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Folate and vitamin B-12 status in relation to anemia, macrocytosis, and cognitive impairment in older Americans in the age of folic acid fortification[1–4]

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ABSTRACT

Background: Historic reports on the treatment of pernicious anemia with folic acid suggest that high-level folic acid fortification delays the diagnosis of or exacerbates the effects of vitamin B-12 deficiency, which affects many seniors. This idea is controversial, however, because observational data are few and inconclusive. Furthermore, experimental investigation is unethical.

Objective: We examined the relations between serum folate and vitamin B-12 status relative to anemia, macrocytosis, and cognitive impairment (ie, Digit Symbol-Coding score <34) in senior participants in the 1999–2002 US National Health and Nutrition Examination Survey.

Design: The subjects had normal serum creatinine concentrations and reported no history of stroke, alcoholism, recent anemia therapy, or diseases of the liver, thyroid, or coronary arteries ($n = 1459$). We defined low vitamin B-12 status as a serum vitamin B-12 concentration <148 pmol/L or a serum methylmalonic acid concentration >210 nmol/L—the maximum of the reference range for serum vitamin B-12–replete participants with normal creatinine.

Results: After control for demographic characteristics, cancer, smoking, alcohol intake, serum ferritin, and serum creatinine, low versus normal vitamin B-12 status was associated with anemia [odds ratio (OR): 2.7; 95% CI: 1.7, 4.2], macrocytosis (OR: 1.8; 95% CI: 1.01, 3.3), and cognitive impairment (OR: 2.5; 95% CI: 1.6, 3.8). In the group with a low vitamin B-12 status, serum folate >59 nmol/L (80th percentile), as opposed to ≤59 nmol/L, was associated with anemia (OR: 3.1; 95% CI: 1.5, 6.6) and cognitive impairment (OR: 2.6; 95% CI: 1.1, 6.1). In the normal vitamin B-12 group, ORs relating high versus normal serum folate to these outcomes were <1.0 ($P_{interaction} < 0.05$), but significantly <1.0 only for cognitive impairment (0.4; 95% CI: 0.2, 0.9).

Conclusion: In seniors with low vitamin B-12 status, high serum folate was associated with anemia and cognitive impairment. When vitamin B-12 status was normal, however, high serum folate was associated with protection against cognitive impairment. *Am J Clin Nutr* 2007;85:193–200.

KEY WORDS Aging, anemia, cognition disorders, folate, fortified food, nutrition surveys, vitamin B-12 deficiency

INTRODUCTION

Since January of 1998, the US Food and Drug Administration has required the folic acid fortification of all enriched cereal-grain products. The main goal of this program is the prevention of neural tube birth defects (1). Before the program's inception, concern was expressed over the possibility that an increased folic acid intake might delay the diagnosis of vitamin B-12 deficiency or even exacerbate its neurologic and neuropsychiatric effects (2, 3). The elderly are of particular concern because of age-related declines in vitamin absorption and extraction of vitamin B-12 from protein (4–11) and age-related increases in autoimmunity against intrinsic factor or the gastric parietal cells that produce it (11–13).

Fears of harm from fortification to seniors and other Americans with low vitamin B-12 status are based on early case reports of pernicious anemia that detail the alleviation of anemia but the precipitation or exacerbation of neurologic or neuropsychiatric sequelae after folic acid administration—an early form of treatment based on the mistaken idea that a lack of folate was the problem (8, 14). Modern reports on the effects of high folic acid intakes are infrequent because of the rarity of identified cases of vitamin B-12 deficiency affected by oversupplementation or mistreatment with folic acid. However, close study of the original case reports showed no proof that folic acid therapy exacerbated central nervous system (CNS)—related symptoms (15, 16), and modern case reports and studies suggest that folic acid therapy did not cure anemia either (17–20). Such data support the idea that folic acid can be safely added to foods in moderation, but recent editorials have stressed the need for systematic study of the hypothesis that high folate intakes cause harm (21, 22).

Data collected in the most recent National Health and Nutrition Examination Survey (NHANES) afforded us the opportunity to study interrelations between serum folate and vitamin B-12 status in relation to anemia, macrocytosis, and cognitive impairment in the age of folic acid fortification.

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[2] Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the views or policies of the US Department of Agriculture, nor does mention of trade names, commercial products, or organizations imply endorsement by the US government.

[3] Supported by USDA agreement no. 58-1950-9-001 and NIH no. R03 AG021536-01.

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Received January 12, 2006.

Accepted for publication August 3, 2006.

SUBJECTS AND METHODS

Study population

NHANES monitors the nation's health and nutritional status. The survey is currently implemented as a continuous annual survey and uses a complex multistage probability design to select a representative sample of the noninstitutionalized US civilian population. To increase the precision of estimates derived from the survey, adolescents, the elderly, Mexican Americans, and blacks are oversampled. The protocols for conducting the NHANES were approved by the institutional review board of the National Center for Health Statistics, Centers for Disease Control and Prevention, and informed consent was obtained from all participants (23). Consistent with NHANES analytic guidelines, we combined data from the 2 most recent surveys into a single data set (1999–2002) (24).

Trained interviewers used a computer-assisted personal interview system to interview participants in their homes. The participants were also asked to report to a mobile examination center (MEC) to provide further interview data and undergo a physical examination that included phlebotomy. A detailed description of blood collection and processing can be found in the NHANES Phlebotomy Manual (25). Although the survey included people of all ages, we focused our attention on seniors (ie, those aged ≥60 y)—the only group whose cognitive function was assessed.

We excluded seniors with serum creatinine concentrations (based on the Jaffe reaction) indicative of renal dysfunction (ie, men, >131 μmol/L; women, >115 μmol/L) and who reported recent anemia therapy or a history of stroke, heavy alcohol use, or diseases of the liver, kidney, or coronary arteries. Of 3706 senior survey participants, 1684 were eligible, 1078 were ineligible, and eligibility status could not be determined for 944. Complete information for analyses pertaining to anemia was available for 1458 seniors, and complete information for analyses pertaining to cognitive function was available for 1302 seniors.

Assessment of anemia and macrocytosis

Anemia and macrocytosis were assessed on the basis of hemoglobin concentrations and mean cell volumes, which were measured at the mobile examination center laboratory with a MAXM hematology flow cytometer (Beckman Coulter Inc, Fullerton, CA). Anemia was defined according to World Health Organization criteria (ie, hemoglobin <12 g/dL for women and <13 g/dL for men) (26). We defined macrocytosis as a mean cell volume ≥99 fL.

Assessment of cognitive function

The cognitive function of seniors was assessed by using a version of the Digit Symbol-Coding subtest of the Wechsler Adult Intelligence Scale III—a screening test designed to detect cognitive impairment in adults and children (27). In the test, participants copy symbols that are paired with numbers. Using the key provided at the top of the exercise form, the participant draws the symbol under the corresponding number. The score, which declines with age (28), is the number of correct symbols drawn within 120 s. One point is given for each correctly drawn symbol completed within the time limit for a maximum score of 133. Use of the test in the most recent NHANES was based on its reputation as a more sensitive measure of dementia than the Mini-Mental State Examination (29). According to NHANES documentation, aptitudes needed for a high score are response speed, sustained attention, visual spatial skills, associative learning, and memory (29). However, research suggests that speed is the prime determinant of performance on the test (28). We defined cognitive impairment as having attained a test score <34—the 20th percentile of the distribution.

Biochemical measurements

Blood samples were analyzed at the Inorganic Toxicology and Nutrition Branch of the Division of Laboratory Sciences, National Center for Environmental Health. Serum concentrations of folate and vitamin B-12 were measured by using the Quantaphase II Radioassay Kit (Bio-Rad Laboratories, Anaheim, CA). Serum methylmalonic acid (MMA) was measured by gas chromatography–mass spectrometry with cyclohexanol derivatization (30). Serum homocysteine was analyzed by using a commercially available fluorescence polarization immunoassay kit (Abbott Laboratories, Abbott Park, IL) on the Abbott IMx analyzer (31). Serum ferritin was measured by using the QuantaImmune Ferritin IRMA Kit (Bio-Rad Laboratories). Serum creatinine concentration was based on the Jaffe reaction, and serum glucose was determined by using a hexokinase enzymatic method.

Classification of subjects according to vitamin B-12 and folate status

We defined low vitamin B-12 status as a low serum vitamin B-12 or elevated serum MMA concentration. We defined low serum B12 as a value below the conventionally applied cutoff for deficiency of 148 pmol/L. We defined elevated MMA as a serum MMA concentration above the recently published reference range (ie, 60–210 nmol/L) for serum vitamin B-12–replete survey participants with normal serum creatinine concentrations (32). It should be noted that a similar strategy applied to data from the previous NHANES led to a cutoff point of 370 nmol/L (33). Pfeiffer et al (32) attributed the different MMA values obtained in the 2 surveys to differences in the laboratories conducting the analyses and the matrices and methods used. For powerful tests of interactions between vitamin B-12 status and serum folate in relation to anemia, macrocytosis, and cognitive impairment, we used serum folate as a continuous variable. In other analyses we defined high folate status as a serum folate concentration >59 nmol/L—the 80th percentile of the distribution of the seniors.

Statistical analyses

Data analyses were performed by using SUDAAN release 9.0 (Research Triangle Institute, Research Triangle Park, NC) with appropriate 4-y sampling weights to account for the survey's complex sampling design (24). $P < 0.05$ was considered statistically significant for all tests. Except for the percentages displayed in **Table 1**, and where otherwise indicated, the results were obtained after multivariate adjustment for age, sex, race-ethnicity (as determined by combining responses to questions on race and Hispanic origin;23), education (<high school diploma, high school diploma, >high school diploma), current cigarette smoking status, alcohol intake, self-reported history of cancer, and serum concentrations of creatinine and ferritin.

We first used SUDAAN PROC REGRESS, SUDAAN PROC CROSSTAB, and SUDAAN PROC RLOGIST to describe the

TABLE 1
Characteristics of eligible senior participants in the National Health and Nutrition Examination Survey (1999–2002) by vitamin B-12 status[1]

Characteristic	Vitamin B-12 status: Normal (n = 1113)	Vitamin B-12 status: Low[2] (n = 346)	OR (95% CI)	P
Age (y)	70 ± 0.30[3]	72 ± 0.39[3]	1.05 (1.03, 1.07)	<0.001
Female (%)	62	66	1.6 (1.1, 2.5)	0.022
Non-Hispanic white (%)	81	85	1.0	Referent
Non-Hispanic black (%)	8.0	4.4	0.4 (0.3, 0.6)	<0.001
Mexican American (%)	3.1	2.1	0.9 (0.6, 1.3)	0.532
<High school diploma (%)	25	32	1.5 (1.01, 2.3)	0.046
Cigarette smoker (%)	12	17	2.1 (1.4, 3.3)	0.002
Supplement user (%)	71	54	0.4 (0.3, 0.7)	<0.001
Serum folate (nmol/L)	39 ± 0.7[4]	34 ± 1.4[4]	—	0.019
Serum creatinine (μmol/L)	67 ± 0.9[4]	74 ± 1.3[4]	—	<0.001
Serum ferritin (μg/L)	102 ± 2.3[4]	94 ± 5.6[4]	—	0.146
Cancer diagnosis (%)	13	14	1.1 (0.7, 1.7)	0.76
Macrocytosis (%)[5]	2.7	6.5	1.8 (1.02, 3.1)	0.041
Anemia (%)[6]	3.2	8.3	2.7 (1.7, 4.4)	<0.001
Cognitive impairment (%)[7]	15	32	2.5 (1.6, 3.8)	<0.001

[1] Subjects with high serum creatinine concentrations and those who reported stroke, alcoholism, recent anemia therapy, or diseases of the liver, thyroid, or coronary arteries were excluded. Means, odds ratios (ORs), and P values were generated from a multivariate model that included terms for age, sex, race-ethnicity, educational status, cancer history, and serum concentrations of ferritin and creatinine; percentages are sample-weighted.

[2] Defined as a serum vitamin B-12 concentration <148 pmol/L or a serum methylmalonic acid concentration above the reference range (ie, 60–210 nmol/L) for serum vitamin B-12–replete participants with normal serum creatinine.

[3] $\bar{x}$ ± SEM.

[4] Geometric least-squares $\bar{x}$ ± SEM.

[5] Defined as a mean cell volume ≥99 fL.

[6] Defined as a hemoglobin concentration <12 g/dL (women) or <13 g/dL (men).

[7] Defined as a Digit Symbol-Coding Score <34; test results were available for 1302 nonexcluded seniors.

study subjects via least-squares means and proportions and to perform comparisons between those with low and normal vitamin B-12 status.

Our primary data analyses addressed the hypothesis that the effects of vitamin B-12 and folate on study outcomes modified each other. We tested this hypothesis by evaluating statistical interactions between vitamin B-12 status and folate status in relation to macrocytosis, anemia, and cognitive impairment using multivariate models for the 3 outcome variables that included terms for vitamin B-12 category, serum folate (continuous), and their interaction along with the potentially confounding factors identified above (full model). For subjects in the 2 vitamin B-12–status categories, we also used SUDAAN PROC RLOGIST to estimate the odds ratios (ORs) and associated 95% CIs relating high versus normal serum folate to anemia and cognitive impairment. We generated these estimates from both the full multivariate model and a model controlled only for age, sex, and race-ethnicity (basic model). To summarize the interactions we found, we created a single variable with 4 levels (ie, abnormal for serum folate alone, abnormal for vitamin B-12 status alone, abnormal for both vitamins, and normal for both vitamins). We then used multivariate logistic regression models to estimate ORs (95% CI) for anemia and cognitive impairment that compared each abnormal group with the group that was normal for both vitamins.

To shed light on homocysteine's influence on our findings, we used SUDAAN PROC REGRESS and the full model plus terms for diabetes and serum glucose (factors inversely related to homocysteine and cognition) to estimate the multivariate-adjusted prevalence of hyperhomocysteinemia (>13 μmol/L) in each of the 4 B vitamin status categories. We then estimated the ORs (95% CI) relating B vitamin status to anemia and cognitive impairment after controlling for hyperhomocysteinemia.

Finally, we used SUDAAN ROC REGRESS and the full multivariate model to examine the association between serum vitamin B-12 and log-transformed serum folate as well as potential effect modification by both supplement use and low-versus-normal vitamin B-12 status as previously defined. For this purpose we divided subjects according to self-reported supplement use (yes or no) and determined quartiles of serum vitamin B-12 separately for each group. For trend tests and tests of interaction, we modeled serum vitamin B-12 as a continuous variable created by assigning each subject the median of his or her quartile category. We also examined the interaction between supplement use and low-versus-normal vitamin B-12 status as previously defined. To graphically display the association between serum vitamin B-12 and serum folate, we plotted least-square geometric mean serum folate for the serum vitamin B-12 quartile categories.

RESULTS

Subject description: characteristics and study outcomes

The mean age of the participants was 70 ± 0.32 y, and the mean (±SEM) Digit Symbol-Coding score was 49 ± 0.7. Sixty-seven percent reported using dietary supplements, 14% were smokers, and 20.7% had a serum folate concentration >59 nmol/L. Anemia affected 4.5% of the seniors, and 4% were macrocytic. Just under 3% had serum vitamin B-12 concentrations <148 pmol/L, but 25% met our definition of low vitamin

B-12 status (ie, serum vitamin B-12 <148 pmol/L or serum MMA >210 nmol/L, the upper limit of the reference range for serum vitamin B-12–replete participants with normal serum creatinine). Seniors with a combination of low vitamin B-12 status and high serum folate concentrations accounted for ≈4% of the participants.

Subject characteristics in relation to anemia, macrocytosis, and cognitive impairment

After multivariate adjustment, anemia was marginally significantly related to cancer ($P = 0.07$) and significantly related to age, serum creatinine, non-Hispanic black race-ethnicity, and nonsmoking status. (The masking of anemia by cigarette smoking is consistent with previously reported findings from NHANES II; 34.) Macrocytosis was marginally significantly related to alcohol intake ($P = 0.06$) and significantly related to age, cigarette smoking, and serum creatinine. Macrocytosis was inversely related to serum ferritin. Cognitive impairment was significantly associated with age, nonwhite race-ethnicity, and not having attained a high school diploma.

Vitamin B-12 status in relation to subject characteristics and study outcomes

Non-Hispanic black subjects were significantly less likely than were non-Hispanic white subjects to have low vitamin B-12 status. Subject characteristics and health problems directly associated with low vitamin B-12 status after multivariate adjustment were age, female sex, not having attained a high school diploma, cigarette smoking, nonuse of dietary supplements, serum creatinine, anemia, macrocytosis, and cognitive impairment (Table 1).

Interaction between vitamin B-12 status and serum folate in relation to anemia and cognition

Vitamin B-12 status interacted significantly with serum folate concentration in relation to anemia ($P = 0.03$) and cognitive impairment ($P < 0.001$), but not with macrocytosis ($P = 0.14$). In all subjects combined, high folate status was not significantly associated with macrocytosis (OR: 1.7; 95% CI: 0.7, 4.0).

Among subjects classified as having normal vitamin B-12 status, high serum folate compared with normal serum folate was associated with protection from cognitive impairment. Specifically, the multivariate-adjusted OR (95% CI) relating serum folate >59 nmol/L versus a lower value to cognitive impairment was 0.4 (0.2, 0.9). The OR (95% CI) relating high versus normal serum folate to anemia was 0.6 (0.1, 2.4). Among subjects classified as having low vitamin B-12 status, high serum folate compared with normal serum folate was directly related to both anemia (OR: 3.1; 95% CI: 1.5, 6.6) and cognitive impairment (OR: 2.6; 95% CI: 1.1, 6.1). These estimates were very similar to those generated from the basic model. With the basic model, ORs (95% CI) relating high serum folate to anemia and cognitive impairment were 0.5 (0.2, 1.6) and 0.4 (0.2, 0.7) for the group with normal vitamin B-12 status and 3.0 (1.4, 6.2) and 2.6 (1.1, 6.1) for the group with low vitamin B-12 status.

Data displayed in **Table 2** summarize the interaction between serum folate and vitamin B-12 status in relation to anemia and cognitive impairment and address the potential role of homocysteine in the associations. The tabulated data show that, compared with having normal status for both vitamins, having high serum folate status alone was associated with a reduced prevalence of anemia and a significantly reduced prevalence of cognitive impairment. Furthermore, having low vitamin B-12 status, regardless of serum folate, was associated with a significantly increased prevalence of both anemia and cognitive impairment. The worst combination was low vitamin B-12 status and high serum folate. Specifically, anemia and cognitive impairment were observed ≈5 times as often in the group with that combination as they were in the group with normal vitamin B-12 status and normal serum folate.

After multivariate adjustment, hyperhomocysteinemia was associated with an increased prevalence of both cognitive impairment (OR: 1.9; 95% CI: 1.1, 3.4) and anemia (OR: 2.0; 95% CI: 1.01, 3.8). When hyperhomocysteinemia was controlled for, associations between low vitamin B-12 status alone and study outcomes were slightly reduced in magnitude and marginally statistically significant. However, the ORs relating the combination of low vitamin B-12 status and high serum folate to anemia and cognitive impairment remained large and statistically significant (Table 2). Furthermore, although hyperhomocysteinemia was common in the group that was abnormal for both vitamins, the prevalence of hyperhomocysteinemia in that group was significantly lower than that in the group with low vitamin B-12 status but normal serum folate ($P < 0.001$).

Association between serum vitamin B-12 and serum folate

We observed a direct association ($P_{trend} < 0.001$) between serum vitamin B-12 and serum folate (**Figure 1**) regardless of supplement use ($P_{interaction} = 0.751$) or low-versus-normal vitamin B-12 status as previously defined ($P_{interaction} = 0.502$). Geometric mean serum folate was 34.1 nmol/L (95% CI: 31.6, 36.9) for subjects classified as having a low vitamin B-12 status based on both serum vitamin B-12 and serum MMA. It was 39.2 nmol/L (95% CI: 37.7, 40.8) for subjects with normal vitamin B-12 status, and this difference ($P = 0.003$) was not significantly affected by supplement use ($P_{interaction} = 0.228$).

DISCUSSION

In this study of older Americans in the age of folic acid fortification, we found direct associations between high serum folate and both anemia and cognitive impairment in subjects with low vitamin B-12 status. Among subjects with normal vitamin B-12 status, on the other hand, high serum folate was associated with protection from cognitive impairment.

Our findings were somewhat consistent with predictions of harm to vitamin B-12–deficient seniors from the US government's folic acid fortification program (2, 3). However, the positive association we found between high folate status and anemia among older Americans with low vitamin B-12 status was unexpected.

Two related ideas have been expressed in the literature about impaired CNS function in the elderly from folic acid fortification. Both scenarios follow from hypothesized effects of unmetabolized folic acid in the circulation. Normally, folate circulates in the body as 5-methyltetrahydrofolate (5-MTHF) (11). Folic acid, the form of folate in supplements and in fortified foods, can be converted to 5-MTHF as it passes through the intestinal mucosa (11). However, capacity for this conversion is limited, such

TABLE 2
Interaction between vitamin B-12 status and serum folate in relation to anemia and cognitive impairment in eligible senior participants in the National Health and Nutrition Examination Survey (1999–2002)[1]

Outcome	Vitamin status		No. of subjects	Percentage with outcome[4]	Percentage with high homocysteine[4–6]	OR (95% CI)		
	B-12[2]	Folate[3]				Basic model[7]	Full model 1[8]	Full model 2[9]
				%	%			
Anemia[10]	Normal	Normal	913	3.5	12	1.0	1.0	1.0
Anemia	Normal	High	198	2.5	7.8	0.6 (0.2, 2.2)	0.6 (0.2, 2.4)	0.6 (0.2, 2.4)
Anemia	Low	Normal	297	6.9	31	2.0 (1.1, 3.5)	2.1 (1.1, 3.7)	1.9 (1.01, 3.6)
Anemia	Low	High[11]	49	15	23	5.2 (2.5, 10.6)	4.9 (2.3, 10.6)	4.8 (2.3, 10.4)
Cognitive impairment[12]	Normal	Normal	826	18	11	1.0	1.0	1.0
Cognitive impairment	Normal	High	180	11	7.8	0.5 (0.2, 0.9)	0.4 (0.2, 0.9)	0.5 (0.2, 0.96)
Cognitive impairment	Low	Normal	253	25	31	1.9 (1.1, 3.1)	1.7 (1.01, 2.9)	1.6 (0.95, 2.8)
Cognitive impairment	Low	High[13]	42	45	25	4.9 (2.6, 9.2)	5.0 (2.7, 9.5)	4.9 (2.6, 9.2)

[1] Subjects with high serum creatinine concentrations and those who reported stroke, alcoholism, recent anemia therapy, or diseases of the liver, thyroid, or coronary arteries were excluded.
[2] Low serum vitamin B-12 status defined as a concentration <148 pmol/L or a serum methylmalonic acid concentration above the reference range (ie, 60–210 nmol/L) for serum vitamin B-12–replete participants with normal serum creatinine.
[3] High status defined as a serum folate concentration >59 nmol/L (80th percentile).
[4] Adjusted for age, sex, race-ethnicity, educational status, cancer history, diabetes status, and serum concentrations of ferritin, creatinine, and glucose.
[5] High homocysteine concentration defined as >13 nmol/L.
[6] All percentages within an outcome group are significantly different from each other.
[7] Adjusted for age, sex, and race-ethnicity.
[8] Adjusted for age, sex, race-ethnicity, educational status, cancer history, diabetes status, and serum concentrations of ferritin, creatinine, and glucose.
[9] Adjusted for age; sex; race-ethnicity; educational status; cancer history; diabetes status; serum concentrations of ferritin, creatinine, and glucose; and hyperhomocysteinemia.
[10] Defined as a hemoglobin concentration <12 g/dL (women) or <13 g/dL (men).
[11] In a comparison of this group with the group with low vitamin B-12 status and normal serum folate, $P = 0.01$ with the basic model, $P = 0.028$ with full model 1, and $P = 0.025$ with full model 2. In comparisons between this group and all other groups, regardless of the model used, $P \leq 0.001$.
[12] Defined as a Digit Symbol-Coding Score <34.
[13] In a comparison of this group with the group with low vitamin B-12 status and high serum folate, $P = 0.013$ with the basic model, $P = 0.008$ with full model 1, and $P = 0.005$ with full model 2. In comparisons between this group and all other groups, regardless of the model, $P < 0.001$.

that repeated high dosing with folic acid can result in the appearance of unmetabolized folic acid in the bloodstream (35). Circulating unmetabolized folic acid has been suggested to either merely delay diagnosis by curing vitamin B-12 deficiency anemia (6, 16, 19, 35, 36), a key clinical sign (the so-called "masking" effect), or cause rapid deterioration of CNS function (8). The simultaneous curing of anemia and exacerbation of CNS effects is potentially explicable by a hypothesized stimulatory effect of unmetabolized folic acid on DNA synthesis. According to the methyl-folate trap hypothesis (14), the hematologic and neuropsychiatric consequences of vitamin B-12 deficiency result from the loss of vitamin B-12's function as a cofactor for the enzyme methionine synthase—the catalyst for the remethylation of homocysteine to methionine. The result is not only a lack of *S*-adenosyl methionine (SAM), the CNS methyl donor (37), but also the failure of methyl folate to be converted to tetrahydrofolate (THF), and consequently, a deficiency of folate precursors needed for DNA synthesis and red blood cell maturation (38).

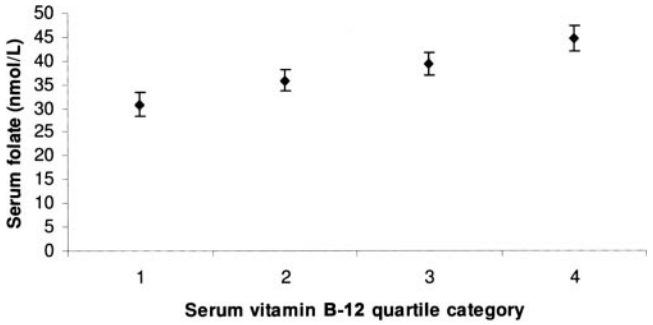

FIGURE 1. Association between serum vitamin B-12 and serum folate in senior participants in the National Health and Nutrition Examination Survey (1999–2002) who had no evidence of renal dysfunction or history of stroke, alcoholism, recent anemia therapy, or diseases of the liver, thyroid, or coronary arteries ($n = 1457$). Points represent least-squares geometric means adjusted for age, sex, race-ethnicity, education, cancer, smoking, alcohol intake, and serum concentrations of ferritin and creatinine for supplement users and nonusers combined. Error bars represent 95% CIs. Quartile categories 1, 2, 3, and 4 are <271, 271–366, 367–484, and >484 pmol/L for supplement users and <208, 208–281, 282–350, and >350 pmol/L for nonusers, respectively. The direct association between serum vitamin B-12 and serum folate ($P_{trend} < 0.001$) did not vary with supplement use ($P_{interaction} < 0.751$).

According to theory, this deficiency can be overcome by the direct conversion of folic acid to THF (11), but the cost is further depletion of SAM because of the demand for methionine imposed by protein synthesis (14).

Advocates of the addition to food of more folic acid than the US government currently requires continue to question the importance of masking to the consequences of vitamin B-12 deficiency (39, 40), particularly in light of increasing knowledge of the clinical heterogeneity of vitamin B-12 deficiency and the predominance of atypical presentations in the elderly (41). More recently, the phenomenon of masking, itself, has been questioned—one case report shows that macrocytosis persisted and anemia worsened after folic acid treatment of sickle cell disease (18) and another study reported no effect of fortification on the proportion of vitamin B-12–deficient veterans with anemia (17).

The idea that high folic acid intake exacerbates neurologic and neuropsychiatric effects of vitamin B-12 deficiency is also controversial. Dickinson failed to find evidence of this phenomenon after closely scrutinizing the historical pernicious anemia case reports (15, 16). However, on the basis of not only case reports, but also on the basis of animal data and the known metabolic interaction between folate and vitamin B-12, the Food and Nutrition Board of the Institute of Medicine called the evidence "suggestive" (42).

Our findings are most consistent with Reynolds's (8) conclusion from both original data and case reviews that folic acid precipitates both hematologic and neuropsychiatric manifestations of vitamin B-12 deficiency. Thus, our results are at odds with the idea that folic acid stimulates cell division at the expense of homocysteine remethylation, particularly in light of the lower prevalence of hyperhomocysteinemia in subjects with a low vitamin B-12 status and high serum folate compared with those with a low vitamin B-12 status and normal serum folate.

Our findings also support the often-expressed idea that many seniors would actually benefit from more folate (16, 43, 44). Despite a marked increase in the folate status of Americans as a result of fortification (45, 46), we found a strong inverse relation between high folate status and cognitive impairment among vitamin B-12–replete subjects. Perhaps because of the diverse cognitive function tests used and the different domains assessed, previous studies of relations between folate status (47–50) or hyperhomocysteinemia (48, 49, 51–54) and cognition have yielded mixed results. In one recently published study, the subjects aged >65 y whose total folate intake at baseline exceeded 400 μg/d had a more rapid cognitive decline over 6 y of follow-up than did the participants with intakes <201 μg/d (55). However, other prospective studies linked low folate intake (56) or low circulating folate concentrations (48, 49, 57, 58) with an elevated risk of cognitive decline.

If folate causally affects cognitive function, its benefits, like those of vitamin B-12, may relate to its role in homocysteine remethylation. Remethylation leads to SAM production. Furthermore, homocysteine or its metabolites may damage neurons or cause vascular disease (59)—a leading cause of dementia (60). In subjects with a normal vitamin B-12 status, high serum folate was associated with protection from hyperhomocysteinemia, but this did not entirely explain the inverse relation between high serum folate and cognitive impairment.

Given our study's cross-sectional design, a direct association between folate status and cognition could, theoretically, reflect the adverse effects of cognitive impairment on diet. However, such reverse causation could not explain the link between high folate status and poor cognition among subjects with low vitamin B-12 status. Although the methyl-folate trap hypothesis predicts normal or high serum folate in vitamin B-12 deficiency (61), this phenomenon probably does not explain our results either. The higher serum folate supposedly results from a failure of polyglutamation, a modification of THF that facilitates intracellular folate retention (62, 63). Indeed, folate was lost from tissues in rats made vitamin B-12 deficient by nitrous oxide (64). However, in our study, as in previous human investigations (19, 65), serum concentrations of folate and vitamin B-12 were directly, not inversely, related, regardless of vitamin B-12 status and dietary supplement use.

In addition to its large size and general population base, the strengths of our study included the availability of data on MMA and our control of key confounders. Although some data were self-reported, such that residual confounding might remain, we tried to exclude low intelligence (as indicated by educational level), stroke, and coronary artery disease as causes of poor cognition; iron deficiency and cancer as causes of anemia; and renal impairment as a cause of high MMA (66–70).

The lack of a gold standard indicator of low vitamin B-12 status presents a challenge to all investigators of this nutritional problem (71). It is currently accepted that clinically significant vitamin B-12 deficiency can occur in the elderly at serum vitamin B-12 concentrations >148 pmol/L (41), and the serum MMA concentration is considered to be a sensitive and specific diagnostic tool (72–76) that is particularly helpful in identifying so-called preclinical or subtle cases (77–79). Although the cognitive function test administered in the NHANES is not specific for the cognitive impairment that results from vitamin B-12 deficiency (73, 80–82), the strong association we found between low scores and low vitamin B-12 status attests to its ability to capture cognitive impairment due to this cause. On the other hand, the availability of this single marker prevented us from evaluating associations between folate status and other neurologic and neuropsychiatric effects. We also cannot say definitively that the associations we found were due to unmetabolized folic acid, because only serum total folate was measured.

In conclusion, we undertook this investigation to shed light on long-held but evolving ideas about the effects of folic acid fortification on the elderly. We found a higher prevalence of both anemia and cognitive impairment in association with high serum folate in older Americans with a low vitamin B-12 status. We encourage further study of these relations and their underlying mechanisms and hope our findings both inform the continuing debate about folic acid fortification and influence efforts to detect and treat low vitamin B-12 status in seniors.

JS was responsible for the study concept. MSM and JS were responsible for the research design. MSM designed and carried out the data analyses and drafted the manuscript. MSM, PFJ, IHR, and JS critically revised the article for important intellectual content. None of the authors had a conflict of interest.

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