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Am J Clin Nutr. Author manuscript; available in PMC 2020 May 01.

Published in final edited form as:

Am J Clin Nutr. 2019 May 01; 109(5): 1452–1461. doi:10.1093/ajcn/nqz027.

Defining the plasma folate concentration associated with the red blood cell folate concentration threshold for optimal neural tube defects prevention: a population-based, randomized trial of folic acid supplementation

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Abstract

Background: For women of reproductive age, a population-level red blood cell (RBC) folate concentration below the threshold 906 nmol/L or 400 ng/mL indicates folate insufficiency and suboptimal neural tube defect (NTD) prevention. A corresponding population plasma/serum folate concentration threshold for optimal NTD prevention has not been established.

Objective: The aim of this study was to examine the association between plasma and RBC folate concentrations and estimated a population plasma folate insufficiency threshold (pf-IT) corresponding to the RBC folate insufficiency threshold (RBCf-IT) of 906 nmol/L.

Methods: We analyzed data on women of reproductive age (n = 1673) who participated in a population-based, randomized folic acid supplementation trial in northern China. Of these women, 565 women with anemia and/or vitamin B-12 deficiency were ineligible for folic acid intervention (nonintervention group); the other 1108 received folic acid supplementation for 6 mo (intervention group). We developed a Bayesian linear model to estimate the pf-IT corresponding to RBCf-IT by time from supplementation initiation, folic acid dosage, methyltetrahydrofolate reductase (*MTHFR*) genotype, body mass index (BMI), vitamin B-12 status, or anemia status.

Results: Using plasma and RBC folate concentrations of the intervention group, the estimated median pf-IT was 25.5 nmol/L (95% credible interval: 24.6, 26.4). The median pf-ITs were similar between the baseline and postsupplementation samples (25.7 compared with 25.2 nmol/L) but differed moderately (±3–4 nmol/L) by *MTHFR* genotype and BMI. Using the full population-based baseline sample (intervention and nonintervention), the median pf-IT was higher for women with vitamin B-12 deficiency (34.6 nmol/L) and marginal deficiency (29.8 nmol/L) compared with the sufficient group (25.6 nmol/L).

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Supplemental Tables 1–5 and Supplemental Figures 1–3 are available from the “Supplementary data” link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/ajcn/.

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Conclusions: The relation between RBC and plasma folate concentrations was modified by BMI and genotype and substantially by low plasma vitamin B-12. This suggests that the threshold of 25.5 nmol/L for optimal NTD prevention may be appropriate in populations with similar characteristics, but it should not be used in vitamin B-12 insufficient populations. This trial was registered at NCT00207558.

Keywords

neural tube defects; folic acid; plasma folate; red blood cell folate; vitamin B-12; Bayesian

Introduction

Low folate status is associated with increased risk of adverse health outcomes, including megaloblastic anemia and neural tube defect (NTD)-affected pregnancies (1, 2). Folate is essential for DNA synthesis, cell growth and differentiation, as well as the formation and maturation of red blood cells (RBCs) (3, 4). Although essential throughout life, folate is particularly critical during early stages of human development. Low folate status in pregnancy has also been associated with other adverse health outcomes, including congenital heart defects, oral clefts, fetal growth restriction, low birth weight, and preterm delivery (1, 2). Evidence from clinical trials and observational studies in multiple settings has shown that folic acid intake in the periconceptional period reduces the occurrence of NTD-affected pregnancies (5–7). To prevent folate-sensitive NTDs, women of reproductive age are encouraged to consume folate-rich foods and 400 μg of synthetic folic acid daily (8, 9). Successful folic acid fortification programs have been documented in several countries, including Canada, Costa Rica, Chile, South Africa, and the United States, resulting in a 31–50% reduction in the prevalence of NTD-affected pregnancies, with the percentage reduction dependent on folic acid dosage, intake, and baseline NTD rates (10–15).

To promote optimal NTD risk reduction at the population level, WHO recently recommended that the population RBC folate concentrations should be above a threshold of 906 nmol/L (400 ng/mL) in women of reproductive age (16). In contrast, the threshold for preventing megaloblastic anemia is much lower (i.e., 305 nmol/L) (17). The RBC folate insufficiency threshold (RBCf-IT) of 906 nmol/L per WHO guidance has been used in countries such as the United States (18) and Guatemala (19) to evaluate the impact of fortification programs for the prevention of NTDs. Many countries, however, do not have RBC folate concentration data available; instead, they have plasma or serum folate concentration data for their populations because these are more widely utilized due to lower cost and the easier process of obtaining them. Although RBC and plasma/serum folate are both associated with folic acid intake (20, 21), they result from different biologic processes and are not interchangeable. It is generally accepted that plasma/serum folate concentrations reflect very recent intake, whereas RBC folate concentrations reflect both the long-term average of intake over the life span of RBCs and folate stores in the liver (22). Although ideal, currently there are no studies that link plasma/serum folate concentrations before or at the time of conception with NTD risk (studies in the second trimester or later are problematic due to hemodilution in pregnancy). Our objectives were to examine the association between paired plasma and RBC folate concentrations and to estimate a

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population plasma folate insufficiency threshold (pf-IT) corresponding to the RBCf-IT of 906 nmol/L.

Methods

Population

We used data from a population-based, double-blind randomized trial of folic acid supplementation conducted from 2003 to 2005 in northern China. All participants provided informed consent, and the project was approved by the institutional review boards at both the US Centers for Disease Control and Prevention and Peking University Health Sciences Center, Beijing, People's Republic of China. The study design, participant eligibility, blood collection procedure, and methods for biochemical measurements and methyltetrahydrofolate reductase (*MTHFR*) genotyping have been previously described (23, 24). Briefly, 1673 women of reproductive age underwent baseline assessments of hematologic and vitamin status (Supplemental Figure 1). Of this group, 565 women with anemia and/or vitamin B-12 deficiency were ineligible for folic acid intervention. The other 1108 women were asked to take folic acid supplements for 6 mo and were randomly assigned to dosages of 25 μg 4 times a day, 100 μg 1 time a day, 100 μg 4 times a day, 400 μg 1 time a day, 4000 μg 1 time a day, or 4000 μg 1 time per week. Fasting blood samples of RBC folate, plasma folate, vitamin B-12, homocysteine, and hemoglobin were collected at baseline; months 1, 3, and 6 during the supplementation trial; and month 9, which was 3 mo postsupplementation. Plasma and RBC folate concentrations were measured by the Molloy method with microbiological assays (chloramphenicol-resistant strain and folic acid calibrator) (25). For our purpose, we used data at baseline and 6 mo after supplementation (month 6), the time points when plasma and RBC folate concentrations were at or approaching a steady state (24). Our primary analysis used the intervention group data at baseline (n = 1108) and month 6 (n=977).Our secondary analysis used data from the intervention (n = 1108) and nonintervention (n = 565) groups at baseline. All analyses combined the subgroups of 25 μg 4 times a day and 100 μg 1 time a day into the 100 μg/d group and the subgroups of 100 μg 4 times a day and 400 μg 1 time a day into the 400 μg/d group because previous studies of this trial showed that the subgroups with different schedules but same overall doses have similar dose–response relations (24).

The primary outcomes for the original trial were to assess the changes in plasma folate, RBC folate, and homocysteine due to different intakes of folic acid with secondary analysis of the impact of *MTHFR* genotypes, and these have been reported previously (23, 24). The current analysis takes advantage of the well-characterized population-based sample of paired RBC folate and plasma folate concentration data to assess the association between paired plasma and RBC folate concentrations and to estimate a population pf-IT corresponding to the RBCf-IT of 906 nmol/L between different groups. The baseline sample was population-based and included healthy, anemic, and B-12–deficient individuals. Because anemic individuals were excluded from receiving the folic acid intervention and were referred to medical care, the 6-mo postintervention sample included only nonanemic or B-12–deficient individuals. Groups were stratified by age, body mass index (BMI, in kg/m^2), and *MTHFR* genotype.

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Statistical analysis

Descriptive analysis.—We described the data used for modeling by calculating the baseline characteristics for age group (<25, 25 to <35, and ≥35 years), *MTHFR* 677 genotypes (*CC*, *CT*, and *TT*), BMI (<25, 25 to <30, and ≥30), plasma folate, RBC folate, plasma hemoglobin, and plasma vitamin B-12. We used the natural logarithm of plasma and RBC folate concentrations in all analyses to normalize the distribution of folate concentrations, and back-transformed to calculate the geometric mean (GM). We calculated and compared these characteristics for the baseline data by intervention and nonintervention groups using chi-square tests or nonparametric Kruskal–Wallis tests. In addition, we used the intervention group data at baseline and month 6 and estimated the GM and the 2.5th and 97.5th percentiles of plasma and RBC folate concentrations overall, by folic acid dosage groups, *MTHFR* 677 genotypes (*CC*, *CT*, and *TT*), and BMI subgroups. We used Pearson's correlation coefficient (*r*) to assess correlations between plasma and RBC folate concentrations. Similarly, we used baseline data only and calculated the GM and 2.5th and 97.5th percentiles as well as *r* of plasma and RBC folate concentrations by group (intervention compared with nonintervention), vitamin B-12 status (deficient <148 pmol/L, marginal deficiency 148–221 pmol/L, and sufficient >221 nmol/L) (26), and anemia status (hemoglobin <120 g/L compared with ≥120 g/L).

Bayesian linear model.—We modeled RBC folate concentration as a function of plasma folate concentration using a linear model within the Bayesian framework. We chose the Bayesian framework because of the flexibility, direct inferences through the posterior distribution for quantities of interest, and usability of all available information. Flexibility allows us to model RBC folate concentration as a function of plasma folate concentration and obtain the posterior for the plasma distribution that corresponds to the RBC folate concentration threshold. We are able to use all available information for RBC folate concentration, plasma folate concentration, and covariates of interest in model development and treat missing data as additional parameters to be estimated in the modeling process. We investigated several models to estimate the plasma folate concentration corresponding to RBC folate threshold. We set 906 nmol/L as the RBC folate concentration threshold for optimal NTD prevention based on the WHO recommendation and previous studies (15, 27).

We used data from baseline and month 6 for the intervention group models and baseline data for the combined nonintervention and intervention models. We estimated intervention group models overall and by *1*) baseline and month 6, *2*) supplementation group, *3*) genotype, and *4*) BMI. In addition, we estimated an overall pf-IT using a multivariable model that controlled for age, *MTHFR* genotype, and BMI. We performed sensitivity analyses to evaluate the impact of missing information on genotypes and BMI in the multivariable models, using Bayesian methods to impute missing values.

To estimate the pf-IT for the combined nonintervention and intervention groups, we developed Bayesian linear models (BLMs) overall and by *1*) intervention and nonintervention group, *2*) vitamin B-12 status, and *3*) anemia status. We also developed an adjusted BLM including age, *MTHFR* genotypes, BMI, vitamin B-12 status, and anemia status as covariates.

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We used the following standard procedure for all BLMs. Our estimation procedure consisted of computing the joint posterior probability distribution of all parameters using a Markov chain Monte Carlo computational approach. We used a burn-in of 10,000 samples, drew 500,000 samples post burn-in, and thinned by retaining every 50th sample, which resulted in a sample of 10,000 for our parameter estimates. We used mildly informative priors of $N(0, 10)$ for all parameters. In addition, for our multivariable models, we estimated the parameters by removing missing data and using a full Bayesian approach by treating the missing data as additional parameters to be estimated jointly with our model parameters of interest. We assumed all missing data were missing at random. In addition, for all models that used baseline and 6-mo data, we treated the individual as a random effect to account for the correlation within a person. For each model, we summarized the 10,000 posterior samples of the estimated pf-IT that corresponds to the RBCf-IT of 906 nmol/L. In addition, when appropriate, we calculated and summarized the difference of the pf-IT estimates by subgroup. We report the medians and 95% credible intervals (CIs) of the posterior distributions of the estimated pf-ITs and differences in pf-ITs. A credible interval is Bayesian statistics and defines a plausible range of values, within which an unobserved parameter value falls with a particular probability. We estimated all models using SAS MCMC (version 9.4; SAS Institute).

Results

Descriptive analysis

Baseline characteristics of women in the intervention and nonintervention groups are presented in Table 1. Women in the intervention group were more likely to be in the age group <25 y ($P = 0.03$) and had higher plasma folate, RBC folate, hemoglobin, and vitamin B-12 concentrations than those in the nonintervention group ($P < 0.001$). *MTHFR 677* genotype *TT* was more prevalent in the nonintervention group (43.1% compared with 35.1%), whereas *CC* and *CT* were more prevalent in the intervention group ($P = 0.04$).

The GM of plasma and RBC folate concentrations did not differ substantially by dosage groups at baseline but differed at month 6 (Supplemental Table 1). The correlation coefficient (r) for plasma and RBC folate concentrations increased in the intervention group from 0.46 at baseline to 0.67 at month 6 (Figure 1A, Supplemental Table 1). The increase in r after supplementation was observed in all dosage groups except the 4000 μg/wk group (Figure 1C, D, E and F, Supplemental Table 1), whereas the r in the 100 μg/d group remained the lowest compared to other dosage groups.

Among the intervention group, women with *MTHFR* 677 genotype *TT* had lower plasma and RBC folate concentrations at baseline and month 6 compared with the *CC* and *CT* groups (Supplemental Table 1). The increase in r between plasma and RBC folate concentrations after supplementation was observed in all genotype groups (Figure 2A, B, and C, Supplemental Table 1). At baseline or month 6, the r did not differ substantially between genotype groups. For BMI subgroups, at baseline and month 6, the obese women (BMI ≥30) had higher RBC folate and lower plasma folate concentrations than nonoverweight/nonobese women (BMI <25) (Figure 2D, E, and F, Supplemental Table 1).

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The *r* was lower in the obese women than in the nonoverweight/nonobese women and remained low at month 6 (Figure 2D, E, and F, Supplemental Table 1).

We found the *r* between plasma and RBC folate concentrations to be lower in the nonintervention group (0.41) compared to the intervention group (0.46) (Figure 1A, B, Supplemental Table 2) and also lower in the anemic group (0.35) compared with the nonanemic group (0.48).

Plasma threshold analysis

When using data from the intervention group, the estimated median pf-IT was 25.5 nmol/L (95% CI: 24.6, 26.4) (Table 2, Figure 3). Results of the subgroup analyses for the intervention group are summarized in Table 2 and Supplemental Figure 2. The estimated median pf-IT at month 6 (25.2 nmol/L) was similar to baseline (25.7 nmol/L). The estimated median pf-IT for the 100 μg/d group (38.4 nmol/L) was higher than that of the other three dosage groups, whereas those in the other three dosage groups were closer to one another (400 μg/d: 24.4 nmol/L; 4000 μg/d, 22.0 nmol/L; and 4000 μg/wk, 25.6 nmol/L). The higher estimated median pf-IT in the 100 μg/d group was observed both at baseline and at month 6 (Supplemental Table 3). For the 4000 μg/d group, a lower estimated median pf-IT was observed at month 6 (13.2 nmol/L) (Supplemental Table 3); however, when removing a few influential observations, the 6-mo estimated median pf-IT (19.5 nmol/L) was not substantially different from the baseline estimated median pf-IT(22.8nmol/L).

The estimated median pf-IT was higher for the *TT* group (26.3 nmol/L) than the *CC* and *CT* groups (*CC*: 23.2 nmol/L; *CT*: 25.1 nmol/L) (Table 2, Supplemental Figure 2). The estimated median pf-ITs were not different by genotype groups at month 6 alone (Supplemental Table 3). Among BMI subgroups, the estimated median pf-IT was lowest for the obese group (21.9 nmol/L), followed by the overweight group (23.3 nmol/L) and the nonoverweight/nonobese group (26.7 nmol/L) (Table 2, Supplemental Figure 2). When using only baseline or 6-mo data, the estimated median pf-IT for the obese group was ~5–6 nmol/L lower than that for the non-overweight/non-obese group (Supplemental Table 3). Differently, the estimated median pf-IT for the overweight group was higher at baseline but lower at month 6 compared to that for the nonoverweight/nonobese group. When comparing the unadjusted and adjusted models, we did not observe meaningful differences in all the threshold values (Supplemental Table 4).

Using data from all women at baseline, the estimated median pf-IT was 27.2 nmol/L (95% CI: 24.6, 30.9) (Table 3). The estimated median pf-IT for the intervention group was lower than that for the nonintervention group (25.7 nmol/L compared with 32.5 nmol/L), with a difference of 6.7 nmol/L (95% CI: −0.6, 18.2) (Table 3, Supplemental Figure 3). The estimated median pf-IT was higher for vitamin B-12–deficient and marginal deficiency women (34.6 and 29.8 nmol/L; respectively) compared with vitamin B-12–sufficient women (25.6 nmol/L) (Table 3). The difference between vitamin B-12–deficient and – sufficient groups was 8.9 nmol/L (95% CI: 0.4, 22.9) (Table 3, Supplemental Figure 3). The estimated median pf-IT was also higher for the anemic group (28.5nmol/L) than for the nonanemic group (26.4 nmol/L), but the difference was small (2.1 nmol/L; 95% CI: −6.3, 23.9) (Table

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3). When comparing the unadjusted and adjusted models, we did not observe meaningful differences in the threshold values (Supplemental Table 5).

Discussion

We observed a moderate positive correlation of plasma and RBC folate concentrations in nonpregnant women of reproductive age by using data from a population-based, randomized trial of folic acid supplementation in northern China. In this population, 21% were vitamin B-12 deficient and 15% were anemic. We also generated population pf-ITs corresponding to RBCf-IT for optimal NTD prevention. Among nonanemic, non-vitamin B-12–deficient women, the estimated median pf-IT corresponding to RBCf-IT of 906 nmol/L was 25.5 nmol/L. The estimated median pf-IT did not change after supplementation. However, the estimated median pf-ITs in vitamin B-12–deficient or marginal deficiency women were higher than with that in vitamin B-12–sufficient women. Our results suggest that the threshold of 25.5 nmol/L may be inappropriate for populations with high prevalence of vitamin B-12 deficiency or marginal deficiency.

Association between plasma and RBC folate concentrations

We found a moderate linear relation between plasma and RBC folate concentrations at baseline ($r = 0.46$ in the intervention group and $r = 0.41$ in the nonintervention group). The correlations were not substantially different by *MTHFR* genotype or vitamin B-12 status, but they were lower in obese and anemic groups. We also found the correlation increased after folic acid intervention ($r = 0.67$), implying that the individual variation of blood folate concentrations in the population decreased after folic acid intervention (e.g., food fortification). Note that the correlations are lower before intervention compared to after the folic acid intervention due to higher variance in the same subjects at baseline and not due to a difference in the slope orconclusionsconclusions intercept of the association such that there is more scatter around the same line (Figure 1).Another way to demonstrate that the slope of the association is similar between the pre- and post-intervention data is by comparing the median plasma folate concentration that correlates with the 906 nmol/L RBC folate concentration, which is almost identical [baseline, 25.7 nmol/L (95% CI: 23.0, 29.7); 6-mo folic acid, 25.2 nmol/L (95% CI: 23.9, 26.5); Table 2], just with wider credible intervals at baseline that reflect the higher variance in the baseline data. It is not surprising to have less variance in crude r^2 correlation at low concentrations when a small absolute change produces a larger percentage change and that variance decreases when the population is homogenized by 6 mo of folic acid supplementation and much higher concentrations, where the same absolute difference is a much smaller percentage change. Previous Irish studies with blood folate samples collected among pregnant women who attended the antenatal clinic showed the correlation coefficient of RBC and plasma folate to be 0.61–0.71 (28), which is consistent with the postsupplementation data. However, it is difficult to compare the reported correlation coefficients with our estimates due to difference in pregnancy status and lack of information on folic acid supplementation, baseline folate concentrations, and genetic or biologic characteristics.

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Population plasma folate concentration insufficiency threshold

In our study, for nonanemic, non-vitamin B-12–deficient women, the estimated median pf-IT corresponding to RBCf-IT of 906 nmol/L for optimal NTD prevention (< 8 NTD per 10,000 births) was 25.5 nmol/L. A previous nested case–control study of women in the second trimester found that the subpopulation with plasma folate concentration >15.9 nmol/L was associated with a lower risk of NTDs (< 9 per 10,000 births) (29). Due to hemodilution of pregnancy, which leads to lower plasma/serum folate concentration in normal pregnancy (30), it may be inappropriate to compare this plasma concentration with the one derived from nonpregnant women in our study. The difference in plasma folate concentration thresholds might also be influenced by the limited number of participants in the previous study and by biological factors such as race/ethnicity or environmental factors such as different food sources between the two different populations.

Effect of folic acid supplementation.—This analysis finds that folate metabolism and possibly transportation into the cell change the plasma folate equivalent of the 906 nmol/L threshold (B-12, *MTHFR*, and BMI), whereas 6 mo of folic acid supplement in and of itself does not change the pf-IT (such that those whose RBC folate concentration is 906 nmol/L have similar plasma folate concentrations at baseline as those who have consumed folic acid supplements and have RBC folate concentrations of 906 nmol/L). When stratifying by folic acid dosage groups, the pf-IT for the 100 μg/d group was higher than those of the other three dose groups. However, the additional analysis on the 100 μg/d group revealed that the pf-ITs did not differ by baseline and 6 mo of supplementation, suggesting that factors other than supplementation affect the relation between plasma and RBC folate concentrations in this subgroup. Given that the women in the original trial were randomly allocated to each dose group and there was no difference in baseline characteristics between dose groups (24), it might be an unmeasured confounder that results in the difference in plasma threshold estimates for the 100 μg/d group. Because our model was intended to reflect true population variance of plasma and RBC folate concentrations, we retained the 100 μg/d group data in the final overall model.

Effect of MTHFR genotype.—The *MTHFR* genotype variation (667 *C*> *T* transition) is a determinant of folate status in women of reproductive age (31). Studies have shown that the genetic variant *TT* was associated with lower plasma/serum and RBC folate concentrations (23) and increased risk of NTDs (32). Previous modeling showed that *MTHFR* genotype variation does not modify the relation of RBC folate concentration threshold and NTD risk in the population (33), although additional folate intake would be needed for a population with the *MTHFR T* alleles to achieve a specific RBC folate concentration. Genotype *TT*, which links to lower concentrations of RBC and plasma folate concentrations, had a higher pf-IT than *CC* and *CT* groups (trend *TT*> *CT*> *CC*). When further stratifying data of genotype subgroups by different time points, the trend of the pf-ITs among genotype subgroups was found at baseline but not after 6 mo of folic acid supplementation. Our findings suggest that the differences between RBC folate and plasma folate concentrations due to genotype are moderated after folic acid supplementation.

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Effect of BMI.—Our study revealed an inverse trend between BMI and pf-IT: higher BMI is associated with a lower plasma threshold. This association is consistent with results from previous studies among US women showing that higher BMI was associated with lower serum folate but higher RBC folate concentrations (34, 35). A possible explanation for this observation could be that the altered distribution of folate as body size increases leads to a change in the body pool of freely available plasma/serum folate and folate in the cell (36, 37). Obesity is also thought to affect folate metabolism (e.g., higher cellular uptake of the developing erythrocytes) (38), but the effect has not yet been determined. In addition, a US study (35) found that the use of folic acid supplements modified the inverse association between serum folate concentration and BMI: lower serum folate concentrations were associated with higher BMI among supplement nonusers but not among users. However, in our study, the inverse association between BMI and pf-IT remained after 6 mo of supplementation. Further studies are needed to delineate the associations between BMI, folic acid intake, and the two different folate status indicators (plasma and RBC folate).

Effect of vitamin B-12.—We found that lower vitamin B-12 status is associated with much higher pf-IT. Vitamin B-12 is directly involved in folate metabolism and required for folate retention in developing RBC, thus leading to functional folate deficiency and impaired erythropoiesis (1). Vitamin B-12 serves as a coenzyme in methionine synthase reaction [i.e., the conversion of 5-methyl-tetrahydrofolate (5-methyl-THF) to THF]. Vitamin B12 deficiency can lead to reduced methionine synthase reaction, causing 5-methyl-THF to accumulate in the “methyl trap” (39). The effect of vitamin B-12 status on the relation between RBC folate and plasma folate highlights the differences between the two folate biomarkers and suggests caution in using a pf-IT in the context of lower vitamin B-12 intakes. Vitamin B-12 deficiency should be corrected because there are clinical consequences beyond those associated with folate deficiency (e.g., neurological damage). In populations with a high prevalence of vitamin B-12 deficiency and insufficiency, correction of both vitamin B-12 deficiency and low folate status could be considered and RBC folate, plasma/serum folate, and vitamin B-12 monitored.

Strengths and limitations

This study has several strengths. This was a large population-based study with multiple biomarkers available for each subject. There was high compliance with folic acid supplementation, allowing us to show the correlations and pf-IT estimates between different subgroups receiving different dosages. Furthermore, *MTHFR* genotype information was available, allowing us to examine if genotype affects plasma folate concentration thresholds.

One study limitation is that we used folic acid concentration measured after 6 mo of supplementation in the analysis. This assumes that blood folate, particularly RBC folate, has reached a steady state. Studies have shown that it takes ~6–12 mo for RBC to reach a steady state, whereas it takes less time for plasma/serum to do so (40–44). The timing to reach a steady state also depends on the dosage of folic acid supplementation (43–45). RBC folate concentrations in women who take high doses (e.g., >1 mg/d) may plateau earlier than those in lower dose groups. Our previous study, which used the same China data, showed that the plasma concentration plateaued at 3 mo but RBC concentration had not plateaued by 6 mo

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(24). Because in the trial folic acid supplementation was withdrawn after 6 mo, we could not determine whether the RBC folate concentration increased after this period or whether it reached a plateau between 3 and 6 mo. However, an assumption of steady state may not be so important because we found no substantial difference in the pf-ITs between baseline and after 6 mo of supplementation.

This study is also limited in that this correlation is limited to the microbiological assay and is not generalizable to other assays. In addition, the folate measurements in this study were taken under fasting conditions, which limited our ability to assess the effect of fasting. Last, this study only included women of reproductive age in northern China, with a background of high (35.1%) prevalence of *TT* genotype (23). More studies using data from other populations in different settings will help determine if our findings of the association between plasma and RBC folate concentrations and the pf-ITs can be applied to other populations.

Implications

RBC and plasma folate concentrations, although highly correlated, are not identical biomarkers of folate status, and their relation is affected by BMI, *MTHFR* genotype, and especially vitamin B-12 status. Previous studies in various populations have consistently shown that as RBC folate concentration increases, NTD risk decreases; for example, a 4-fold increase in RBC concentration, from 300 nmol/L to 1200 nmol/L, was associated with up to a 10-fold decreased risk of NTDs (33). WHO has established that the RBC folate insufficiency threshold for optimal NTD prevention is 906 nmol/L, and this threshold (adjusted for folate assay method) helped Guatemala evaluate its fortification program. Fortification was found to be relatively effective at increasing blood folates in urban areas (~19% of the population under the RBCf-IT) but was not effective in reaching rural and indigenous populations (~ 81% under the RBCf-IT in Norte region) (19). The pf-IT could be used by programs to determine what percentage of their population is likely to be folate insufficient for optimal NTD prevention. High rates of plasma folate insufficiency would indicate a need to implement an appropriate folic acid fortification or supplementation program. Although RBC folate concentration distributions are still needed to predict NTD prevalence, use of a population plasma folate insufficiency threshold could help programs with existing plasma folate data plan and initiate fortification programs.

Conclusions

We estimated that the population-based median pf-IT corresponding to RBCf-IT of 906 nmol/L is ~25.5 nmol/L. We observed no substantial change in estimated median pf-IT after folic acid supplementation, and only moderate changes by *MTHFR* genotype and BMI. The estimated median pf-IT among vitamin B-12–deficient women was much higher than that of vitamin B-12–sufficient women, suggesting the threshold may be inappropriate for populations with vitamin B-12 insufficiency or other characteristics different from those of our study population.

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Supplementary Material

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Acknowledgments

We thank Christine Pfeiffer for the interpretation of folate cutoffs. We also thank the staff members and participating women of the original trial.

The authors' responsibilities were as follows—M-YC: analyzed and interpretedthedataanddraftedthemanuscript;CER:designedandperformed the statistical analysis and drafted the statistical methods; YPQ, JLW, LFY, RJB, LH, and MJC: interpreted the data and critically revised the manuscript; KSC: conceived and designed the study, interpreted the data, critically revised the manuscript, and had primary responsibility for final content; and all authors: read and approved the final manuscript. None of the authors reported a conflict of interest related to this study.

The authors reported no funding received for this study.

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Abbreviations used:

BLM	Bayesian linear model
CI	Bayesian credible interval
GM	geometric mean
MTHFR	methyltetrahydrofolate reductase
NTD	neural tube defect
Pf-IT	plasma folate insufficiency threshold
RBC	red blood cell
RBCf-IT	red blood cell folate insufficiency threshold
THF	tetrahydrofolate

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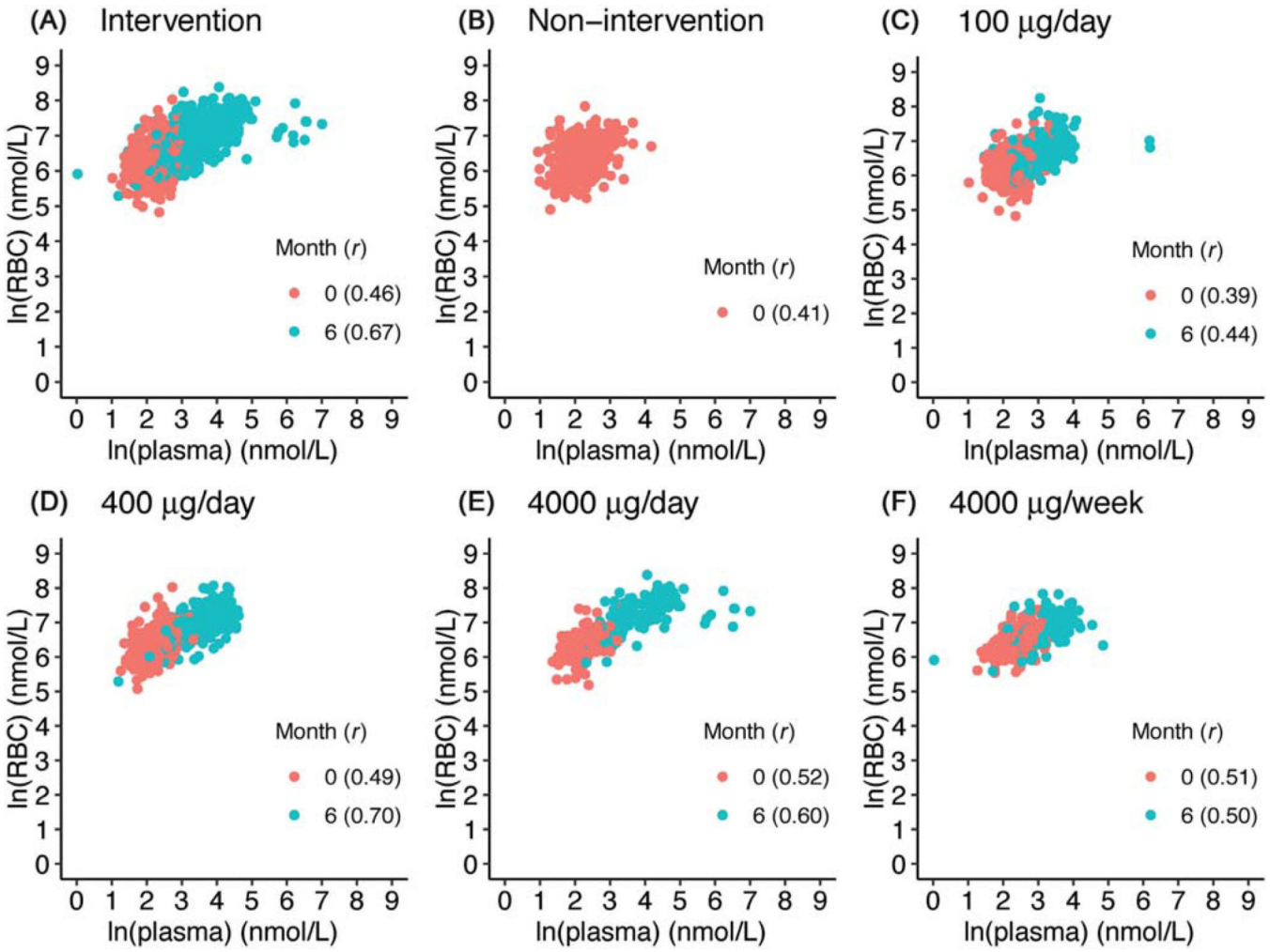

FIGURE 1.

Correlation plots between plasma and RBC concentrations at baseline (month 0) and after 6 mo of supplementation. Plots show the natural log-transformed plasma and RBC folate concentrations for (A) the intervention group (baseline $n = 1108$, 6 mo $n = 977$), (B) the non-intervention group ($n = 565$), and different folic acid dosage groups (C: baseline $n = 368$, 6 mo $n = 330$; D: baseline $n = 371$, 6 mo $n = 332$; E: baseline $n = 183$, 6 mo $n = 162$; F: baseline $n = 186$, 6 mo $n = 153$). The values of correlation coefficients (r) are shown in each panel. RBC, red blood cell

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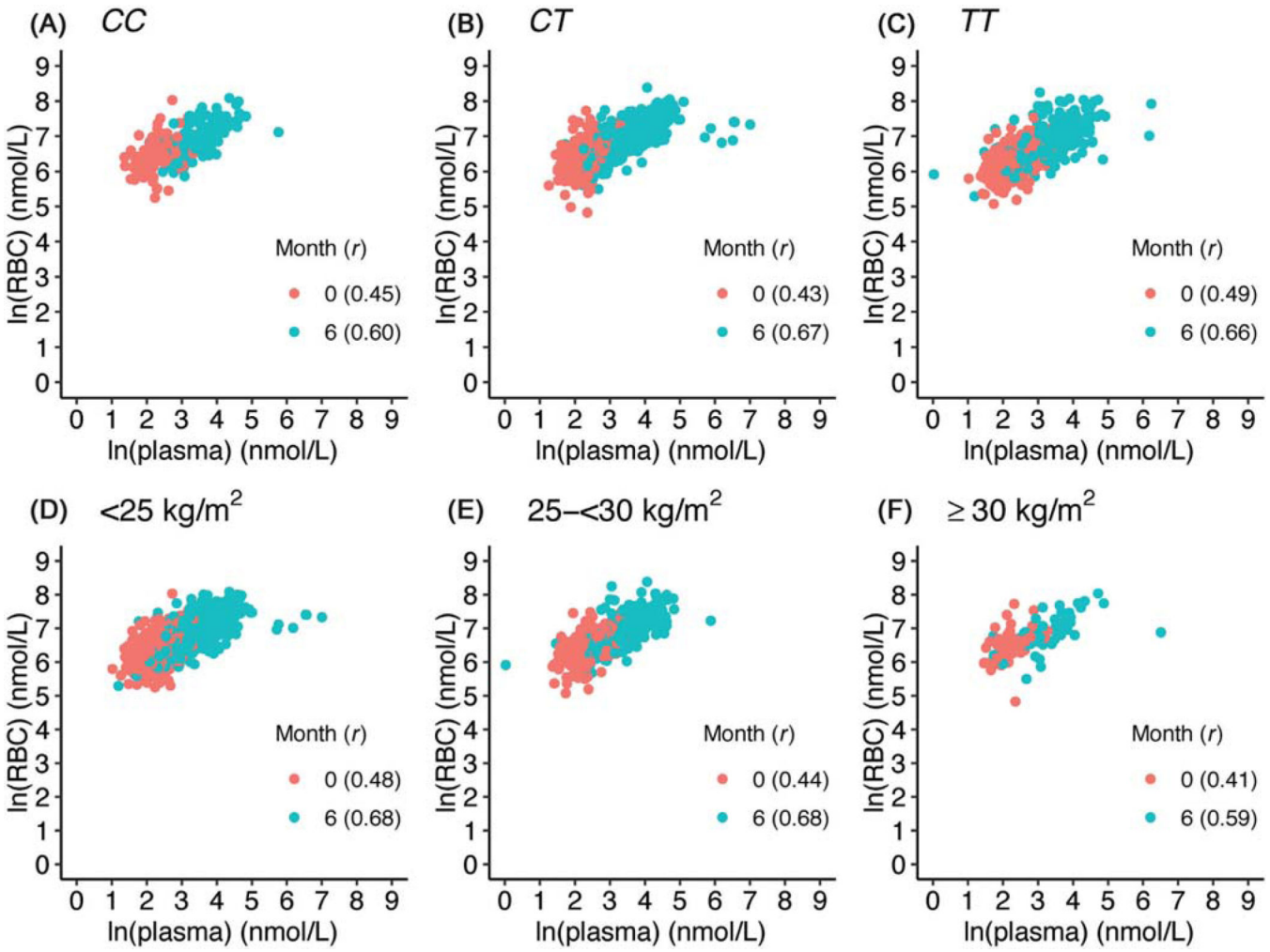

FIGURE 2.

Correlation plots between plasma and RBC concentrations at baseline (month 0) and after 6 mo of supplementation. Plots show the natural log-transformed plasma and RBC folate concentrations for *MTHFR* 677 genotype groups (A: baseline $n = 163$, 6 mo $n = 159$; B: baseline $n = 448$, 6 mo = 434; C: baseline $n = 330$, 6 mo $n = 361$) and BMI groups (D: baseline $n = 670$, 6 mo $n = 584$; E: baseline $n = 287$, 6 mo $n = 260$; F: baseline $n = 69$, 6 mo $n = 60$). Data included only women who received the folic acid intervention. The estimated correlation coefficients (r) are shown in each panel. RBC, red blood cell.

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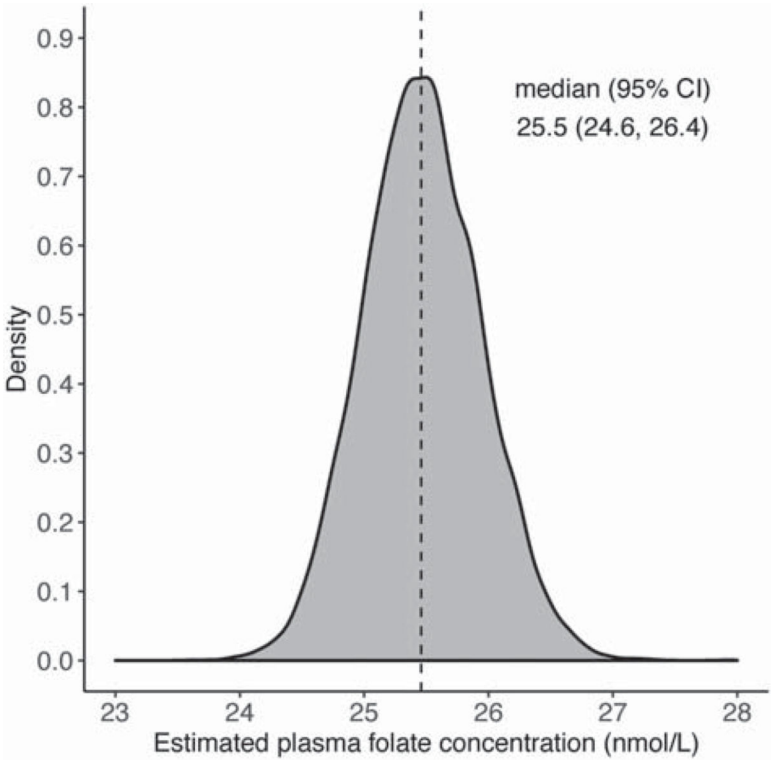

FIGURE 3.

Estimated plasma folate concentration corresponding to the RBC folate concentration of 906 nmol/L using data from the intervention group (includes baseline and 6 mo). The dashed line represents the median, and the values of the estimated median and 95% credible interval are shown. CI, Bayesian credible interval; RBC, red blood cell.

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TABLE 1

Baseline characteristics of the women of reproductive age in a randomized trial of folic acid supplementation stratified by intervention status, northern China (n = 1673)[1]

Characteristic	Intervention (n = 1108)	Non-intervention[2] (n = 565)	P value[3]
Age group, y	—	—	0.03
<25	101 (9.1%)	31 (5.5%)	
25 to <35	812 (73.3%)	428 (75.7%)	
≥35	195 (17.6%)	106 (18.8%)	
MTHFR 677 genotype[4]	—	—	0.04
CC	163 (17.3%)	33 (13.0%)	
CT	448 (47.6%)	111 (43.9%)	
TT	330 (35.1%)	109 (43.1%)	
BMI,[4] kg/m^2	—	—	0.71
< 25	670 (65.3%)	350 (66.0%)	
25–<30	287 (28.0%)	140 (26.4%)	
≥30	69 (6.7%)	40 (7.5%)	
Plasma folate, nmol/L[5]	9.5 (4.7, 24.1)	8.5 (4.2, 22.8)	<0.001
RBC folate, nmol/L[5]	611 (272, 1271)	533 (253, 1244)	<0.001
Hemoglobin, g/L[4]	134 (121, 155)	122 (87, 151)	<0.001
Plasma vitamin B-12, pmol/L	250 (154, 527)	137 (54.6, 442.9)	<0.001

[1] Values are n (%) or medians (2.5th, 97.5th percentiles). *MTHFR*, methyltetrahydrofolate reductase; RBC, red blood cell.

[2] Ineligible for folic acid intervention due to anemia (hemoglobin <120 g/L) or vitamin B-12 deficiency (<148 pmol/L).

[3] Chi-square statistics were used to test the proportions between intervention and non-intervention groups. Nonparametric Kruskal–Wallis tests were used to test the difference in medians between intervention and non-intervention groups.

[4] Genotype data were missing in 167 women in the intervention group and 312 women in the non-intervention group. BMI data were missing in 82 women in the intervention group and 35 women in the non-intervention group. Hemoglobin data were missing in one woman in the non-intervention group.

[5] Measured by the Molloy method (microbiologic assay with chloramphenicol-resistant strain and folic acid calibrator).

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TABLE 2

Estimated plasma folate concentrations corresponding to the red blood cell folate concentration of 906 nmol/L using data from the intervention group, stratified by subgroups[1]

	Median plasma folate concentration in nmol/L (95% CI)	Median difference (95% CI)
Overall	25.5 (24.6, 26.4)	—
Time		
Baseline	25.7 (23.0, 29.7)	Reference
Month 6	25.2 (23.9, 26.5)	−0.5 (−4.7, 2.6)
Folic acid dosage		
100 μg/d	38.4 (33.3, 45.8)	13.9 (8.7, 21.5)
400 μg/d	24.4 (23.1, 25.9)	Reference
4000 μg/d	22.0 (20.5, 23.6)	−2.4 (−4.4, −0.4)
4000 μg/wk	25.6 (23.0, 28.8)	1.0 (−1.8, 4.5)
MTHFR 677 genotype		
CC	23.2 (21.2, 25.5)	Reference
CT	25.1 (23.8, 26.6)	1.9 (−0.7, 4.4)
TT	26.3 (24.6, 28.2)	3.1 (0.2, 5.9)
BMI, kg/m^2		
<25	26.7 (25.5, 28.1)	Reference
25 to <30	23.3 (21.9, 25.0)	−3.3 (−5.3, −1.4)
≥30	21.9 (19.0, 26.1)	−4.5 (−8.0, −0.5)

[1] Includes data from the intervention group at baseline and 6 mo (n = 2085). CI, Bayesian credible interval; *MTHFR*, methylenetetrahydrofolate reductase.

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TABLE 3

Estimated plasma folate concentrations corresponding to the red blood cell folate concentration of 906 nmol/L using data from all women at baseline, stratified by subgroups[1]

	Median plasma folate concentration in nmol/L (95% CI)	Median difference (95% CI)
Overall	27.2 (24.6, 30.9)	—
Subgroup		
Intervention	25.7 (23.0, 29.7)	Reference
Non-intervention	32.5 (26.4, 43.6)	6.7 (−0.6, 18.2)
Vitamin B-12, pmol/L		
<148	34.6 (27.6, 48.2)	8.9 (0.4, 22.9)
148–221	29.8 (24.1, 40.8)	4.2 (−3.4, 15.5)
>221	25.6 (22.3, 30.7)	Reference
Hemoglobin, g/L		
120	28.5 (20.9, 50.1)	2.1 (−6.3, 23.9)
≥120	26.4 (23.8, 29.8)	Reference

[1] Includes data from the intervention and the non-intervention groups at baseline (n = 1673). CI, Bayesian credible interval.