# Folate, vitamin B-12, and cognitive function in the Boston Puerto Rican Health Study

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**ABSTRACT**

**Background:** There is evidence that low plasma vitamin B-12 and folate individually, as well as an imbalance of high folic acid and low vitamin B-12 status, may be associated with lower cognitive function.

**Objectives:** We examined dietary and plasma folate and vitamin B-12 status, and their interaction, in relation to cognitive function in a cohort of older Puerto Rican adults.

**Methods:** The design is cross-sectional, with 1408 participants from the Boston Puerto Rican Health Study (mean ± SD age: 57.1 ± 7.9 y). Cognitive function was assessed with a comprehensive test battery and a global composite score was derived. Plasma folate, vitamin B-12, and methylmalonic acid (MMA) were assessed in fasting blood samples.

**Results:** After adjusting for covariates, high plasma folate and high plasma vitamin B-12 were each positively associated with global cognitive score ($\beta$: 0.063; 95% CI: −0.0008, 0.127; $P = 0.053$ and $\beta$: 0.062; 95% CI: 0.009, 0.12; $P = 0.023$, respectively, for logged values, and $\beta$: 0.002; 95% CI: 0.00005, 0.004; $P$-trend = 0.044 and $\beta$: 0.00018; 95% CI: 0.00001, 0.0003; $P$-trend = 0.036, respectively, across tertiles). Nine percent of participants had vitamin B-12 deficiency (plasma vitamin B-12 < 148 pmol/L or MMA > 271 nmol/L), but none were folate deficient (plasma folate < 4.53 nmol/L). Deficient compared with higher vitamin B-12 was significantly associated with lower cognitive score ($\beta$: −0.119; 95% CI: −0.208, −0.029; $P = 0.009$). We could not examine the interaction for vitamin B-12 deficiency and high plasma folate, because there were too few individuals (<1% of the cohort) in this category to draw conclusions.

**Conclusions:** Low plasma vitamin B-12 and low plasma folate were each associated with worse cognitive function in this population. Vitamin B-12 deficiency was prevalent and clearly associated with poorer cognitive function. More attention should be given to identification and treatment of vitamin B-12 deficiency in this population. Additional, larger studies are needed to examine the effect of vitamin B-12 deficiency in the presence of high exposure to folic acid. *Am J Clin Nutr* 2021;113:179–186.

**Keywords:** vitamin B-12, folate, cognition, Puerto Rican adults, diet, folic acid

## Introduction

The prevalence of cognitive impairment and dementia is growing with the aging of the US population. It has been estimated that dementia prevalence may range from 14% in those older than age 70 y, to 37% among those age 90 y and older (1, 2), The number of persons with dementia is expected to double every 20 y, resulting in an increase from 42.3 million individuals in 2020 to 81.1 million in 2040 (3). B-vitamins in particular have been associated with cognition, because they are critical for the synthesis of monoamine neurotransmitters and the formation of RBCs (4).

Vitamin B-12 deficiency has been associated with cognitive impairment (1, 5). Further, evidence suggests that vitamin B-12 inadequacy, which may develop owing to malabsorption, dietary inadequacy, or lack of intrinsic factor, may also be a risk factor (5, 6) for cognitive impairment. Vitamin B-12 deficiency is common among older populations, because the ability to absorb vitamin B-12 declines with the reduction in gastric cell function (6). The prevalence of subclinical vitamin B-12 deficiency has been shown to be high in older populations and in vegetarians (6).

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Data Availability: Data described in the article, code book, and analytic code will be made available upon request pending application and approval.

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Abbreviations used: APOE, apolipoprotein E; GCS, global cognitive function composite score; MMA, methylmalonic acid; PLP, pyridoxal-5-phosphate; UL, upper limit.

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Older adults with low folate status have been shown to be at higher risk of cognitive impairment and dementia (7, 8). A study reported that RBC folate was directly associated with cognitive function score and, inversely, with dementia in elderly Latinos (7). A few studies have also raised the hypothesis that high exposure to folic acid may accelerate the effects of vitamin B-12 inadequacy (9, 10). In an analysis of national data, older adults with low vitamin B-12 status and high serum folate concentration had greater odds of cognitive impairment, whereas among those with normal vitamin B-12 status, high serum folate was found to be protective (9). Therefore, we sought to examine the associations between plasma folate, dietary intake of folic acid, vitamin B-12 status, and cognitive function, and to assess their interaction, in the Boston Puerto Rican Health Study.

## Methods

### Participants

This analysis was conducted within the Boston Puerto Rican Health Study, which included 1502 self-identified Puerto Rican adults, aged 45–75 y at baseline (2004–2009), residing in the Boston, MA metropolitan area. As described previously, study participants were recruited through door-to-door enumeration and community approaches (11). Study protocols were approved by the Institutional Review Board at Tufts Medical Center and by the University of Massachusetts Lowell.

### Data collection

Trained bilingual interviewers administered questionnaires to participants in their homes (11). Many of the questionnaires and measures followed procedures used in the NHANES (12) and the MacArthur Studies of Successful Aging (13). Retraining and review sessions, including checks on scoring of tests and scales, were conducted periodically. Completed interviews were self- and peer-reviewed before data entry. Participants provided information on age and education and were asked to self-report whether they had been diagnosed with chronic conditions. Detailed information on prescription and over-the counter medications was collected by asking participants to show medication bottles. Frequency, history, and type of alcohol consumption and smoking were assessed. A physical activity score was calculated as the weighted sum of hours spent on activities during a typical 24-h period. Diabetes was defined as fasting glucose $\geq$126 mg/dL or use of diabetes medication. Genetic polymorphisms, including apolipoprotein E (APOE), were identified with applied Biosystems TaqMan SNP genotyping systems. Dietary intake was assessed with a semiquantitative FFQ, adapted and validated against 24-h recalls and several plasma measures such as vitamin B-12, vitamin E, and plasma carotenoids for this population. Based on the National Cancer Institute—Block format, the FFQ was revised to include foods, portion sizes, and recipes appropriate to this population (14–18).

### Blood samples

Fasting blood samples (12-h) were drawn from participants by a certified phlebotomist on the morning after the interview, or as soon as possible thereafter, in the participant's home. Blood was collected into evacuated tubes containing EDTA and immediately centrifuged at 964 $\times$ *g* at 4°C for 15 min. All tubes were shielded from light during specimen collection, processing, and handling. All samples were brought to the Nutrition Evaluation Laboratory at the Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University for further processing and storage. Samples were stored in cryogenic tubes at −80°C until further analysis. Plasma folate and vitamin B-12 concentrations were measured with a competitive, liquid-phase, ligand-labeled, protein-binding chemiluminescent assay with a commercially available kit from Diagnostics Products Corporation (IMMULITE 1000). Plasma pyridoxal-5-phosphate (PLP) was measured with a radioenzymatic assay on a Beckman LS 6500 Scintillation Counter. The intra- and interassay CVs (19) for each of these analytes were as follows: plasma PLP, 5% and 7%, respectively; plasma folate, 4.2% and 6.7%, respectively; and plasma vitamin B-12, 5.3% and 11.4%, respectively. Total homocysteine was measured with an improved method based on principles described by Araki and Sako (20). This analysis employed a C18 column on a Waters HPLC instrument equipped with a WISP automatic injector and attached to a fluorimeter (Krato from Applied Sciences Inc); detection was via a Perkin Elmer 650-15 Fluorescence Spectrophotometer (Perkin Elmer). Methylmalonic acid (MMA) in serum was determined after treatment with EDTA and protein precipitation solution by LC/MS/MS (API 5500 QTRAP, AB Sciex LLC; Agilent 1100 LC series, Agilent) according to Lakso et al. (21). MMA was measured with a range of 90–279 nmol/L, and intra- and interassay CVs were <10%. We defined deficient vitamin B-12 as plasma cobalamin <148 pmol/L or MMA > 271 nmol/L and deficient folate as <4.53 nmol/L, based on CDC recommendations (22), and high plasma folate as >59 nmol/L, based on a previously published cutoff defined by the 80th percentile in the 1999–2002 NHANES (9).

### Cognitive function

Participants completed a battery of neuropsychological tests intended to assess various aspects of cognitive functioning. All cognitive testing was conducted in the participant's language of choice (98% in Spanish) at the participants' home by trained interviewers, in consultation with a clinical neuropsychologist (TMS), and included the Mini-Mental State Examination as a measure of overall cognitive function (23); a 16-word list learning task for verbal memory; Digit Span forward and backward for attention and working memory (24); the Stroop test for processing speed, cognitive flexibility, and response to inhibition (24); clock drawing (25) and figure copying (26) for visual-spatial organization; and verbal fluency to assess verbal ability and executive function (24). Individual test scores were transformed to *z* scores and a global cognitive function composite score (GCS) was computed as the arithmetic mean of the individual *z* scores. The GCS was used as the outcome variable in this study, as done in prior work (27, 28). If a participant did not complete an individual test, the given score was imputed using the minimum *z* score of the same individual test for the rest of the cohort, unless the missing values were due to illiteracy, hearing impairment, or

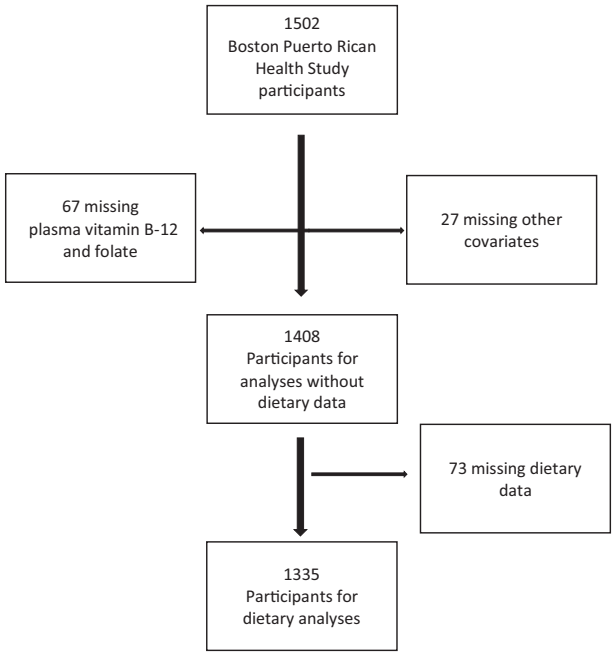


**FIGURE 1** Participant flowchart.

poor vision. In these cases, only the existing individual test values were averaged.

### Statistical analysis

Statistical analyses were conducted using the SAS System for Windows (version 9.1; SAS Institute, Inc). Descriptive analyses were performed to examine baseline characteristics across tertiles of plasma vitamin B-12 and plasma folate, by age, sex/estrogen status (female postmenopausal with no estrogen use, female premenopausal or postmenopausal with estrogen use, or male), BMI (in kg/m$^2$), physical activity score, level of education, APOE $\varepsilon$4 allele, diabetes status, vitamin B-6 (as PLP in nmol/L), smoking status (past, current, or never), and alcohol intake (heavy, moderate, or none). Multivariable linear regression models were used to estimate $\beta$ coefficients and 95% CIs for the association between plasma folate and vitamin B-12 and GCS. Of 1502 participants, we excluded 67 participants owing to missing plasma vitamin B-12 or folate measures and 27 owing to missing data on other covariates, leaving a sample size of 1408 for analyses (**Figure 1**). In analyses of dietary folic acid intake, we excluded an additional 73 individuals who reported intakes of <600 kcal or >4800 kcal or had >12 line items blank on the FFQ, as having invalid dietary data, leaving an analytical sample of 1335 for those analyses.

Models were adjusted for variables previously observed to be associated with cognitive function. These included age, sex/estrogen status, BMI, physical activity score, education (less than high school, high school, or higher), diabetes (yes/no), smoking (past, current, or none), alcohol use (heavy, none, or moderate), and, when appropriate, energy intake. Additional models were adjusted also for APOE ($\varepsilon$4 carrier) and plasma PLP (nmol/L), as well as mutually adjusted for vitamin B-12 and folate. PLP was included as a confounder owing to its relation with the other B vitamins and homocysteine, and possible effects on cognitive function (29).

## Results

The mean ± SD age of study participants at baseline was 57.1 ± 7.9 y and women comprised 70% of the sample (**Table 1**). The prevalence of diabetes was 40% and 48% had less than a high school education. Importantly, 124 of 1408 (9%) participants had vitamin B-12 deficiency, based on the CDC definition (plasma vitamin B-12 < 148 pmol/L or MMA > 271 nmol/L) (22), and none were folate deficient (<4.53 nmol/L). Following Morris et al. (10), based on NHANES data for high plasma folate (>59 nmol/L), 16.1% had high plasma folate. Although the true value may be underestimated with the FFQ, only 0.3% had folic acid intake above the upper limit (UL) of 1000 $\mu$g/d (**Table 2**).

After adjusting for covariates [age, sex (postmenopausal women with no estrogen use, premenopausal or postmenopausal women with estrogen use, or male), APOE ($\varepsilon$4 carrier), BMI, physical activity score, diabetes status (yes/no), education level (less than high school, high school, or higher), smoking (past, current, or none), alcohol use (heavy, none, or moderate), and PLP (nmol/L)], we observed positive associations of both log plasma folate and vitamin B-12, modeled as linear variables, with GCS ($\beta$: 0.063; 95% CI: −0.0008, 0.127; $P$ = 0.053 and $\beta$: 0.062; 95% CI: 0.009, 0.12; $P$ = 0.023, respectively). Individuals in the highest tertile of plasma vitamin B-12 had significantly higher GCS than those in the lowest tertile, with a mean difference of 0.121 $z$ score units ($P$ = 0.033; $P$-trend = 0.036) (**Figure 2**). Individuals in the highest tertile of plasma folate also had higher GCS than those in the lowest tertile, with a mean difference of 0.083 $z$ score units ($P$ = 0.053; $P$-trend = 0.044) (**Figure 3**). Plasma folate, vitamin B-12, and PLP concentrations were intercorrelated in our participants, and further, those in the highest tertiles of plasma folate and of vitamin B-12 also had the highest plasma concentration of PLP (Table 1). Deficient vitamin B-12 status, relative to adequate vitamin B-12, was significantly associated with lower GCS ($\beta$: −0.119; 95% CI: −0.208, −0.029; $P$ = 0.009) (**Table 3**). There were too few individuals with high folate and low vitamin B-12 status (<1% of the cohort) to test for interaction.

Among the 10 individuals with high folate and low vitamin B-12, 7 were postmenopausal women, 3 were taking a folic acid supplement, 3 a B-complex, 2 multivitamins, and only 1 a vitamin B-12 supplement (**Table 4**). Interestingly, despite high plasma folate, the mean homocysteine concentration was significantly higher in this group than in those with adequate vitamin B-12 (Table 4).

## Discussion

As the population continues to age rapidly, dementia is of growing concern. Low concentrations of folate and vitamin B-12 appear to have important roles in the pathogenesis of cognitive impairment in the elderly population. Our findings support the hypothesis that higher plasma folate and vitamin B-12 concentrations are each associated with better GCSs, including linear associations above deficiency states. The $\beta$ coefficients

**TABLE 1** Baseline characteristics, by tertile of plasma folate and vitamin B-12 status, among 1408 Boston Puerto Rican Health Study participants[1]

| | Plasma folate, nmol/L | | | | Plasma vitamin B-12, pmol/L | | | |
|---|---|---|---|---|---|---|---|---|
| | Tertile 1 | Tertile 2 | Tertile 3 | *P* | Tertile 1 | Tertile 2 | Tertile 3 | *P* |
| Median | 26.3 | 39.2 | 58.5 | | 223 | 344 | 596 | |
| *n* | 467 | 473 | 468 | | 474 | 468 | 466 | |
| Age, y | 56.1 ± 7.6 | 57.1 ± 7.5 | 57.9 ± 7.3 | 0.001 | 56.8 ± 7.8 | 56.9 ± 7.3 | 57.2 ± 7.4 | 0.67 |
| Sex | | | | <0.0001 | | | | 0.63 |
| Male | 37.3 | 25.6 | 24.6 | | 31.0 | 27.0 | 29.4 | |
| Female pre- or postmenopausal with estrogen use | 14.0 | 14.6 | 12.0 | | 13.4 | 14.6 | 12.7 | |
| Female postmenopausal with no estrogen use | 48.8 | 59.8 | 63.5 | | 55.5 | 58.7 | 57.8 | |
| BMI | 31.9 ± 7.2 | 32.0 ± 6.6 | 31.7 ± 6.0 | 0.69 | 32.2 ± 6.9 | 31.9 ± 6.6 | 31.5 ± 6.3 | 0.19 |
| Physical activity[2] | 31.2 ± 4.4 | 31.6 ± 5.0 | 31.9 ± 4.9 | 0.09 | 31.4 ± 4.7 | 31.8 ± 4.9 | 31.6 ± 4.8 | 0.44 |
| Current smoking | 32.8 | 19.2 | 20.5 | <0.0001 | 23.1 | 26.9 | 22.7 | 0.23 |
| Alcohol use | | | | 0.57 | | | | 0.51 |
| Nondrinker | 27.8 | 28.5 | 31.6 | | 28.8 | 29.7 | 29.9 | |
| Heavy drinker[3] | 43.0 | 41.2 | 38.0 | | 43.5 | 38.1 | 40.7 | |
| Diabetes | 32.8 | 42.3 | 42.1 | 0.003 | 41.0 | 36.5 | 40.0 | 0.36 |
| ≤8$^{th}$-grade education | 46.2 | 48.8 | 44.9 | 0.24 | 52.2 | 44.7 | 43.4 | 0.05 |
| APOE ε4[4] | 20.8 | 18.8 | 16.9 | 0.02 | 19.7 | 17.8 | 18.4 | 0.91 |
| Vitamin B-12, pmol/L | 346 ± 163 | 396 ± 216 | 457 ± 221 | <0.0001 | | | | |
| Plasma folate, nmol/L | | | | | 38.2 ± 16.6 | 41.8 ± 17.2 | 48.8 ± 22.4 | <0.0001 |
| PLP,[5] nmol/L | 41.5 ± 38.9 | 53.0 ± 50.0 | 74.8 ± 77.9 | <0.0001 | 41.7 ± 28.6 | 52.5 ± 49.1 | 75.4 ± 83.1 | <0.0001 |
| Global cognitive *z* score | −0.050 ± 0.6 | −0.047 ± 0.6 | 0.034 ± 0.6 | 0.03 | −0.084 ± 0.6 | −0.015 ± 0.6 | 0.037 ± 0.5 | 0.004 |
| Supplement use | | | | | | | | |
| Multivitamins | 11.8 | 18.6 | 28.2 | <0.0001 | 14.8 | 17.5 | 26.4 | <0.0001 |
| Folic acid | 0.43 | 1.3 | 4.7 | <0.0001 | 1.9 | 1.3 | 3.2 | 0.11 |
| Vitamin B-12 | 1.72 | 1.69 | 2.35 | 0.71 | 1.9 | 1.3 | 2.6 | 0.36 |
| B complex | 3.65 | 5.51 | 7.69 | 0.027 | 5.1 | 3.8 | 7.9 | 0.021 |
| Total folate intake, DFE | 662 ± 336 | 738 ± 398 | 860 ± 414 | <0.0001 | 715 ± 373 | 741 ± 381 | 807 ± 418 | 0.001 |

[1] Values are means ± SDs or percentages unless otherwise indicated. APOE, apolipoprotein E; DFE, Dietary Folate Equivalents; PLP, pyridoxal-5-phosphate.
[2] Physical activity score (range: 24.3–62.6), weighted for total time sleeping and lying down, sitting activity, light activity, moderate activity, and vigorous activity.
[3] >1 drink/d for women or >2 drinks/d for men.
[4] APOE ε4 carrier.
[5] Vitamin B-6.

**TABLE 2** Vitamin B-12 and folate status among Puerto Rican adults[1]

| | Vitamin B-12 status | | |
|---|---|---|---|
| | Deficient[2] | Not deficient | Total |
| Plasma folate status | | | |
| Deficient[3] | 0 | 0 | 0 |
| Not deficient | 114 (8.1) | 1067 (75.8) | 1181 (83.9) |
| High[4] | 10 (0.71) | 217 (15.4) | 227 (16.1) |
| Total | 124 (8.8) | 1284 (91.2) | 1408 (100) |
| Intake of folic acid, $\mu$g/d | | | |
| ≤1000 | 118 (8.9) | 1213 (90.9) | 1331 (99.7) |
| >1000[5] | 0 | 4 (0.3) | 4 (0.3) |
| Total | 118 (8.9) | 1217 (91.1) | 1335 (100) |

[1] $n = 1408$ for plasma; $n = 1335$ for dietary intake. Values are $n$ (%).
[2] Vitamin B-12 deficiency was defined as <148 pmol/L or MMA > 271 nmol/L.
[3] Folate deficiency was defined as <4.53 nmol/L.
[4] High plasma folate was defined as >59 nmol/L.
[5] The upper limit for folic acid intake is >1000 $\mu$g/d.

of 0.063 SD units for log plasma folate and 0.062 for log plasma vitamin B-12 may be comparable with 1 y of global cognitive decline among community-dwelling adults in their mid-70s, without dementia (30). These findings are consistent with other studies, including several studies showing significant associations of low folate and vitamin B-12 with accelerated cognitive decline (7, 29, 31–34) and development of Alzheimer disease (35). It should be noted that although individuals in the highest tertile of plasma vitamin B-12 also had the highest plasma concentrations of folate and PLP, a significant relation between vitamin B-12 and cognition remained after adjusting for the other 2. A recent study identified the presence of microstructural damage in the brains of patients with asymptomatic vitamin B-12 deficiency (36). Importantly, Feldman et al. (37) suggested that, if detected early enough, dementia caused by vitamin B-12 deficiency could be reversible (38). A randomized crossover study reported that whey protein isolate high in vitamin B-12 improved cognitive function in older Australians with low vitamin B-12 status (39).

Vitamin B-12 deficiency appears to be an important problem in this population, with 124 participants (9%) deficient. Malabsorption of vitamin B-12 is thought to be the most common cause of vitamin B-12 deficiency in older adults, because inflammation of the gastric mucosa increases with age and results in a reduction of stomach acid, which is required to cleave vitamin B-12 from protein (6). Vitamin B-12 is an important coenzyme for the methyl donation from 5-methyltetrahydrofolate to tetrahydrofolate and it plays an important role in methionine synthesis (40). A number of methylation reactions take place in the brain, including DNA-methylation, synthesis and degradation of neurotransmitters, and of membrane phospholipids. In addition, vitamin B-12 is essential in the maintenance of the myelin sheath of the central and peripheral nervous systems (41, 42). In either folate or vitamin B-12 deficiency, the methionine synthase reaction will be eventually impaired. Hyperhomocysteinemia in vitamin B-12 or folate deficiency is important as a sign of hypomethylation, for example, of DNA, RNA, myelin, phospholipids, or neurotransmitters. The result is reduced methionine synthesis, with subsequent lowering of the S-adenosyl methionine concentration (42). With insufficient vitamin B-12, methylmalonyl CoA (MMA) accumulates and may result in memory loss due to an accumulation of abnormal fatty acids in the membranes of neural tissue (5, 43). Therefore, vitamin B-12 is central in protecting cognitive function and against dementia in the aging population.

Plasma folate was also associated linearly with higher cognitive function in this population, despite the fact that none of the participants had folate deficiency based on the CDC cutoff point. Folate deficiency sometimes includes consideration of homocysteine. However, high homocysteine in this population was more likely due to the low vitamin B-12 and low PLP, because these were common, whereas none had deficient folate. Folate works with vitamin B-12 in the

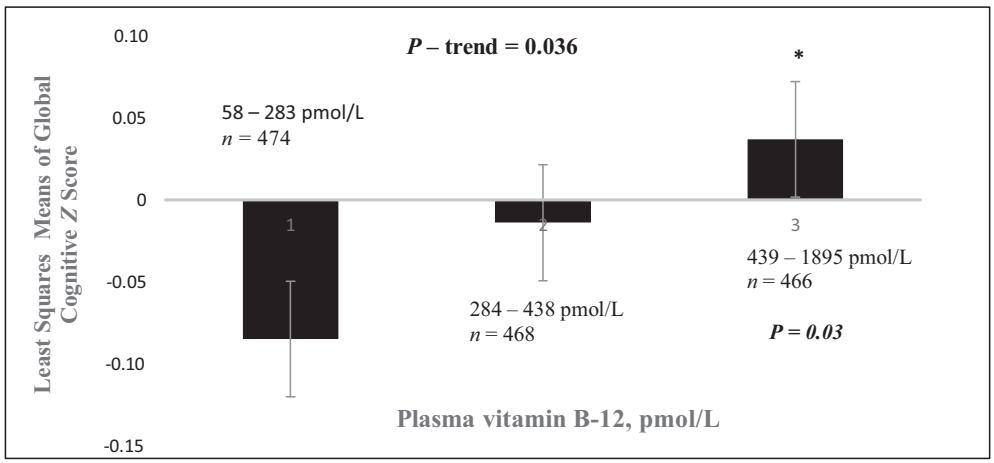


**FIGURE 2** Global cognitive function by tertile of plasma vitamin B-12, among 1408 Boston Puerto Rican Health Study participants at baseline. Adjusted for age (y), sex (premenopausal or postmenopausal female with estrogen use, female postmenopausal with no estrogen use, or male), BMI (in kg/m$^2$), physical activity score, diabetes (yes/no), education level (≤8$^{th}$ grade, 9$^{th}$–12$^{th}$ grade, or college), smoking (past, current, or none), alcohol use (heavy, none, or moderate), apoE $\varepsilon$4 carrier (yes/no), vitamin B-6 (pyridoxal-5-phosphate; nmol/L), and folate (nmol/L). *Significantly different from tertile 1 ($P = 0.03$).

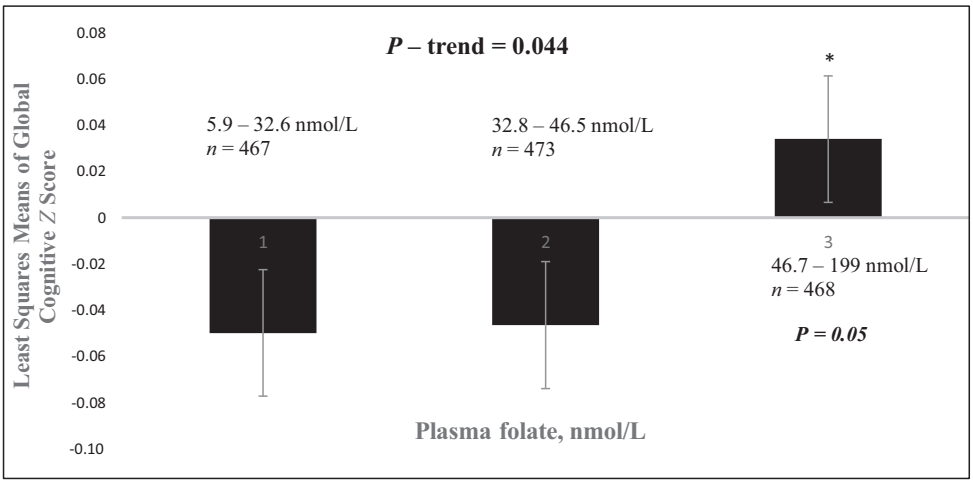


**FIGURE 3** Global cognitive function by tertile of plasma folate, among 1408 Boston Puerto Rican Health Study participants at baseline. Adjusted for age (y), sex (premenopausal or postmenopausal female with estrogen use, female postmenopausal with no estrogen use, or male), BMI (in kg/m$^2$), physical activity score, diabetes (yes/no), education level ($\leq$8$^{th}$ grade, 9$^{th}$–12$^{th}$ grade, or college), smoking (past, current, or none), alcohol use (heavy, none, or moderate), apoE $\varepsilon$4 carrier (yes/no), vitamin B-6 (pyridoxal-5-phosphate; nmol/L), and vitamin B-12 (pmol/L). *Significantly different from tertile 1 ($P = 0.05$).

methionine synthase reaction important for the production of DNA and RNA in cells and tissues (4, 42). Several studies have previously identified a relation between plasma folate and cognitive function cross-sectionally (8, 44, 45) and longitudinally (5, 29, 46).

Since the introduction of folic acid in the American food supply in the 1990s via fortification of cereal grain products, along with the promotion of folic acid for protection against neural tube defects in women of childbearing age, exposure to folic acid has increased in the entire population. The UL for folic acid, established by the National Academies Food and Nutrition Board, is 1000 $\mu$g/d (47). With 400 $\mu$g in a typical vitamin supplement, 400 $\mu$g in a serving of fortified breakfast cereal, and an estimated 200 $\mu$g/d from fortified grains (47), it is easy to exceed this limit, raising concern about potential negative effects of high folic acid in combination with vitamin B-12 deficiency. In this cohort, few individuals exceeded 1000 $\mu$g/d ($n = 4$, 0.3%), although this may be underestimated due to the semiquantitative nature of the FFQ.

**TABLE 3** Cognitive function (global $z$ score) by plasma vitamin B-12 deficiency (yes/no) among 1408 Boston Puerto Rican Health Study participants at baseline[1]

| Vitamin B-12 | $\beta$ (95% CI) | $P$-trend |
|---|---|---|
| Model 1 | −0.135 (−0.224, −0.047) | 0.003 |
| Model 2 | −0.128 (−0.217, −0.038) | 0.005 |
| Model 3 | −0.119 (−0.208, −0.029) | 0.009 |

[1] Vitamin B-12 deficiency was defined as <148 pmol/L or MMA > 271 nmol/L. Model 1: adjusted for age (y), sex (premenopausal or postmenopausal female with estrogen use, female postmenopausal with no estrogen use, or male), BMI (in kg/m$^2$), physical activity score, diabetes (yes/no), education level ($\leq$8$^{th}$ grade, 9$^{th}$–12$^{th}$ grade, or college), smoking (past, current, or none), alcohol use (heavy, none, or moderate), and apoE $\varepsilon$4 carrier (yes/no). Model 2: Model 1 + plasma vitamin B-6 (pyridoxal-5-phosphate; nmol/L). Model 3: Model 2 + plasma folate (nmol/L).

In this sample, there were too few individuals with vitamin B-12 deficiency and high plasma folate or folic acid intake above the UL, limiting the power to conduct interaction analysis. These results are, therefore, unable to support those reported from the Framingham study (5), as well as other studies, which showed more pronounced metabolic evidence of cognitive impairment with vitamin B-12 deficiency in the presence of high intakes of folic acid, than with lower intakes of folic acid (10, 48, 49). Among the small group with high folate and vitamin B-12 deficiency, supplement use was relatively high for both vitamin B-12 and folate, yet total homocysteine was high, which may suggest that this category contains individuals with vitamin B-12 malabsorption. However, the numbers were insufficient to draw conclusions and the evidence of an adverse impact of high folic acid exposure combined with low vitamin B-12 status on cognitive function must be further studied to evaluate the hypothesis that neurodegenerative consequences of vitamin B-12 deficiency may be exacerbated by high folic acid intake.

Strengths of our study include a comprehensive battery of culturally appropriate tests of cognition, and a thorough assessment of B vitamin status, diet, and relevant covariates. Limitations of our study include insufficient numbers of individuals in the combination of low vitamin B-12 and high folate or high folic acid intake, leading to inconclusive results. Reliance on the FFQ may have led us to underestimate the number of individuals with folic acid exposure > 1000 $\mu$g/d. Further limitations include the cross-sectional design, limiting our ability to examine the temporal evidence of cause–effect relations. This cohort study includes Puerto Rican older adults, with on average a lower education than that of the general population, limiting generalizability to other ethnicities and socioeconomic backgrounds.

**TABLE 4** Characteristics of 1408 Boston Puerto Rican Health Study participants, by vitamin B-12 deficiency and high plasma folate status[1]

| | Deficient B-12/high folate | Deficient B-12/not high folate | Adequate B-12/high folate | Adequate B-12/not high folate | *P* |
|---|---|---|---|---|---|
| *n* | 10 | 114 | 217 | 1067 | |
| Age, y | 58.8 ± 7.9 | 59.4 ± 7.8 | 59.07 ± 7.0 | 56.4 ± 7.4 | <0.0001 |
| Sex | | | | | 0.002 |
| Male | 2 (20.0) | 43 (37.7) | 44 (20.3) | 321 (30.1) | |
| Pre- or postmenopausal female with estrogen use | 1 (10.0) | 7 (6.1) | 26 (12.0) | 156 (14.6) | |
| Postmenopausal female with no estrogen use | 7 (70.0) | 64 (56.1) | 147 (67.7) | 590 (55.3) | |
| Physical activity[2] | 29.7 ± 3.6 | 30.7 ± 4.2 | 32.2 ± 5.4 | 31.5 ± 4.7 | 0.02 |
| PLP,[3] nmol/L | 42.0 ± 21.2 | 34.9 ± 21.2 | 79.5 ± 67.4 | 54.2 ± 59.5 | <0.0001 |
| Homocysteine, $\mu$mol/L | 15.8 ± 14.7 | 12.7 ± 7.2 | 7.9 ± 2.8 | 8.8 ± 3.9 | <0.0001 |
| Global cognitive *z* score | −0.269 ± 1.1 | −0.224 ± 0.6 | 0.043 ± 0.5 | −0.009 ± 0.5 | <0.0001 |
| Multivitamin supplement | 2 (20.0) | 15 (13.1) | 75 (34.6) | 183 (17.1) | <0.0001 |
| Folic acid supplement | 3 (30.0) | 2 (1.8) | 13 (6.0) | 12 (1.1) | <0.0001 |
| Vitamin B-12 supplement | 1 (10.0) | 1 (0.9) | 5 (2.3) | 20 (1.9) | 0.23 |
| B complex supplement | 3 (30.0) | 5 (4.4) | 19 (8.8) | 52 (4.9) | 0.0008 |

[1]Values are means ± SDs or *n* (%) unless otherwise indicated. Groups did not differ significantly in BMI, education, alcohol use, smoking, diabetes, or apoE $\varepsilon$4. Vitamin B-12 deficiency was defined as <148 pmol/L or MMA > 271 nmol/L. High plasma folate was defined as >59 nmol/L. PLP, pyridoxal-5-phosphate.

[2]Physical activity score (range: 24.3–62.6), weighted for total time sleeping and lying down, sitting activity, light activity, moderate activity, and vigorous activity.

[3]Vitamin B-6.

In conclusion, low plasma vitamin B-12 and low plasma folate were each associated with worse global cognitive function in this high-risk population of Puerto Rican adults. Importantly, vitamin B-12 deficiency, which remained significantly associated with poorer cognitive function after full adjustment for confounders including other B vitamins, was prevalent and should be monitored in aging populations. Larger studies are needed to test the possible detrimental impact of a combination of high folate and vitamin B-12 deficiency status on cognitive decline.

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