weight gain up until 26–28 wk of gestation, educational level, and GDM. Infant sex was also included in the adjusted model because it can influence the birth outcomes. In addition, we investigated the effect of mutual adjustment of folate, vitamin B-12, and vitamin B-6. Missing values of the covariates were imputed by using median values [for pregnancy weight gain up until 26–28 wk of gestation (n = 92) and maternal height (n = 21)] or by using a predictive formula derived by regressing the covariate as a dependent variable with several closely related variables [for prepregnancy weight (n = 87)]. For educational level, we assumed that participants with missing values (n = 27) had a postsecondary education (the middle and most common category). For participants with unknown GDM status (n = 66), we assumed that they did not have GDM.

We conducted several sensitivity analyses. We further adjusted for cigarette smoking, alcohol consumption, physical activity, supplement use, and total energy intakes in multivariable regression models. In addition, we restricted the analyses to women who had spontaneous labor (n = 482; defined as labor beginning without pharmacologic or mechanical stimulation) for B-vitamin vs. gestational age analysis. We also assessed the associations between B vitamins and spontaneous preterm birth by excluding all medically indicated preterm birth. In addition, we limited our analysis to participants with no missing data (n = 853) to evaluate whether the imputation of missing data may have affected the results. Gestational age in our population was left-skewed, and the assumptions of linear regression may have been violated. We did not apply statistical transformation on gestational age for easier interpretation and to maintain consistency with the analyses for other outcomes. Nonetheless, we additionally conducted bootstrap analysis, a nonparametric approach for performing statistical inference, with 1000 bootstrap replications to obtain the 95% CI for the variable associated with gestational age. Furthermore, we used the likelihood ratio test to test for potential nonlinear relations between folate status and gestational age and preterm birth by comparing the spline regression models with the regression models where the exposure variable was specified as a continuous predictor variable.

All statistical analyses were performed by using the statistical software package STATA 11.2 (StataCorp). Two-sided P values <0.05 were considered significant. Values described in the text are means ± SDs unless otherwise indicated.

### Results

### Characteristics of participants.

Table 1 shows maternal characteristics according to quintiles of folate, vitamin B-12, and vitamin B-6. The age of the study population was 30.6 ± 5.2 y. The median (IQR) maternal plasma concentrations at 26–28 wk of gestation were 34.4 (24.5–44.6) nmol/L for folate, 209 (167–258) pmol/L for vitamin B-12, and 61.8 (25.9–113) nmol/L for vitamin B-6. The proportions of participants who were deficient for the B vitamins, based on widely used clinical cutoffs (36, 37), were 3% for folate [<6.8 nmol/L; 11% if including participants with “marginal deficiency” (<13.6 nmol/L)]

| TABLE 1 | Maternal characteristics for all participants (n = 986) and according to quintile of maternal folate, vitamin B-12, and vitamin B-6 concentration
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All participants</td>
<td>Folate</td>
<td>Vitamin B-12</td>
<td>Vitamin B-6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(n = 986)</td>
<td>Q1</td>
<td>Q5</td>
<td>P-trend</td>
<td>Q1</td>
<td>Q5</td>
<td>P-trend</td>
</tr>
<tr>
<td>Plasma folate, nmol/L</td>
<td>34.4 (24.5–44.6)</td>
<td>12.5 (8.6–17.0)</td>
<td>57.1 (51.2–72.7)</td>
<td>—</td>
<td>NA</td>
<td>NA</td>
<td>—</td>
</tr>
<tr>
<td>Plasma vitamin B-12, pmol/L</td>
<td>209 (167–250)</td>
<td>NA</td>
<td>NA</td>
<td>—</td>
<td>131 (113–144)</td>
<td>320 (293–357)</td>
<td>—</td>
</tr>
<tr>
<td>Plasma vitamin B-6, nmol/L</td>
<td>61.8 (25.9–113)</td>
<td>NA</td>
<td>NA</td>
<td>—</td>
<td>15.4 (12.4–18.8)</td>
<td>152 (135–177)</td>
<td>—</td>
</tr>
<tr>
<td>Age, y</td>
<td>30.6 ± 5.2</td>
<td>27.9 ± 5.0</td>
<td>32.1 ± 4.5</td>
<td>&lt;0.001</td>
<td>30.4 ± 5.0</td>
<td>30.3 ± 5.1</td>
<td>0.65</td>
</tr>
<tr>
<td>Height, cm</td>
<td>158 ± 58</td>
<td>158 ± 58</td>
<td>158 ± 55</td>
<td>0.65</td>
<td>158 ± 5.9</td>
<td>158 ± 6.1</td>
<td>0.71</td>
</tr>
<tr>
<td>Prepregnancy BMI, kg/m²</td>
<td>22.7 ± 4.4</td>
<td>23.0 ± 4.7</td>
<td>22.4 ± 4.3</td>
<td>0.11</td>
<td>24.1 ± 4.7</td>
<td>21.3 ± 3.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Weight gain at week 26, kg</td>
<td>8.6 ± 4.6</td>
<td>8.4 ± 5.2</td>
<td>8.6 ± 4.4</td>
<td>0.63</td>
<td>8.6 ± 4.6</td>
<td>8.2 ± 4.1</td>
<td>0.39</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Chinese</td>
<td>537 (54)</td>
<td>70 (36)</td>
<td>129 (65)</td>
<td>91 (47)</td>
<td>113 (57)</td>
<td>89 (45)</td>
<td>117 (59)</td>
</tr>
<tr>
<td>Malay</td>
<td>262 (27)</td>
<td>82 (42)</td>
<td>32 (16)</td>
<td>45 (23)</td>
<td>60 (30)</td>
<td>67 (34)</td>
<td>44 (22)</td>
</tr>
<tr>
<td>Indian</td>
<td>187 (19)</td>
<td>44 (22)</td>
<td>37 (19)</td>
<td>58 (30)</td>
<td>25 (13)</td>
<td>41 (21)</td>
<td>37 (19)</td>
</tr>
<tr>
<td>Educational status</td>
<td>&lt;0.001</td>
<td>0.11</td>
<td>0.05</td>
<td>0.85</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Primary/secondary</td>
<td>298 (30)</td>
<td>81 (41)</td>
<td>40 (20)</td>
<td>59 (30)</td>
<td>55 (28)</td>
<td>65 (33)</td>
<td>63 (32)</td>
</tr>
<tr>
<td>Postsecondary</td>
<td>360 (37)</td>
<td>76 (39)</td>
<td>68 (34)</td>
<td>74 (38)</td>
<td>67 (34)</td>
<td>69 (35)</td>
<td>72 (36)</td>
</tr>
<tr>
<td>University</td>
<td>328 (33)</td>
<td>39 (20)</td>
<td>90 (45)</td>
<td>61 (31)</td>
<td>76 (38)</td>
<td>63 (32)</td>
<td>63 (32)</td>
</tr>
<tr>
<td>Physical activity³ (h/wk)</td>
<td>0.18</td>
<td>0.11</td>
<td>0.40</td>
<td>0.18</td>
<td>0.11</td>
<td>0.40</td>
<td>0.18</td>
</tr>
<tr>
<td>&lt;3</td>
<td>285 (29)</td>
<td>52 (27)</td>
<td>56 (28)</td>
<td>44 (23)</td>
<td>51 (26)</td>
<td>55 (28)</td>
<td>50 (26)</td>
</tr>
<tr>
<td>3–9.9</td>
<td>400 (41)</td>
<td>73 (38)</td>
<td>88 (43)</td>
<td>81 (42)</td>
<td>87 (45)</td>
<td>75 (38)</td>
<td>84 (43)</td>
</tr>
<tr>
<td>≥10</td>
<td>286 (29)</td>
<td>67 (35)</td>
<td>56 (28)</td>
<td>68 (35)</td>
<td>57 (29)</td>
<td>65 (33)</td>
<td>60 (31)</td>
</tr>
<tr>
<td>Smoked during pregnancy</td>
<td>25 (3)</td>
<td>5 (3)</td>
<td>5 (3)</td>
<td>0.76</td>
<td>3 (2)</td>
<td>8 (4)</td>
<td>0.49</td>
</tr>
<tr>
<td>Used alcohol during pregnancy</td>
<td>20 (2)</td>
<td>6 (3)</td>
<td>3 (2)</td>
<td>0.27²</td>
<td>2 (1)</td>
<td>8 (4)</td>
<td>0.025³</td>
</tr>
</tbody>
</table>

¹Values are means ± SDs, medians (IQRs), or n (%). NA, not applicable; Q, quintile.
²Physical activity includes strenuous, moderate, and gentle exercise.
³Tests may not be valid due to violation of test assumption (>20% of the cells have expected cell counts <5).

Maternal vitamin B status and birth outcomes 115
and 16% for both vitamins B-12 (<148 pmol/L) and B-6 (<20 nmol/L). Approximately half of the participants were Chinese (54%), with the remainder of Malay (27%) or Indian (19%) ethnicity. Participants with lower folate status tended to be younger, of Malay ethnicity, and less likely to have a higher educational level. Participants with lower vitamin B-12 status tended to have a higher prepregnancy BMI and more likely to be of Indian ethnicity but were less likely to consume alcohol during pregnancy and to be primigravida. Participants with lower vitamin B-6 status tended to be younger. Birth weight was $3101 \pm 449$ g and gestational age was $38.6 \pm 1.4$ wk. Ninety-seven infants (9.8%) were born SGA and 85 births (8.6%) were preterm, including 46 spontaneous preterm births.

**Associations with gestational age and preterm birth.** After adjustment for potential confounders, higher plasma folate concentrations were associated with a longer gestational age (0.12 wk per SD increase in folate; 95% CI: 0.02, 0.21) (Table 2). Furthermore, an inverse relation of borderline significance between folate concentration and risk of preterm birth was observed (OR: 0.79; 95% CI: 0.63, 1.00) (Table 2). Similar results were observed in analyses stratified by ethnicity (P-interaction = 0.45 for gestational age and 0.41 for preterm birth). In the corresponding quintile analysis, being in the highest quintile of folate concentrations (median concentration: 57.1 nmol/L; IQR: 51.2–72.7 nmol/L) was associated with a 0.33-wk longer gestational age (95% CI: 0.03, 0.62) compared with the lowest quintile (median concentration: 12.5 nmol/L; IQR: 8.6–17.0 nmol/L) (Figure 1).

There were trends toward associations between higher maternal vitamin B-12 concentrations and longer gestational age (0.09 wk per SD increase in vitamin B-12; 95% CI: 0.00, 0.19) and lower preterm birth risk (OR: 0.81; 95% CI: 0.64, 1.03) (Table 2). Maternal vitamin B-6 status was not associated with gestational age and preterm birth risk (Table 2). Similar results were observed in the corresponding quintile analysis, and there were no interactions of vitamin B-12 or vitamin B-6 with ethnicity in relation to gestational age or preterm birth (data not shown).

**Associations with birth weight and birth length.** Higher maternal plasma folate concentrations were associated with higher birth weight and birth length in univariate analysis (Table 3).

**TABLE 2** Associations of maternal plasma folate, vitamin B-12, and vitamin B-6 status with gestational age and preterm birth

<table>
<thead>
<tr>
<th></th>
<th>Gestational age, wk (n = 986)</th>
<th>Preterm birth (n = 986)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β (95% CI) P</td>
<td>OR (95% CI) P</td>
</tr>
<tr>
<td>Folate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted</td>
<td>0.13 (0.04, 0.22) 0.003</td>
<td>0.77 (0.62, 0.95) 0.015</td>
</tr>
<tr>
<td>Multivariable model2</td>
<td>0.12 (0.02, 0.21) 0.017</td>
<td>0.79 (0.63, 1.00) 0.05</td>
</tr>
<tr>
<td>Vitamin B-12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted</td>
<td>0.09 (0.00, 0.18) 0.047</td>
<td>0.83 (0.67, 1.03) 0.10</td>
</tr>
<tr>
<td>Multivariable model2</td>
<td>0.09 (0.00, 0.19) 0.05</td>
<td>0.81 (0.64, 1.03) 0.09</td>
</tr>
<tr>
<td>Vitamin B-6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted</td>
<td>0.05 (−0.04, 0.14) 0.24</td>
<td>0.95 (0.77, 1.19) 0.68</td>
</tr>
<tr>
<td>Multivariable model2</td>
<td>0.95 (−0.04, 0.14) 0.23</td>
<td>0.96 (0.77, 1.20) 0.73</td>
</tr>
</tbody>
</table>

1 Effect sizes are expressed for 1-SD higher log-transformed concentrations of folate, vitamin B-12, and vitamin B-6.
2 Adjusted for infant sex, ethnicity, maternal age, gravidity, maternal height, prepregnancy BMI, weight gain up until 26 wk, educational level, and gestational diabetes mellitus.

These associations were no longer significant after adjustment for covariates. Overall, folate concentrations were also not associated with SGA risk (Table 3). However, we observed an interaction between ethnicity and folate concentrations (P-interaction = 0.004) in relation to SGA risk, where higher folate status was associated with a lower risk of SGA only in the Chinese participants (OR: 0.65; 95% CI: 0.46, 0.91). There was, however, no interaction (P = 0.42) between folate and ethnicity in relation to birth weight as a continuous outcome.

Maternal vitamin B-12 and vitamin B-6 concentrations were not associated with birth length, birth weight, or risk of SGA (Table 3). There were no interactions between vitamins B-12 or B-6 and ethnicity in relation to birth weight, SGA, or birth length.

**Sensitivity analyses.** Results remained largely the same with mutual adjustment of folate, vitamin B-12, and vitamin B-6 concentrations. Furthermore, no interactions were found between folate, vitamin B-12, and vitamin B-6 in relation to the studied birth outcomes. The results did not change substantially with further adjustment for maternal smoking, alcohol consumption, physical activity, total energy intakes, and supplement use. When the analyses were restricted to spontaneous labors (n = 482), the association of plasma folate concentrations with gestational age (β: 0.07 wk; 95% CI: −0.07, 0.22) remained largely unchanged, although the number of subjects was lower and the CI wider. Furthermore, being in the highest quintile of folate concentrations was still significantly associated with longer gestational age (β: 0.46 wk; 95% CI: 0.01, 0.90 wk), compared with the lowest quintile in an analysis restricted to spontaneous labors. In contrast, the association of vitamin B-12 with duration of gestation at birth (β: 0.03 wk; 95% CI: −0.11, 0.18 wk) largely disappeared. Similarly, in our analysis involving spontaneous preterm birth only (excluding all indicated preterm...
Table 3: Associations of maternal plasma folate, vitamin B-12, and vitamin B-6 status with birth weight, being born small for gestational age, and birth length

<table>
<thead>
<tr>
<th></th>
<th>Small for gestational age</th>
<th>Birth weight, g</th>
<th>Birth length, cm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 986)</td>
<td>(n = 986)</td>
<td>(n = 940)</td>
</tr>
<tr>
<td><strong>Folate</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted</td>
<td>0.94 (0.76, 1.16)</td>
<td>28.8 (0.9, 56.8)</td>
<td>0.21 (0.08, 0.34)</td>
</tr>
<tr>
<td>Multivariable model²</td>
<td>0.97 (0.77, 1.22)</td>
<td>20.5 (–7.8, 48.9)</td>
<td>0.12 (–0.02, 0.25)</td>
</tr>
<tr>
<td><strong>Vitamin B-12</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted</td>
<td>1.08 (0.87, 1.33)</td>
<td>–19.4 (–47.4, 8.6)</td>
<td>–0.07 (–0.29, 0.06)</td>
</tr>
<tr>
<td>Multivariable model²</td>
<td>1.02 (0.82, 1.28)</td>
<td>–1.2 (–29.1, 26.6)</td>
<td>–0.04 (–0.17, 0.09)</td>
</tr>
<tr>
<td><strong>Vitamin B-6</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted</td>
<td>0.95 (0.77, 1.17)</td>
<td>5.8 (–22.1, 33.7)</td>
<td>–0.03 (–0.16, 0.11)</td>
</tr>
<tr>
<td>Multivariable model²</td>
<td>0.98 (0.79, 1.22)</td>
<td>–3.9 (–30.7, 22.9)</td>
<td>–0.08 (–0.21, 0.05)</td>
</tr>
</tbody>
</table>

1 Effect sizes are expressed for a 1-SD higher log-transformed concentrations of folate, vitamin B-12, and vitamin B-6.
2 Adjusted for infant sex, ethnicity, maternal age, gravidity, maternal height, prepregnancy BMI, weight gain up until 26 wk, educational level, and gestational diabetes mellitus.

Discussion

In this prospective birth cohort study, higher maternal folate concentrations at approximately the start of the third trimester (26th–28th week) of pregnancy were significantly associated with a longer duration of gestation and tended to be associated with a lower risk of preterm birth. Overall, maternal folate, vitamin B-12, and vitamin B-6 concentrations were not independently associated with birth weight and risk of SGA.

As reflected by median plasma concentrations, our population was generally replete with the studied B vitamins. However, deficiencies of these vitamins, especially of vitamin B-12 and vitamin B-6, were still observed in a substantial proportion of the participants. The high prevalence of folate-containing supplement use at any time during pregnancy (90%) in our population may have explained the relatively low percentage of participants with folate deficiency.

Comparison with other studies. In concordance with our results, several recent cohort studies in Western countries suggested that higher maternal folate concentrations (mainly measured during early pregnancy) were associated with longer gestational age or lower preterm birth risk (14–17, 19). In these earlier studies, median serum folate concentrations were usually higher (15, 19) or similar (16) to that of our population. In combination with our study, these data suggest that the achievement of high plasma folate concentrations, probably through folic acid supplement use rather than merely avoiding deficiencies, may be needed to minimize the risk of preterm birth. In contrast, no association was observed between folate concentrations and gestational age or risk of preterm birth in 3 other prospective studies (20–22). The discrepancy in results could be due to difference in blood sampling period (midpregnancy or preconception in the 3 studies compared with late pregnancy in our study) or lack of power due to a small number of cases of preterm birth in 2 of the studies (20, 22). A Cochrane review of 3 clinical trials concluded that folic acid supplementation starting at mid- or late pregnancy through delivery had no effect on preterm birth risk (38–41). Nonetheless, the authors of the Cochrane review commented that their findings were likely to have been influenced by bias and confounding due to methodologic limitations of the original studies (41). For instance, these trials were at high risk of selection bias due to inadequate random sequence generation and allocation concealment, which may lead to biased allocation of participants to interventions (39–41). Furthermore, incomplete reporting of study methodology in these studies did not permit judgment for other biases such as attrition bias (incomplete outcome data) and reporting bias (selective outcome reporting) (38–41).

The lack of association between maternal folate concentrations and birth weight outcomes independent of gestational age in our study agrees with most (16, 17, 21, 22, 26–28) but not all previous studies (14, 23–25). The association between higher folate status and higher birth weight or lower SGA risk in some of these earlier studies, median serum folate concentrations were usually higher (15, 19) or similar (16) to that of our population. In combination with our study, these data suggest that the achievement of high plasma folate concentrations, probably through folic acid supplement use rather than merely avoiding deficiencies, may be needed to minimize the risk of preterm birth. In contrast, no association was observed between folate concentrations and gestational age or risk of preterm birth in 3 other prospective studies (20–22). The discrepancy in results could be due to difference in blood sampling period (midpregnancy or preconception in the 3 studies compared with late pregnancy in our study) or lack of power due to a small number of cases of preterm birth in 2 of the studies (20, 22). A Cochrane review of 3 clinical trials concluded that folic acid supplementation starting at mid- or late pregnancy through delivery had no effect on preterm birth risk (38–41). Nonetheless, the authors of the Cochrane review commented that their findings were likely to have been influenced by bias and confounding due to methodologic limitations of the original studies (41). For instance, these trials were at high risk of selection bias due to inadequate random sequence generation and allocation concealment, which may lead to biased allocation of participants to interventions (39–41). Furthermore, incomplete reporting of study methodology in these studies did not permit judgment for other biases such as attrition bias (incomplete outcome data) and reporting bias (selective outcome reporting) (38–41).

The lack of association between maternal folate concentrations and birth weight outcomes independent of gestational age in our study agrees with most (16, 17, 21, 22, 26–28) but not all previous studies (14, 23–25). The association between higher folate status and higher birth weight or lower SGA risk in some studies (14, 23–25) may be due to chance or different participant characteristics such as lower folate status of the study population (14, 24). In our study, higher folate concentrations were associated with a lower risk of SGA only in participants of Chinese ethnicity, but this result should be interpreted with caution because it reflects a subgroup finding and no association was observed for birth weight as a continuous variable.

We did not observe a significant association between vitamin B-12 and birth weight, which is in line with most previous studies (14, 18, 27, 28). We found that higher maternal vitamin B-12 concentrations tended to be associated with longer gestational age and lower preterm birth risk, but the associations largely
disappeared when the analysis was restricted to spontaneous labors. Similarly, no association was observed between maternal vitamin B-12 concentrations and preterm birth or gestational age in previous studies (14, 18). There are limited data on the relation of vitamin B-12 and birth length. In contrast to our study, vitamin B-12 status was not found to be associated with birth length in a Turkish study (28) and a Norwegian study (42). The inverse association between vitamin B-12 and birth length in our study therefore requires replication in future studies.

Similar to most previous observational (20, 22, 29, 43) or intervention (44, 45) studies, we did not find any significant associations between maternal vitamin B-6 status and birth weight or gestational age–related outcomes. In one clinical trial, vitamin B-6 supplementation was associated with reduced birth weight of offspring compared with placebo (46). However, this was a small trial (total n = 33) with unclear descriptions of randomization and allocation concealment methods.

**Mechanisms.** The exact biological mechanisms underlying a putative effect of higher folate concentrations on longer gestational age and lower preterm birth risk are not clear. There is an increased requirement for folate during pregnancy due to rapid maternal and fetal cellular growth and development (19). Folate plays critical roles in nucleotide (purine and thymidine) synthesis, which can subsequently affect DNA synthesis and mitotic cell division (19, 22). In addition, due to its effect on S-adenosylmethionine (an important methyl group donor) synthesis, a low folate concentration may perturb DNA methylation (14, 19). Folate may also affect placenta implantation and vascular remodeling through its role as a superoxide scavenger in antioxidant defenses (14). Moreover, higher folate concentrations may also reduce preterm birth risk by conferring protection against intrauterine infection, one of the major causes (accounting for 25–40%) of preterm birth (47). In animal studies, folic acid supplementation during pregnancy protected against preterm delivery induced by LPS (an agent used to mimic maternal infection) exposure in mice, probably through its anti-inflammatory effects (48). For instance, folic acid was shown to inhibit LPS-stimulated inflammatory cytokines such as IL-6 in amniotic fluid (48) and in macrophages (49) in vitro and animal studies. In addition, pretreatment with folic acid in mice also attenuated LPS-induced cyclooxygenase 2–mediated production of prostaglandins, an enzyme that is essential in the initiation and progression of the labor process (48, 50). Last, an inadequate folate concentration can lead to a high homocysteine concentration, which is associated with placental vasculopathy and other vascular-related pregnancy complications, such as preeclampsia, that may, in turn, restrict fetal growth and elevate the risk of preterm birth (14, 51). Taken together, it is plausible that better maternal folate status prolongs gestational age and reduces preterm birth risk through its actions on placental and fetal growth and development (14, 18).

**Strengths and limitations of the study.** Strengths of this study included the prospective design, reducing the likelihood of reverse causation, and the Asian study population. There were differences in the prevalence of methylenetetrahydrofolate reductase (MTHFR) gene polymorphisms (involved in folate metabolism) in women of different ethnic groups, suggesting that folate metabolism and requirements may be different in Asians due to genetic variation (52, 53). Moreover, lifestyle and other dietary factors, which could potentially influence B-vitamin metabolism, are likely to be different in Asian populations. Plasma folate, vitamin B-12, and vitamin B-6 concentrations were available in this study and may reflect the “internal dose,” the amount of nutrient available after metabolism and absorption, better than self-reported dietary assessments (54, 55). Furthermore, we simultaneously investigated the associations of folate, vitamin B-12, and vitamin B-6 with birth outcomes, evaluating possible interaction and confounding among the vitamins. Our study also had several limitations. We only assessed folate, vitamin B-12, and vitamin B-6 concentrations once during late pregnancy. Although this is the period when most of fetal growth occurs, longitudinal exposure data would allow investigation of possible different relations during different periods of pregnancy between folate, vitamin B-12, and vitamin B-6 concentrations and birth outcomes. Nonetheless, misclassification due to the use of only a single measurement of B vitamins would weaken rather than strengthen the observed associations. Furthermore, we measured total B vitamins in the blood, and information on the vitamins of the B vitamins and functional biomarkers such as homocysteine was not available in our study. Moreover, our data on supplement use were self-reported with no dose and compliance information, thus offering less insight into the possible sources of the B vitamins. Because our analyses were restricted to participants with B-vitamin status assessed during 26–28 wk of gestation, participants with very early preterm birth were not included in our analysis, which may have created “left truncation bias.” However, in our cohort only 2 participants had very early preterm birth (gestational duration <28 wk) and thus we do not expect that their exclusion would have substantially affected our results. Another limitation of our study is that we cannot rule out the possibility that residual confounding affected the observed associations due to the observational nature of our study.

**Conclusions.** Higher maternal folate concentrations at approximately the start of the third trimester of pregnancy were associated with longer gestational age and tended to be associated with lower preterm birth risk in this Asian population. Because the association between higher folate status and lower preterm birth risk is often seen in folate-replete populations, pregnant women may have to consume additional folic acid supplements on top of their diet to lower preterm birth risk. In contrast, the results of our study suggested little or no benefit of higher folate concentrations for reducing the risk of SGA or of higher vitamin B-6 and vitamin B-12 concentrations for reducing the risk of preterm birth or SGA. Supplementation with folic acid during pregnancy is recommended worldwide during the periconceptional period and the first trimester to prevent neural tube defects, but the effect of supplementation beyond the first trimester is not clear (56). A recent trial found that continued supplementation with 400 μg folic acid/d in the second and third trimester can prevent the decline in maternal folate status due to an increase in folate requirements during the pregnancy period. However, the trial was not powered to investigate the supplementation effects on birth outcomes (56). A larger trial is warranted to confirm that folic acid supplementation beyond the first trimester can lower the risk of preterm birth.

**Acknowledgments**

We thank the GUSTO study group, which includes Pratihiha Agarwal, Arijit Biswas, Choon Looi Bong, Birit FP Broekman, Shirong Cai, Jerry Kok Yen Chan, Yiong Huak Chan, Cornelia Yin Ing Chee, Helen YH Chen, Yin Bun Cheung, Audrey Chia, Amutha Chinnadurai, Chai Kiat Chng, Shang Chee Chong, Mei Chen Chua, Chun Ming Ding, Eric Andrew Finkelstein, Doris Fok, Marielle Fortier, Anne Eng Neo Goh, Yam Thiam Daniel
Goh, Joshua J Gooley, Wee Meng Han, Mark Hanson, Christiani Jayakumar Henry, Joanna D Holbrook, Chin-Ying Hsu, Hazel Inskeep, Jeevesh Kapur, Ivy Yee-Man Lau, Bee Wah Lee, Ngee Lek, Sok Bee Lim, Yen-Ling Low, Iliana Magiati, Lourdes Mary Daniel, Michael Meaney, Cheryl Ngo, Krishnamoorthy Naiduvaje, Wei Wei Pang, Anqi Qiu, Boon Long Quah, Victor Samuel Rajadurai, Mary Rauff, Salome A Rebello, Jenny L Richmond, Anne Rifkin-Graboi, Lynette Pei-Chi Shek, Allan Sheppard, Borys Shuter, Leher Singh, Shu-E Soh, Walter Stunkel, Lin Lin Su, Kok Hian Tan, Oon Hoe Teoh, Hugo PS van Bever, Inez Bik Yun Wong, PC Wong, and George Seow Seong Yeo. We also thank Stephen Young, Medical Research Council Human Nutrition Research, for assistance with the vitamin B-6 assays.

L-WC, KMG, MF-FC, and RMvD designed the research; Y-SC, PDG, KMG, KK, SS-MS, FY, and YSL designed and led the GUSTO study; ALL coordinated blood samples analysis; MC, M-TT, and AMA contributed to data collection and analysis; CST provided statistical input; L-WC analyzed the data; and L-WC, MF-FC, and RMvD wrote the manuscript and had primary responsibility for final content. MF-FC and RMvD are joint last authors. All authors read and approved the final manuscript.

References