Do high blood folate concentrations exacerbate metabolic abnormalities in people with low vitamin B-12 status?[1–3]

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ABSTRACT

Background: In elderly individuals with low serum vitamin B-12, those who have high serum folate have been reported to have greater abnormalities in the following biomarkers for vitamin B-12 deficiency: low hemoglobin and elevated total homocysteine (tHcy) and methylmalonic acid (MMA). This suggests that folate exacerbates vitamin B-12–related metabolic abnormalities.

Objective: We determined whether high serum folate in individuals with low serum vitamin B-12 increases the deleterious effects of low vitamin B-12 on biomarkers of vitamin B-12 cellular function.

Design: In this cross-sectional study, 2507 university students provided data on medical history and exposure to folic acid and vitamin B-12 supplements. Blood was collected to measure serum and red blood cell folate (RCF), hemoglobin, plasma tHcy, and MMA, holotranscobalamin, and ferritin in serum.

Results: In subjects with low vitamin B-12 concentrations (<148 pmol/L), those who had high folate concentrations (>30 nmol/L; group 1) did not show greater abnormalities in vitamin B-12 cellular function in any area than did those with lower folate concentrations (≤30 nmol/L; group 2). Group 1 had significantly higher holotranscobalamin and RCF, significantly lower tHcy, and nonsignificantly lower ($P = 0.057$) MMA concentrations than did group 2. The groups did not differ significantly in hemoglobin or ferritin. Compared with group 2, group 1 had significantly higher mean intakes of folic acid and vitamin B-12 from supplements and fortified food.

Conclusions: In this young adult population, high folate concentrations did not exacerbate the biochemical abnormalities related to vitamin B-12 deficiency. These results provide reassurance that folic acid in fortified foods and supplements does not interfere with vitamin B-12 metabolism at the cellular level in a healthy population. *Am J Clin Nutr* 2011;94:495–500.

INTRODUCTION

Fortification of enriched cereal grains with folic acid to prevent neural tube defects has proved controversial. Although effective, fortification has caused a dramatic increase in blood folate concentrations (1). High folate concentrations have raised concerns ranging from masking pernicious anemia (2) to promoting the growth of malignant or premalignant cells (3). Moreover, several studies have shown that people who have low serum vitamin B-12 concentrations are more likely to have anemia, elevated plasma total homocysteine (tHcy), and elevated serum methylmalonic acid (MMA) if they also have high serum folate concentrations (4–6). Although one study showed no association, the authors noted that the number of subjects in the low–vitamin B-12 and high-folate group was small (7).

Selhub et al (8) observed trends toward higher tHcy and higher MMA in subjects with low vitamin B-12 status as folate concentrations increased and speculated that there was a negative metabolic interaction between folate and the 2 vitamin B-12–dependent enzymes methionine synthase and methylmalonyl coenzyme A mutase when the balance of folate and vitamin B-12 within the cell were at opposite extremes. Such a metabolic interaction has not been previously considered and would have important clinical, nutritional, and public health implications.

However, it has been noted (9, 10) that subjects in studies that showed that high folate concentrations exacerbated vitamin B-12 deficiency may have developed the combination of low vitamin B-12 and high folate because of coexisting, but unrecognized, medical conditions that affected vitamin B-12 absorption, particularly pernicious anemia. Therefore, to avoid these potential confounding effects, it is necessary to study a population in which comorbidities are not present to determine whether or not high folate concentrations have a deleterious effect on vitamin B-12 metabolism when vitamin B-12 status is poor.

The current study was undertaken to examine the effect of high folate concentrations on markers for vitamin B-12 deficiency in a population of students who were screened for potentially

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Received February 22, 2011. Accepted for publication April 29, 2011.

First published online June 8, 2011; doi: 10.3945/ajcn.111.014621.

confounding factors such as chronic diseases and for whom detailed information on folic acid exposure via supplements and all fortified foods was available.

SUBJECTS AND METHODS

Recruitment

Students attending the University of Dublin, Trinity College, were recruited between February 2003 and 2004. As part of a larger genetic-association study, a total of 3569 students initially applied, and of these students, 2524 individuals who had Irish grandparents and no major medical problems at the time of the study were invited to continue with the study and consented to give a venous blood sample. Of these subjects, 15 students did not return a questionnaire and were excluded from the data analysis. Complete blood data were not available for 2 students, which left 2507 individuals for this investigation. All samples were made anonymous before analysis. Ethical approval was obtained from the Dublin Federated Hospitals Research Ethics Committee, which is affiliated with the University of Dublin, Trinity College, and the study was reviewed by the Office of Human Subjects Research at the National Institutes of Health. Written informed consent was obtained from participants when they enrolled.

Health and lifestyle questionnaire

Information on age, sex, height, weight, medical conditions, smoking, dietary habits, and consumption of alcohol, fortified foods, and supplements was collected.

Supplement and fortified-food intake

Students were asked to report their intakes within the past week and over an average month from a list of commonly used vitamin supplements and fortified foods. Sporadic users were encouraged to record their individual style of intake. A member of the research team used the frequency and quantity data reported to estimate intakes over the week and month time periods. The consumption of all other supplements not listed on the questionnaire was also recorded in the same manner. A total of 306 different types of supplements were reported to be consumed. Active-nutrient information was obtained for each supplement and converted to micrograms of nutrient per day. Exposure to vitamins in fortified food was calculated by providing subjects with a list of fortified foods and definitions of serving sizes. Subjects were asked to report how many serving sizes of each food they had consumed in the past week and in an average month. The quantity of vitamins in each portion was calculated based on concentrations indicated by the manufacturers.

Blood collection

Participants were not required to fast. Each participant gave 30 mL blood, which was collected in clotting tubes and tubes containing EDTA and lithium heparin. All samples were processed within 3 h of collection and stored below −40°C or −80°C, as appropriate, until time of analysis. Blood samples were collected on the day of the interview.

Laboratory methods

Serum folate, red blood cell folate (RCF), and serum vitamin B-12 were measured by microbiological assays as previously described (11, 12). Serum MMA and plasma tHcy were measured by gas chromatography–mass spectrometry (13). Serum ferritin and serum holotranscobalamin assays were performed using an Abbott AxSYM analyzer (Abbott Laboratories, Ireland Ltd, Dublin, Ireland) (14, 15). The company KBiosciences (Hoddesdon, United Kingdom) performed the *MTHFR* (5,10-methylenetetrahydrofolate reductase) 677C→T genotyping by using a competitive allele-specific polymerase chain reaction genotyping system. Interassay CVs were as follows: serum vitamin B-12 <10.6%, serum folate <11%, serum MMA <8.1%, serum ferritin <6.0%, serum holotranscobalamin <9.4%, RCF <10.5%, and plasma tHcy <2.2%. All individuals who performed assays were masked to the hypothesis being tested.

Anemia was based on low hemoglobin concentrations defined as <13.0 g/dL in men and <12.0 g/dL in women. Macrocytosis was defined as a mean corpuscular volume ≥99 fL. Serum ferritin concentrations were considered abnormal if ≤18.7 ng/mL in men and ≤6.9 ng/mL in women. Serum holotranscobalamin concentrations were considered low if <20 pmol/L. Elevated plasma tHcy and serum MMA concentrations were defined as >15 and >0.26 μmol/L, respectively.

Statistical methods

As was done in a previous study (7), the population was divided into the following 4 categories based on vitamin B-12 concentrations <148 compared with ≥148 pmol/L and serum folate concentrations >30 compared with ≤30 nmol/L: group 1, low vitamin B-12 and high folate; group 2, low vitamin B-12 and low or normal folate; group 3, normal or high vitamin B-12 and high folate; and group 4, normal or high vitamin B-12 and low or normal folate. For overall comparisons of characteristics in all 4 groups, the Kruskal-Wallis test was used for continuous variables, and the chi-square test was used for categorical variables. For pairwise comparisons, Wilcoxon's rank-sum test was used to compare continuous variables, and the chi-square and Fisher's exact tests were used for categorical variables. Logistic regression was used to estimate an odds ratio and 95% CI for the association between anemia (yes or no) and being in group 2 compared with being in group 1. Adjusted median differences of biomarker concentrations between groups 1 and 2 were compared by fitting linear regression to Box-Cox–transformed values of biomarker concentrations that were outcome measures. Estimated median differences were obtained by back-transforming the predicted values obtained for the covariate pattern observed in the combined (groups 1 and 2) population. Logistic and linear regression models were adjusted for age (y), sex (male or female), serum ferritin (μg/L), plasma creatinine (μmol/L), *MTHFR* 677C→T genotype (CC, CT, and TT), smoking (yes or no), and alcohol intake (g/d). Statistical analyses were performed by using SAS 9.2 software (SAS Institute, Cary, NC).

RESULTS

The 2507 subjects included 1035 men and 1472 women. Sociodemographic characteristics of the entire population are shown in **Table 1**. Women were more likely ($P < 0.0001$) than

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TABLE 1
Subject characteristics by concentration of serum vitamin B-12 and serum folate

Characteristic	Group 1 (vitamin B-12 <148 pmol/L and folate >30 nmol/L)	Group 2 (vitamin B-12 <148 pmol/L and folate ≤30 nmol/L)	Group 3 (vitamin B-12 ≥148 pmol/L and folate >30 nmol/L)	Group 4 (vitamin B-12 ≥148 pmol/L and folate ≤30 nmol/L)	*P* for comparison between all 4 groups[1]
n	43	85	1206	1173	
Age (y)	22.7 ± 1.9[2]	22.9 ± 1.9	22.5 ± 2.5	22.4 ± 1.7	0.03
P[3]	Reference	NS	NS	NS	
Sex [*n* (%)]					<0.0001
M	8 (18.6)	18 (21.2)	470 (39.0)	539 (46.0)	
F	35 (81.4)	67 (78.8)	736 (61.0)	634 (54.0)	
P[3]	Reference	NS	0.019	0.0009	
BMI (kg/m^2)	22.7 ± 2.1	22.8 ± 2.9	22.9 ± 2.9	23.1 ± 3.2	0.48
P[3]	Reference	NS	NS	NS	
Smoking [*n* (%)]	17 (39.5)	30 (35.3)	388 (32.2)	425 (36.2)	0.16
P[3]	Reference	NS	NS	NS	
Alcohol intake (g/d)	24.4 ± 27.2	22.6 ± 18.9	23.0 ± 20.1	26.2 ± 22.3	0.0012
P[3]	Reference	NS	NS	NS	
Creatinine (μmol/L)	59.8 ± 11.6	60.6 ± 13.3	65.8 ± 13.1	66.9 ± 13.2	<0.0001
P[3]	Reference	NS	0.02	0.0033	
MTHFR 677C→T [*n* (%)]					<0.0001
CC	23 (53.5)	32 (37.7)	546 (45.3)	468 (39.9)	
CT	17 (39.5)	34 (40.0)	524 (43.4)	493 (42.0)	
TT	2 (4.7)	15 (17.6)	92 (7.6)	168 (14.3)	
Missing	1 (2.3)	4 (4.7)	44 (3.7)	44 (3.8)	
P[3]	Reference	NS	NS	NS	
Vegetarian diets (vegan or lacto-ovo) [*n* (%)]	4 (9.3)	6 (7.1)	70 (5.8)	27 (2.3)	<0.0001
P[3]	Reference	NS	NS	NS	
Folic acid intake from supplements and fortified food (μg/d)[4]	228.4 ± 162.9	104.4 ± 119.4	327.1 ± 885.1	200.5 ± 789.1	<0.0001
P[3]	Reference	<0.0001	NS	<0.001	
Vitamin B-12 intake from supplements and fortified food (μg/d)[4]	2.5 ± 7.4	0.8 ± 0.8	16.9 ± 159.1	11.0 ± 140.9	<0.0001
P[3]	Reference	0.027	0.017	NS	

[1] *MTHFR*, 5,10-methylenetetrahydrofolate reductase. Kruskal-Wallis test for overall comparison of continuous variables adjusted for 3 comparisons by Bonferroni adjustment and the chi-square test for overall comparison of categorical variables in the 4 groups.
[2] Mean ± SD (all such values).
[3] Wilcoxon's rank-sum test for pairwise comparisons of continuous variables, chi-square test for pairwise comparisons of the *MTHFR* 677C→T genotype distribution, and Fisher's exact test for pairwise comparisons of other categorical variables. Pairwise tests used the group with concentrations of serum vitamin B-12 <148 pmol/L and serum folate >30 nmol/L (group 1) as the reference group.
[4] Self-reported intake in the week before collection of blood samples.

were men to take supplements that contained either folic acid or vitamin B-12. Mean hemoglobin concentrations in group 1 (13.9 ±1.8 g/dL) only differed significantly ($P = 0.01$) from concentrations in group 4 (14.3 ± 1.8 g/dL). Mean ferritin concentrations were significantly lower in group 1 (29.06 ± 25.70 μg/L) than in groups 3 (42.82 ± 37.56 μg/L) and 4 (43.32 ± 34.84 μg/L) but did not differ significantly from concentrations in group 2 (33.58 ± 28.74 μg/L). As expected, subjects with the *MTHFR* 677 TT variant had significantly lower serum and RCF and significantly higher plasma tHcy (all $P < 0.001$). There were no significant differences between groups 1 and 2 in age, sex, body mass index, smoking, alcohol use, plasma creatinine concentrations, or proportion of vegetarians. Group 1 had a lower proportion of subjects who carried the TT variant of *MTHFR* 677 (4.7%) than did group 2 (17.7%), but the difference was of borderline significance ($P = 0.07$).

We compared groups 1 and 2 to test the hypothesis that high serum folate in conjunction with low serum vitamin B-12 (group 1) caused more anemia and abnormal biochemical markers for vitamin B-12 and folate function than did normal or low serum folate in conjunction with low serum vitamin B-12 (group 2) by using group 1 as the reference group (**Table 2**). Group 1 did not differ significantly from group 2 in serum vitamin B-12, hemoglobin, or serum ferritin concentrations. Group 1 had significantly lower plasma tHcy concentrations and higher serum holotranscobalamin and RCF concentrations than did group 2. Serum MMA concentrations were lower in group 1, but the difference did not reach statistical significance ($P = 0.06$). In the examination of groups by percentages of subjects with values outside the normal range, subjects in group 2 were significantly more likely to have abnormal plasma tHcy ($P = 0.0025$) (**Table 3**).

After dividing the groups by sex, women showed the same results; the number of men was probably too small to show a significant difference although men in group 2 had significant higher serum MMA than men in group 1 ($P = 0.03$). After adjusting for age, sex, serum ferritin, plasma creatinine, *MTHFR*

TABLE 2
Comparisons of biomarker concentrations between subjects with concentrations of serum vitamin B-12 <148 pmol/L and serum folate >30 nmol/L (group 1) and subjects with concentrations of serum vitamin B-12 <148 pmol/L and serum folate ≤30 nmol/L (group 2)

Biomarker	Group 1 (n = 43)	Group 2 (n = 85)	P^1
Serum vitamin B-12 (pmol/L)			
Geometric mean	122.9	116.6	0.25
Median (25th–75th percentile)	128.3 (112.5–141.2)	125.0 (105.3–137.0)	—
Hemoglobin (g/dL)			
Geometric mean	13.8	13.8	0.81
Median (25th–75th percentile)	13.5 (12.7–14.7)	13.6 (12.9–14.6)	—
Ferritin (μg/L)			
Geometric mean	20.9	23.6	0.30
Median (25th–75th percentile)	22.3 (9.8–38.5)	28.5 (13.3–45. 9)	—
Plasma total homocysteine (μmol/L)			
Geometric mean	8.3	11.4	<0.0001
Median (25th–75th percentile)	8.3 (6.8–9.7)	10.5 (8.8–13.4)	—
Serum methylmalonic acid (μmol/L)			
Geometric mean	0.20	0.24	0.057
Median (25th–75th percentile)	0.20 (0.14–0.28)	0.22 (0.18–0.31)	—
Serum holotranscobalamin (pmol/L)			
Geometric mean	31.8	26.3	0.035
Median (25th–75th percentile)	27.8 (24.1–39.8)	25.5 (19.7–36.0)	—
Red blood cell folate (nmol/L)			
Geometric mean	999.9	699.9	<0.0001
Median (25th–75th percentile)	1014.3 (792.0–1322.3)	696.8 (562.1–918.9)	—

[1] Wilcoxon's rank-sum test for comparisons of distributions between the 2 groups.

677C→T, smoking, and alcohol use, being in group 1 compared with group 2 was not a significant predictor of anemia (odds ratio: 0.483; 95% CI: 0.130, 1.946; P = 0.3). As in the crude analysis, being in group 2 was associated with a significant upward change in median plasma tHcy and downward change in median serum holotranscobalamin (**Table 4**).

The use of supplements containing folic acid was significantly more common (P = 0.02) in group 1 (34.9%) than in group 2 (15.3%). The use of supplements that contained vitamin B-12 was also more common (P = 0.02) in group 1 (32.6%) than in group 2 (14.1%). The average amount of folic acid and vitamin B-12 received from supplements and fortified food in the week before blood collection was higher in group 1 than in group 2 (Table 1). The median folic acid intake from supplements and fortified food in the week before the collection of blood samples was 197.9 μg/d in group 1 and 67.9 μg/d in group 2 ($P \leq$ 0.0001). The median vitamin B-12 intake was 1.04 μg/d in group 1 and 0.69 μg/d in group 2 (P = 0.009). Four of the 5 vegans in the study used supplements or consumed large quantities of fortified foods; all 5 vegans had serum vitamin B-12 concentrations >148 pmol/L.

The number of individuals who had multiple biochemical or other laboratory abnormalities consistent with vitamin B-12 deficiency was small. No subjects in groups 1 or 2 had macrocytosis (Table 3). Only 5 of 43 subjects (11.6%) in group 1 and 5 of 85 subjects (5.9%) in group 2 had anemia based on low hemoglobin concentrations. Of these subjects, 3 of 43 subjects (7.0%) in group 1 and one of 85 subjects (1.2%) in group 2 had low serum ferritin, which suggested that their anemia was due to iron deficiency rather than to vitamin B-12 deficiency. Among anemic subjects, plasma tHcy was elevated in no subjects in group 1 and in only one subject in group 2. Elevated serum MMA

TABLE 3
Comparisons of proportions of subjects with biomarker concentrations above or below selected normal-range cutoffs between subjects with concentrations of serum vitamin B-12 <148 pmol/L and serum folate >30 nmol/L (group 1) and subjects with concentrations of serum vitamin B-12 <148 pmol/L and serum folate ≤30 nmol/L (group 2)

Biomarker	Reference range	Cutoff	Group 1 (n = 43)	Group 2 (n = 85)	P^1
			n (%)	*n* (%)	
Plasma total homocysteine (μmol/L)	5–15	>15	0 (0.0)	14 (16.5)	0.0025
Serum methylmalonic acid (μmol/L)	≤0.26	>0.26	12 (27.9)	33 (38.8)	0.33
Serum holotranscobalamin (pmol/L)	20–134	<20	4 (9.3)	23 (27.1)	0.022
Hemoglobin (g/dL)	13.0–16.2 for men; 12.0–15.2 for women	<13.0 for men; <12.0 for women	5 (11.6)	5 (5.9)	0.30
Ferritin (μg/L)	18.7–323.0 for men; 6.9–282.5 for women	<18.7 for men; <6.9 for women	3 (7.0)	10 (11.8)	0.54
Mean corpuscular volume (fL)	78–98	≥99	0 (0.0)	0 (0.0)	—

[1] Fisher's exact test for comparisons of proportions between the 2 groups.

TABLE 4
Adjusted change in biomarker concentration for group 2 compared with group 1[1]

Biomarker	Adjusted change in biomarker concentration	*P*
Hemoglobin (g/dL)	−0.02 (−0.50, 0.47)	0.94
Serum methylmalonic acid (μmol/L)	0.04 (−0.005, 0.09)	0.12
Plasma total homocysteine (μmol/L)	2.20 (1.14, 3.59)	0.002
Serum holotranscobalamin (pmol/L)	−5.15 (−9.03, −0.80)	0.032

[1] All values are medians; 95% CIs in parentheses. Group 1 included subjects with concentrations of serum vitamin B-12 <148 pmol/L and serum folate >30 nmol/L (low vitamin B-12 and high folate); group 2 included subjects with concentrations of serum vitamin B-12 <148 pmol/L and serum folate ≤30 nmol/L (low vitamin B-12 and low or normal folate). Adjusted differences in biomarker concentrations between groups 1 and 2 were compared by fitting linear regression to Box-Cox transformed values of biomarker concentrations that were outcome measures. Estimated differences were obtained by back-transforming predicted values obtained for the covariate pattern observed in the combined (groups 1 and 2) population. Models were adjusted for age, sex, serum ferritin, plasma creatinine, *MTHFR* (5,10-methylenetetrahydrofolate reductase) 677C→T genotype, smoking, and alcohol intake.

was present in 2 anemic subjects (4.7%) in group 1 and in no anemic subjects in group 2. We looked for concurrent biochemical abnormalities in subjects with low vitamin B-12 concentrations. No subjects in group 1 had both elevated plasma tHcy and serum MMA; 9 of 85 subjects in group 2 (10.6%) had both elevated plasma tHcy and serum MMA.

The analysis was repeated to determine whether a higher serum folate concentration of 45 nmol/L as the cutoff for high folate would affect the results. The number of subjects in groups 1 and 2 became 15 and 113 subjects, respectively. The findings were not substantially different with the following exceptions. Serum ferritin concentrations became significantly lower in group 2 ($P = 0.02$), and the proportions of subjects in the 2 groups who had abnormal values for plasma tHcy and serum holotranscobalamin were no longer significantly different.

DISCUSSION

Several studies have reported that individuals who have low vitamin B-12 and high folate concentrations are more likely to have anemia, elevated MMA, and tHcy, with reduced holotranscobalamin and holotranscobalamin:vitamin B-12 ratios (4–6) than are individuals who have low vitamin B-12 and lower normal or low folate concentrations. Our data do not support these findings. Subjects in our population who had low serum vitamin B-12 in conjunction with high serum folate had significantly lower plasma tHcy and higher serum holotranscobalamin than did subjects who had low serum vitamin B-12 in conjunction with lower serum folate. The 2 groups showed no significant difference in hemoglobin, serum MMA, or serum ferritin. Thus, our data do not support the claim that high-folate exposure, as can occur with supplement and fortified-food use, interferes in any way with vitamin B-12 metabolism at a molecular level in individuals who have vitamin B-12 deficiency.

Miller et al (6) have stated that significantly lower holotranscobalamin concentrations in their subjects who had low vitamin B-12 and high folate suggested that this phenomenon may be related to the ability to deliver vitamin B-12 to the tissues rather than an intracellular problem. We showed no detrimental effect of high serum folate. In fact, we showed significantly higher serum holotranscobalamin in group 1 than in group 2. Thus, our findings provided evidence against the theory that high folate concentrations interfere in some way with the ability to deliver vitamin B-12. We did not directly test the hypothesis that the absorption of vitamin B-12 is poorer in the low–vitamin B-12 and high0folate groups as reported in some previous publications. However, we showed that hemoglobin concentrations in group 1 were not significantly different from hemoglobin concentrations in group 2, bearing in mind that those who had the lowest exposure to vitamin B-12 from supplements and fortified food were in the low–vitamin B-12 and low-folate group and not in the low–vitamin B-12 and high-folate group.

Why do our findings disagree with previous reports? As has been noted (9), it is important to rule out medical conditions that could produce other types of anemia or affect folate or vitamin B-12 absorption in evaluating the effect of folate on vitamin B-12–deficient subjects. Our study took care to identify anyone who had a medical problem that could have affected folate or vitamin B-12. There were 4 subjects who had a past history of such problems (ulcerative colitis, pernicious anemia, Crohn's disease, and bowel resection). These subjects reported that they were healthy at the time of the study; none of these subjects had a low serum vitamin B-12 concentration and, therefore, none of these subjects were in groups or 2. We also collected data on all supplement and fortified-food exposures. We studied university students and, thus, a young, healthy group that would not be at high risk of either vitamin B-12 or folate problems, and as expected, relatively few subjects had low serum vitamin B-12 or serum folate concentrations . Previous studies have also shown only a small number of subjects with both low vitamin B-12 and high folate concentrations. This finding suggested that this subgroup in other reports may have contained people who had pernicious anemia or other serious conditions that interfered with vitamin B-12 absorption and, thus, who would raise their folate concentrations but not their vitamin B-12 concentrations by taking multivitamins. This possibility could explain why this subgroup had more severe evidence of vitamin B-12 deficiency (anemia and elevated MMA) than did the larger group of subjects who had both low vitamin B-12 and low or normal folate.

Rates of low vitamin B-12 (5.1%) and low vitamin B-12 with high folate (1.7%) in our subjects were similar to rates (–2.4% and <1%, respectively) reported by Selhub et al (8). We strongly suspected that dietary factors accounted for the low vitamin B-12 concentrations in most of our subjects. Although we did not have food-frequency data, we knew that 12% of this largely female group were vegetarians, and this group took in far less than the Recommended Dietary Allowance in vitamin B-12 as supplements and fortified foods. However, it is possible that some of these

subjects have preclinical problems with vitamin B-12 absorption and could go on to develop symptoms in later life.

Some strengths and weaknesses of our study should be noted. We examined a large group of students who were carefully screened to avoid the confounding problems of vegan diets, poor absorption, or illnesses that could have affected vitamin B-12 or folate, and we collected detailed information on exposure to vitamins from supplements or fortified foods. We analyzed a range of folate- and vitamin B-12–related analytes to characterize their vitamin B-12 and folate status. On the negative side, we did not have food-intake data to complete the picture of folate and vitamin B-12 exposure. Similar to other studies, we had a relatively small number of subjects, who were largely female, in the low–vitamin B-12 and high-folate group.

In conclusion, our results provide reassurance to health care providers and patients that taking folic acid supplements or eating large amounts of food fortified with folic acid will not exacerbate vitamin B-12 deficiency, at least not in a young adult population without underlying problems. Such reassurance is important because blood folate concentrations have risen sharply since food fortification was mandated in the United States in 1998 and because a substantial proportion of the population uses folic acid–containing supplements (16, 17). Our results also provide reassurance to countries in the developing world that are considering to fortify food with folic acid that such a program is not likely to exacerbate problems in individuals who have inadequate access to vitamin B-12. We find no evidence in the vitamin B-12–deficient population that individuals who are exposed to high concentrations of folate have any more problems than do individuals who are not exposed to high concentrations of folate.

The authors' responsibilities were as follows—JLM, AMM, and JMS: designed the research; AMM, ERG, PNK, LCB, PMU, and JMS: collected data and conducted research; TCC and JFT: analyzed data; JLM, TCC, PMU, and AMM: wrote the manuscript; BS, LCB, and JMS: assisted with data interpretation; JLM: had primary responsibility for the final content of the manuscript; and all authors: read and approved the final manuscript. None of the authors had a conflict of interest.

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