Effect of oral vitamin B-12 with or without folic acid on cognitive function in older people with mild vitamin B-12 deficiency: a randomized, placebo-controlled trial[1–3]

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ABSTRACT

Background: Vitamin B-12 deficiency is associated with cognitive impairment in older people. However, evidence from randomized trials of the effects of vitamin B-12 supplementation on cognitive function is limited and inconclusive.

Objective: The objective was to investigate whether daily supplementation with high doses of oral vitamin B-12 alone or in combination with folic acid has any beneficial effects on cognitive function in persons aged ≥70 y with mild vitamin B-12 deficiency.

Design: In a double-blind, placebo-controlled trial, 195 subjects were randomly assigned to receive 1000 μg vitamin B-12, 1000 μg vitamin B-12 + 400 μg folic acid, or placebo for 24 wk. Vitamin B-12 status was assessed on the basis of methylmalonic acid, total homocysteine (tHcy), and holotranscobalamin (holoTC) concentrations before and after 12 and 24 wk of treatment. Cognitive function was assessed before and after 24 wk of treatment with the use of an extensive neuropsychologic test battery that included the domains of attention, construction, sensomotor speed, memory, and executive function.

Results: Vitamin B-12 status did not change significantly after treatment in the placebo group; however, oral vitamin B-12 supplementation corrected mild vitamin B-12 deficiency. Vitamin B-12 + folic acid supplementation increased red blood cell folate concentrations and decreased tHcy concentrations by 36%. Improvement in memory function was greater in the placebo group than in the group who received vitamin B-12 alone ($P = 0.0036$). Neither supplementation with vitamin B-12 alone nor that in combination with folic acid was accompanied by any improvement in other cognitive domains.

Conclusion: Oral supplementation with vitamin B-12 alone or in combination with folic acid for 24 wk does not improve cognitive function. *Am J Clin Nutr* 2006;84:361–70.

KEY WORDS Elderly, vitamin B-12 deficiency, oral supplementation, cognitive function

INTRODUCTION

Vitamin B-12 deficiency is common in older persons and results from the inability to release vitamin B-12 from food proteins (food malabsorption), intestinal malabsorption, or inadequate intake (1–3). Vitamin B-12 is involved in one-carbon metabolism, during which it plays a role in the transfer of methyl groups and methylation reactions that are important for the synthesis and metabolism of neurotransmitters and phospholipids in the central nervous system (4). Moreover, vitamin B-12 is required for nucleic acid synthesis and hematopoiesis (3) and for the metabolism of fatty acids and amino acids in the mitochondrial citric acid cycle (5). In addition to causing anemia, vitamin B-12 deficiency has been linked with several neurologic disorders, such as neuropathy, myelopathy, dementia, depression, memory impairment, and cerebrovascular disease (6, 7). Although prolonged vitamin B-12 deficiency may eventually result in irreversible neurologic damage and cognitive impairment (8, 9), early stages of vitamin B-12 deficiency—detected by elevated concentrations of plasma total homocysteine (tHcy) and methylmalonic acid (MMA) (7) and decreased concentrations of holotranscobalamin (holoTC) (10)—may result in milder forms of cognitive impairment in the absence of anemia (11, 12).

Several cross-sectional and prospective studies in both healthy and cognitively impaired older persons have reported associations between impaired vitamin B-12 status and cognitive function (13–15). Intervention trials of vitamin B-12 supplementation and cognitive function have been performed (9, 16–26), of which only 3 were randomized and placebo-controlled (23–25). The results of these trials are inconclusive, possibly because of variations in study duration, sample size, characteristics of study population, diagnosis and treatment of vitamin B-12 deficiency,

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and assessment of cognitive function. It is possible that beneficial effects of vitamin B-12 supplementation on cognition may be related to the duration and severity of cognitive impairment (8, 27). For example, Martin et al (9) postulated that the time frame to reverse milder forms of cognitive impairment with vitamin B-12 treatment is limited in older persons.

In a recent dose-finding study in older persons to determine the minimum effective dosage of oral vitamin B-12 supplementation to correct mild vitamin B-12 deficiency, we found that a daily dose of 650 to 1000 μg/d was required to correct biochemical signs of impaired vitamin B-12 status (28). The aim of the present trial was to investigate the effects of oral vitamin B-12 supplementation alone or in combination with folic acid for 24 wk on cognitive function in older persons with mild vitamin B-12 deficiency with no to moderate cognitive impairment.

SUBJECTS AND METHODS

Recruitment and eligibility of participants

Free-living older persons and older persons living in care-facility homes aged ≥70 y were recruited from different parts of the Netherlands via mailed health questionnaires. Individuals were excluded if they reported a history of cobalamin deficiency, use of cobalamin (>50 μg/d) or folic acid (>200 μg/d) supplementation or injections, surgery or diseases of the stomach or small intestine, anemia, dementia, life-threatening diseases, or severe hearing or visual problems. Medication interfering with vitamin B-12 absorption (29) was permitted if it had been provided ≥3 mo before the screening of vitamin B-12 status and was intended to be continued for the duration of the trial. Screening for vitamin B-12 status was carried out between April 2003 and March 2004. Individuals who fulfilled the criteria for mild vitamin B-12 deficiency were eligible to enter the run-in period. Mild vitamin B-12 deficiency was defined as *1*) a serum vitamin B-12 concentration between 100 and 200 pmol/L, or 2) a serum vitamin B-12 concentration between 200 and 300 pmol/L, a plasma MMA concentration ≥0.32 μmol/L, and a serum creatinine concentration ≤120 μmol/L, the latter intended to exclude severe impairment of renal function (3). A summary of the recruitment procedure and the flow of participants included in the study is shown in **Figure 1**. The Medical Ethics Committee of Wageningen University approved the study protocol. The management of care-facility homes provided informed consent, and written informed consent was obtained from all individuals before the screening for impaired vitamin B-12 status began.

Study design and protocol

Individuals with mild vitamin B-12 deficiency took placebo for 2 wk before randomization (run-in period). The mean (±SD) time elapsed between the screening and run-in periods was 6 ± 3 wk. During the run-in period, subjects were excluded from further participation if they ingested <90% of the capsules or if they scored <19 points (maximum 30 points) on the Mini-Mental State Examination (MMSE). Eligible participants were randomly assigned to receive 24 wk of treatment in a parallel group design with daily oral doses of *1*) 1000 μg vitamin B-12, *2*) a combination of 1000 μg vitamin B-12 and 400 μg folic acid, or *3*) a placebo capsule (Figure 1). The doses selected for this study were based on previous dose-finding studies for oral vitamin B-12 (28) and folic acid (30). Vitamin B-12 was administered as cyanocobalamin. The capsules given to the separate

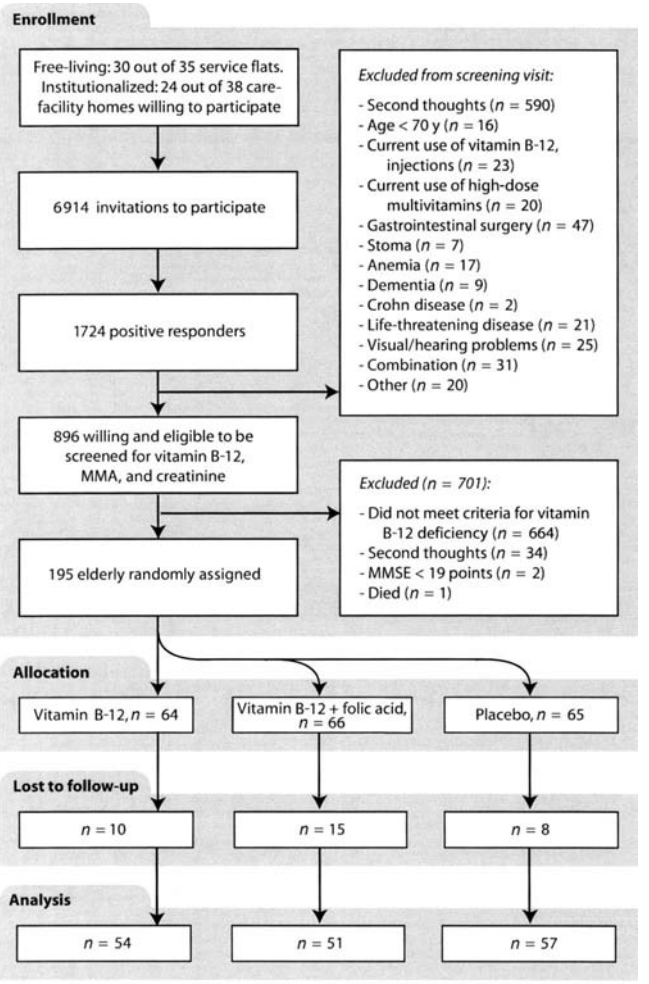

FIGURE 1. Recruitment procedure and flow of participants during the study. MMA, methylmalonic acid; MMSE, Mini-Mental State Examination.

treatment groups were identical in appearance, smell, and taste. The placebo capsules contained AVICEL PH102 (Medipulp GmbH, Aschaffenburg, Germany) as a filler. The mean (±SD) measured doses of vitamin B-12 for the capsules containing vitamin B-12 or vitamin B-12 + folic acid were 986 ± 3.4 and 987 ± 3.8 μg, respectively. The mean (±SD) measured dose of folic acid for the vitamin B-12 + folic acid capsules was 357 ± 6.0 μg.

Sample size calculations indicated that 45 participants per group had 80% power to detect an absolute difference of 3 points between the intervention groups in word fluency scores induced after vitamin B-12 injections, assuming a within-person SD of 4.4 points in word fluency (21). To control for an estimated dropout rate of 23% (31), ≥55 participants were to be enrolled in each group.

Randomization was stratified according to MMA concentration at the screening visit (< and >0.45 μmol/L), age (< and >80 y), sex, and MMSE score (< and >24 points). The study had a double-blind design.

The participants were asked to maintain their regular diet and to record in a diary their daily intake of capsules, use of medication, and occurrence of any new illnesses during the trial. Compliance was checked by counting the number of unused

capsules remaining in capsule dispensers and by verifying pill counts in the participants' diaries. Nurses were asked to monitor the daily capsule intake of the institutionalized participants.

Medical history, lifestyle, and anthropometric measures

The questionnaire collected information on medical history and issues related to vitamin B-12 status and cognitive function (32). The participants were asked to indicate "yes" or "no" to questions about history or presence of myocardial infarction, coronary bypass, stroke, transient ischemic attack, angina pectoris, diabetes mellitus, and hypertension. The participants were also asked about medication use, subjective memory and depressive complaints, smoking status, alcohol consumption, and diet (vegetarian or vegan). Education was classified as "low" (ie, less than primary school or primary school), "intermediate" (ie, less than low vocational training, low vocational training, or mean vocational training), or "high" (ie, high vocational training or university-level training). Body height and weight were measured at baseline while the participants were in a standing position and wearing light clothing and no shoes. Body weight was measured to the nearest 0.5 kg with a calibrated mechanical balance (Seca, Hamburg, Germany), and body height was measured to the nearest 0.1 cm.

Blood

A blood sample was collected at both the screening and randomization visits and after 12 and 24 wk of active treatment. The participants were allowed to eat a light breakfast (without fruit, fruit juices, meat, or eggs) ≥1 h before blood collection. A sample of blood for subsequent measurement of MMA, tHcy, and holoTC was collected into a 10-mL evacuated tube containing EDTA. This blood sample was placed on ice water and centrifuged at 2600 × *g* for 10 min at a temperature of 4 °C within 30 min of collection. All plasma samples were stored at −80 °C before laboratory analyses. Plasma concentrations of MMA were determined by a liquid chromatography electrospray ionization tandem mass spectrometry system (HJ Blom, oral communication, 2005). Plasma tHcy concentrations were measured by using a method based on methylchloroformate derivatization and gas chromatography–mass spectrometry (33), and plasma holoTC was measured by using the AXIS-Shield radioimmunoassay method (34). A second blood sample was collected into a 5-mL gel tube for measurement of serum vitamin B-12 and creatinine. The serum samples for vitamin B-12 measurement were stored at room temperature in the dark for measurement later that day with the IMMULITE 2000 cobalamin method (35). A third blood sample was collected into a 5-mL evacuated tube containing EDTA and stored between 4 and 8 °C before the measurement of red blood cell (RBC) folate on the same day of blood collection and of hematologic variables (hemoglobin, hematocrit, mean cell volume, and hypersegmentation of neutrophils) at the randomization visit.

Cognitive function

Cognitive function was assessed by 6 trained and registered neuropsychologists during the run-in period (baseline) and at week 24 of the intervention during a 1.5–2-h session. The MMSE (36), Clinical Dementia Rating (CDR) Scale (37), and Geriatric Depression Scale (GDS) (38) were used to describe the study population. Individuals with an MMSE score <19 points (maximum 30 points) were excluded. The CDR classified the study population into participants with no cognitive impairment (CDR = 0), mild cognitive impairment (MCI; CDR = 0.5), moderate cognitive impairment (CDR = 1), or severe cognitive impairment (CDR = 2). The neuropsychologists ascribed a score to the CDR according to results of the cognitive test battery described in **Table 1** and an interview based on the criteria developed by Petersen et al (49). Tests that have been shown to be sensitive to the effects of B vitamin treatment and aging in previous studies (14, 21) were used to measure the potential effects of vitamin B-12 supplementation on cognitive function. Because cognitive status can be influenced by depression (50), the presence of depression (defined as a score of ≥5 out of 15 possible points) was assessed by the GDS. The order of the assessment and a description of the tests, including their corresponding cognitive domain and neuropsychologic focus, are listed in Table 1.

Statistical methods

All analyses were carried out, on a per protocol basis, including the 162 participants (84%) who completed the trial. Baseline characteristics between treatment groups were compared by one-factor analysis of variance (ANOVA) for continuous variables and chi-square analysis for categorical variables. The average concentrations of the biochemical variables at the screening and randomization visits were calculated for each participant and defined as "baseline" values. Differences in concentrations of blood variables at baseline and at follow-up were assessed with a 2-factor repeated-measures ANOVA (3 measurements × 3 treatment groups) that included the time × treatment interaction. Tukey's post hoc tests were used to assess differences between intervention groups.

Data on cognitive function were presented as the neuropsychologic domains of attention, construction, sensomotor speed, memory, and executive function. The domains of attention and construction were assessed with the use of a single cognitive test, whereas the other domains were assessed with the use of multiple tests. All crude test scores were transformed to *z* scores by: *z* score = (individual result − mean result at baseline)/SD at baseline. For most of the individual neuropsychologic tests, higher scores indicate a better cognitive performance, except for all tests of sensomotor speed, motor planning task 3, the Stroop test (part C/part A), and the trail making tests (part 3/part 2), for which a higher score indicates lower cognitive performance. To achieve consistency in the interpretation of results, we multiplied the crude test scores from these tests by −1 before transforming them into a *z* score. The multiple tests for the domains of sensomotor speed, memory, and executive function were clustered to provide compound *z* scores to reduce the effects of chance findings and to simplify interpretation of the cognitive data: attention $= Z_{\text{digit span forward}}$; construction $= Z_{\text{Rey, copy}}$; sensomotor speed $= (-Z_{\text{motor planning2}} + -Z_{\text{finger tapping}} + -Z_{\text{trail making, part A}})/3$; memory $= (Z_{\text{15word learning, immediate}} + Z_{\text{15word learning, delayed}} + Z_{\text{15word learning, recognition}} + Z_{\text{Rey, immediate}} + Z_{\text{Rey, delayed}} + Z_{\text{digit span backward}})/6$; executive function $= (-Z_{\text{motor planning 3}} + -Z_{\text{trail making (part C/part A)}} + -Z_{\text{Stroop (part 3/part 2)}} + Z_{\text{similarities (WAIS)}} + Z_{\text{Raven}} + Z_{\text{word fluency(animals)}} + Z_{\text{word fluency(letter)}})/7$.

Tests that were clustered for each cognitive domain correlated well with each other. Spearman's rank correlation coefficients varied from 0.43 to 0.50 ($P < 0.0001$ for all) within the domain

The American Journal of Clinical Nutrition

TABLE 1
Description of neuropsychologic test battery with corresponding domain and neuropsychologic focus[1]

Task	Domain	Neuropsychologic focus	Description
MMSE (36)	All	Global cognitive function	Screening tool. Exclusion from further participation if score <19 points at first visit
Finger tapping, computerized (39)	Sensomotor speed	Simple sensomotor speed	Press a single button as often as possible within 30 s
Motor planning 2, computerized (39)	Sensomotor speed	Simple visuomotor reaction	Press a lit button out of 3 buttons as quickly as possible
Motor planning 3, computerized (39)	Executive function	Complex visuomotor reaction	Inhibit automatic reaction in pressing a button immediately adjacent to a lit button as quickly as possible
Figure of Rey, copy (40)	Construction	Visuoconstruction	Copy the complex figure of Rey from an example
Figure of Rey, immediate recall (40)	Memory	Visual immediate memory	Draw the complex figure of Rey without the example immediately after the copy
15 Word learning, immediate recall (41)	Memory	Verbal immediate memory	Read 15 words 5 times and recall words in between readings
Trail making test, part A (42)	Sensomotor speed	Visuomotor speed	Connect randomly placed numbers with a line as fast as possible
Trail making test, part B (42)	Executive function	Concept shifting	Connect randomly placed numbers and letters alternated with a line as fast as possible
Digit span forward (43)	Attention	Attention	Repeat a string of digits in original order
Digit span backward (43)	Memory	Working memory	Repeat a string of digits in reverse order
Raven (44)	Executive function	Visual reasoning	Choose a design that fits into a matrix
Stroop test (45, 46)	Executive function	Interference	Name the color of ink while inhibiting the automatic response of reading rather than the word (part 3). Part 1: reading names of colors red, green, yellow, and blue; part 2: naming colored blocks red, green, yellow, and blue
Figure of Rey, delayed recall (40)	Memory	Visual delayed memory	Draw the complex figure of Rey without the example 30 min after seeing the copy
15 Word learning, delayed recall (41)	Memory	Verbal delayed memory	Recall the words of the 15-word learning test
15 Word learning, recognition (41)	Memory	Consolidation	Recognize the original 15 words of 30 words read
Similarities, WAIS (47)	Executive function	Verbal reasoning	Mention similarities between 5 pairs of nouns
Word fluency, letter (48)	Executive function	Word generation	List as many nouns beginning with the letter P or G as possible in 2 min
Word fluency, animals (48)	Executive function	Word generation	List as many animals as possible in 1 min
GDS (38)	Emotional status	Depression	Self-rating scale for depression

[1] Tasks ordered by assessment. MMSE, Mini-Mental State Examination; WAIS, Wechsler Adult Intelligence Scale; GDS, Geriatric Depression Scale.

of sensomotor speed, from 0.37 to 0.82 ($P < 0.0001$ for all) within the domain of memory, and from 0.28 to 0.57 ($P < 0.0001$ for all) within the domain of executive function. Some participants were unable to complete all of the tests because of performance difficulties, eg, tiredness. Compound z scores were calculated when data for ≥2, 4, and 5 tests for the domains of sensomotor speed, memory, and executive function, respectively, were available. The compound z scores served as internal z scores from which z scores at baseline and 24 wk by study treatment were derived.

To determine potential treatment effects within and between intervention groups for each cognitive domain, we performed a 2-factor repeated-measures analyses (2 measurements × 3 treatment groups) that included a time × treatment interaction. These analyses were performed with mixed models (SAS PROC MIXED procedure; 51), an extension from the linear regression model that includes random effects. Possible interinvestigator bias of the 6 neuropsychologists was entered as random effects. Tukey's post hoc tests were used to compare mean changes in z scores between treatment groups. All analyses were conducted by using SAS statistical software (version 9.1; SAS Institute Inc, Cary, NC), and the graph was created by using PRISM (version 4; GraphPad Software Inc, San Diego, CA).

RESULTS

We found that 25% (232 of 896) of the older people who were not supplemented had mild vitamin B-12 deficiency. The recruitment, enrollment, and flow of participants during the trial are depicted in Figure 1. Two of the 195 participants who underwent random assignment dropped out during the run-in period, which left data for 193 participants who started supplementation. Thirty-one participants (16%) were unable to complete the trial, mostly because of illness, and the dropout rate was slightly higher in the vitamin B-12 + folic acid group than in the other groups. There were no significant differences in vitamin B-12- and folate status and MMSE scores between the participants who withdrew from the trial and those who completed the trial. However, the participants who withdrew from the trial were slightly more depressed than were those who completed the trial. On the basis of the number of unused capsules in the returned dispensers,

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TABLE 2
Characteristics of older participants with mild vitamin B-12 deficiency, by treatment group[1]

Characteristic	Vitamin B-12	Vitamin B-12 + folic acid	Placebo
Demographic			
Age	82 ± 5[2]	83 ± 6	82 ± 5
Sex, male [*n* (%)]	15 (23)	17 (26)	14 (22)
Institutionalized [*n* (%)]	37 (58)	35 (53)	39 (60)
Education [*n* (%)]			
Low	24 (38)	28 (47)	23 (35)
Intermediate	31 (48)	26 (29)	35 (44)
High	9 (14)	12 (18)	7 (11)
Lifestyle [*n* (%)]			
Smoking status			
Exsmoker	22 (34)	18 (27)	20 (31)
Smoker	2 (3)	8 (12)	6 (9)
Social drinking	19 (30)	30 (46)	33 (51)
Vegetarian	4 (6)	2 (3)	3 (5)
Multivitamin use	14 (22)	11 (17)	14 (22)
Medical history [*n* (%)]			
Myocardial infarction	9 (14)	7 (11)	12 (18)
Coronary artery bypass	1 (2)	5 (8)	5 (8)
Stroke	3 (5)	6 (9)	1 (2)
Transient ischemic attack	9 (14)	16 (25)	14 (22)
Angina pectoris	10 (16)	12 (18)	13 (20)
Diabetes mellitus	5 (8)	4 (6)	9 (14)
Hypertension	15 (23)	22 (33)	16 (25)
H_2 antagonist, proton pump inhibitors	20 (31)	11 (17)	17 (26)
Neurologic symptoms			
MMSE score	26.7 ± 3.1	26.7 ± 3.0	26.8 ± 2.9
19–24 points, cognitive impairment [*n* (%)]	9 (14)	10 (15)	8 (12)
GDS	2.8 ± 2.6	3.2 ± 2.5	2.7 ± 2.7
> 5 points, depression [*n* (%)]	14 (22)	16 (25)	11 (17)
CDR = 0, no cognitive impairment [*n* (%)]	38 (59)	38 (59)	34 (66)
CDR = 0.5, mild cognitive impairment [*n* (%)]	19 (30)	16 (25)	16 (25)
CDR = 1, moderate cognitive impairment	7 (11)	8 (13)	6 (9)
CDR = 2, severe cognitive impairment	0 (0)	2 (3)	0 (0)
Self-perceived memory impairment [*n* (%)]	35 (55)	40 (61)	38 (58)
Self-perceived depression [*n* (%)]	22 (34)	20 (30)	16 (25)
Hematologic symptoms			
Hemoglobin (mmol/L)	8.5 ± 0.7	8.5 ± 0.8	8.5 ± 0.7
Mean cell volume (fL)	91 ± 5	91 ± 6	92 ± 6

[1] MMSE, Mini-Mental State Examination (36); GDS, Geriatric Depression Scale (38); CDR, Clinical Dementia Rating (39). No significant differences between the 3 treatment groups were observed, $P > 0.05$ (one-factor ANOVA for continuous variables and chi-square analysis for categorical variables).
[2] $\bar{x}$ ± SD (all such values).

mean compliance was 99% and 4 participants had a compliance of between 80% and 90%. No adverse effects from study treatment were reported.

Characteristics of participants

A summary of the demographic, lifestyle, comorbidity, and hematologic characteristics of the participants is shown in **Table 2**. These characteristics were not significantly different across the treatment groups. The randomization procedure was successful because age, male-female ratio, MMSE scores (Table 2), and plasma MMA concentrations (**Table 3**) did not differ significantly between the treatment groups. Anemia, defined as hemoglobin concentrations ≤8.1 mmol/L in men and ≤7.4 mmol/L in women, was present in 7% of the participants. Macrocytosis, defined as a mean cell volume (MCV) ≥100 fL, was present in 5% of the participants. Neutrophil hypersegmentation, defined as >5% of neutrophils with ≥5 lobes or the presence of ≥1 neutrophil with ≥6 lobes (52), was present in 54% of the participants. There were no significant differences in the prevalence of anemia, macrocytosis, and hypersegmentation across the treatment groups.

Blood biochemistry

Concentrations of vitamin B-12, MMA, and holoTC were not significantly different across groups, whereas tHcy concentrations increased slightly and RBC folate concentrations decreased slightly between the screening and randomization visit. The concentrations of vitamin B-12, MMA, holoTC, tHcy, and RBC folate at baseline and at 12 and 24 wk of supplementation are presented in Table 3. In the placebo group, no significant changes in blood variables were observed during the study period. There was a significant time × treatment interaction for vitamin B-12, MMA, holoTC, tHcy, and RBC folate ($P < 0.0002$ for all biochemical markers). Mean MMA concentrations were reduced to

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TABLE 3
Vitamin B-12, methylmalonic acid (MMA), holotranscobalamin (holoTC), homocystine, and red blood cell (RBC) folate concentrations in participants with mild vitamin B-12 deficiency, by treatment group at baseline and 12 and 24 wk of supplementation[1]

Marker	Vitamin B-12	Vitamin B-12 + folic acid	Placebo
Vitamin B-12 (pmol/L)			
Baseline	186 ± 56 (52)	199 ± 50 (49)	188 ± 56 (55)
12 wk	477 ± 194[2] (52)	538 ± 167[2] (49)	200 ± 76[3] (54)
24 wk[4]	530 ± 210[2] (52)	627 ± 209[3,5] (51)	185 ± 62 (54)
MMA (μmol/L)			
Baseline	0.47 ± 0.41 (52)	0.43 ± 0.22 (50)	0.46 ± 0.28 (55)
12 wk	0.23 ± 0.06[2] (52)	0.25 ± 0.09[2] (50)	0.46 ± 0.30[3] (54)
24 wk	0.22 ± 0.06[2] (52)	0.25 ± 0.10[2] (50)	0.48 ± 0.33[3] (53)
HoloTC (pmol/L)			
Baseline	58 ± 21 (52)	68 ± 33 (50)	70 ± 39 (54)
12 wk	183 ± 124[2] (52)	222 ± 133[2] (49)	65 ± 43[3] (54)
24 wk[4]	212 ± 118[2] (52)	282 ± 183[2] (51)	64 ± 42 (54)
Homocysteine (μmol/L)			
Baseline	15.6 ± 6.6 (52)	14.5 ± 4.4 (50)	15.8 ± 5.6 (55)
12 wk	13.4 ± 5.7[2] (52)	9.7 ± 2.5[2,6] (49)	15.5 ± 5.6 (54)
24 wk	12.8 ± 4.9[2] (52)	8.9 ± 2.4[2,6] (51)	16.1 ± 6.8 (54)
RBC folate (nmol/L)			
Baseline	578 ± 172 (52)	591 ± 203 (48)	680 ± 280 (55)
12 wk	694 ± 250[2] (52)	1179 ± 333[2,6] (49)	745 ± 353 (54)
24 wk	696 ± 271[2] (52)	1433 ± 418[2,5,6] (51)	670 ± 276 (54)

[1] All values are $\bar{x}$ ± SD; *n* in parentheses. A significant time × treatment interaction was observed for all biochemical markers, $P < 0.0002$ (ANOVA). No significant differences between the 3 treatment groups were observed at baseline for all biochemical markers, $P > 0.05$ (ANOVA with Tukey's post hoc tests).
[2] Significantly different from baseline, $P < 0.05$ (repeated-measures ANOVA with least-squares means).
[3] Significantly different from the vitamin B-12 and vitamin B-12 + folic acid groups, $P < 0.05$ (ANOVA with Tukey's post hoc tests).
[4] Significant differences between the 3 treatment groups were observed, $P < 0.05$ (ANOVA with Tukey's post hoc tests).
[5] Significantly different from 12 wk, $P < 0.05$ (repeated-measures ANOVA with least-squares means).
[6] Significantly different from the placebo group and the vitamin B-12 group, $P < 0.05$ (ANOVA with Tukey's post hoc tests).

normal concentrations, ie, <0.26 μmol/L after 12 wk of supplementation with vitamin B-12. Vitamin B-12 and vitamin B-12 + folic acid supplementation lowered mean tHcy concentrations by 16% and 37%, respectively. Concentrations of MMA and tHcy remained stable between 12 and 24 wk of supplementation with vitamin B-12 + folic acid supplementation, whereas concentrations of vitamin B-12, holoTC (borderline significant,) and RBC folate further increased during the last 12 wk.

Folate deficiency, defined as RBC folate < 305 nmol/L, was present in 2 (vitamin B-12 group), 6 (vitamin B-12 + folic acid group), and 3 (placebo group) participants at baseline and in 2 (vitamin B-12 group), 0 (vitamin B-12 + folic acid group), and 4 (placebo group) participants after 24 wk of supplementation.

Cognitive function after B vitamin supplementation

The mean crude scores from the individual tests at baseline and 24 wk of supplementation for the 3 intervention groups are shown in **Table 4**. Cognitive function improved slightly in all 3 groups, but most changes were not statistically significant. Significant improvement occurred mainly in the domain of memory for all 3 treatment groups.

The mean baseline scores for all of the compound cognitive domains did not differ significantly between treatment groups. There were no significant changes in cognitive function for the domains of attention and construction between the intervention groups after 24 wk of supplementation. Of the 162 participants who completed the trial, data for 141, 158, and 151 participants were included in the analysis of the domains of sensomotor speed, memory, and executive function, respectively, because some participants were unable to complete all of the tests. There was a significant time × treatment interaction for the domain of memory ($P = 0.0142$). The changes in these domains after 24 wk of supplementation are shown in **Figure 2**. The figure confirms the improvement in memory function in all 3 treatment groups. The improvement in the placebo group was significantly better than the improvement in the vitamin B-12 group ($P = 0.0036$). However, separate analysis of the results of the 6 memory tests indicated that only function on the digit span backward ($P = 0.0014$) and the 15 word learning (recognition) tests ($P = 0.0376$) showed this effect. Vitamin B-12 with or without folic acid supplementation did not result in improved function on the domains of sensomotor speed and executive function. No significant changes in GDS scores for emotional status were observed between the intervention groups after 24 wk of supplementation ($P = 0.316$).

DISCUSSION

The present randomized, double-blind, placebo-controlled trial in older persons with mild vitamin B-12 deficiency showed no improvement in cognitive function after 24 wk of vitamin B-12 supplementation when administered alone or in combination with folic acid compared with placebo.

Originally, a 2 × 2 factorial trial with placebo, vitamin B-12, folic acid, and vitamin B-12 + folic acid had been planned to assess the independent effects of vitamin B-12 and folic acid and any interaction between them. However, it was not possible to conduct such a trial because of the theoretical risk of masking

The American Journal of Clinical Nutrition

TABLE 4
Crude scores from neuropsychologic tests of cognitive function at baseline and after 24 wk of B vitamin supplementation in older persons with mild vitamin B-12 deficiency, by treatment group[1]

Test, maximum score	Vitamin B-12	Vitamin B-12 + folic acid	Placebo
Construction			
Complex figure of Rey, copy, 36 points			
Baseline	28.0 ± 8.7 (54)	27.5 ± 9.5 (50)	27.7 ± 8.7 (56)
24 wk	30.0 ± 7.5 (47)	28.5 ± 9.0 (48)	29.2 ± 7.0 (49)
Attention			
Digit span forward, 16 points			
Baseline	7.4 ± 1.5 (54)	7.5 ± 1.7 (51)	7.6 ± 1.7 (57)
24 wk	7.5 ± 1.7 (53)	7.4 ± 1.5 (51)	7.8 ± 1.6 (56)
Sensomotor speed			
Motor planning 2, milliseconds to press a button[2]			
Baseline	627 ± 330 (49)	606 ± 233 (46)	673 ± 318 (53)
24 wk	647 ± 265 (48)	635 ± 295 (44)	618 ± 300 (50)
Finger tapping, milliseconds to press a button[2]			
Baseline	453 ± 270 (49)	442 ± 232 (46)	409 ± 230 (53)
24 wk	412 ± 175 (48)	425 ± 217 (44)	389 ± 168 (50)
Trail making test, part A, seconds to complete the task[2]			
Baseline	75.4 ± 37.3 (54)	76.9 ± 51.9 (49)	72.0 ± 39.3 (56)
24 wk	77.5 ± 52.3 (53)	69.8 ± 49.0 (48)	73.9 ± 43.9 (56)
Memory			
15 Word learning, immediate recall, 75 points			
Baseline	30.9 ± 11.7 (54)	30.1 ± 10.1 (51)	30.0 ± 10.3 (57)
24 wk	35.2 ± 12.1 (53)	36.3 ± 11.1 (50)	35.7 ± 11.1 (56)
15 Word learning, delayed recall, 15 points			
Baseline	4.8 ± 3.6 (54)	4.6 ± 3.7 (51)	5.1 ± 3.0 (57)
24 wk	5.5 ± 3.9 (53)	6.1 ± 4.2 (50)	6.1 ± 3.9 (55)
15 Word learning, recognition, 30 points[3]			
Baseline	25.9 ± 3.6 (54)	26.6 ± 3.3 (51)	25.3 ± 4.4 (57)
24 wk	26.6 ± 3.7 (53)	27.4 ± 3.2 (50)	27.0 ± 3.6 (55)
Complex figure of Rey, immediate recall, 36 points			
Baseline	10.2 ± 7.1 (54)	10.8 ± 7.1 (50)	9.7 ± 6.8 (55)
24 wk	12.2 ± 7.7 (44)	14.0 ± 7.3 (43)	12.7 ± 7.4 (52)
Complex figure of Rey, delayed recall, 36 points			
Baseline	10.0 ± 6.6 (54)	10.3 ± 7.0 (50)	9.5 ± 6.3 (55)
24 wk	11.4 ± 7.0 (49)	12.2 ± 7.6 (43)	11.9 ± 7.3 (49)
Digit span backward, 14 points[3]			
Baseline	4.9 ± 1.9 (54)	5.1 ± 1.3 (51)	4.7 ± 1.8 (57)
24 wk	4.6 ± 1.6 (53)	4.9 ± 1.3 (51)	5.3 ± 1.7 (56)
Executive function			
Motor planning 3, milliseconds to press a button[2]			
Baseline	898 ± 449 (49)	1053 ± 514 (44)	1012 ± 481 (50)
24 wk	863 ± 376 (45)	1066 ± 637 (43)	990 ± 696 (49)
Trail making test (part C/part A)[4]			
Baseline	2.7 ± 1.2 (52)	2.7 ± 1.0 (47)	2.9 ± 1.0 (54)
24 wk	2.8 ± 1.2 (48)	3.1 ± 2.0 (46)	2.8 ± 1.0 (54)
Stroop test (part 3/part 2)[4]			
Baseline	2.2 ± 0.6 (48)	2.2 ± 0.7 (42)	2.1 ± 0.6 (51)
24 wk	2.2 ± 0.9 (46)	2.2 ± 0.7 (43)	2.8 ± 1.0 (54)
Similarities WAIS, 12 points			
Baseline	5.3 ± 2.7 (54)	4.7 ± 2.9 (51)	4.8 ± 3.1 (57)
24 wk	6.1 ± 2.6 (52)	5.8 ± 2.5 (50)	5.4 ± 2.8 (56)
Raven, 24 points			
Baseline	15.6 ± 3.6 (53)	15.2 ± 3.8 (49)	15.5 ± 4.1 (57)
24 wk	16.6 ± 3.5 (52)	16.7 ± 3.2 (47)	16.5 ± 3.9 (56)
Word fluency, animals, number of nouns			
Baseline	17.6 ± 6.3 (54)	17.6 ± 5.4 (51)	17.4 ± 5.5 (57)
24 wk	17.6 ± 5.5 (53)	17.3 ± 5.3 (50)	16.5 ± 5.9 (56)
Word fluency, letter, number of nouns[3]			
Baseline	16.2 ± 7.4 (54)	15.5 ± 6.7 (51)	15.2 ± 7.1 (57)
24 wk	15.5 ± 7.9 (53)	16.0 ± 7.7 (50)	17.5 ± 8.8 (55)

[1] All values are $\bar{x}$ ± SD; *n* in parentheses. The tests are described in Table 1. WAIS, Wechsler Adult Intelligence Scale (47). No significant differences between the 3 treatment groups were observed at baseline for any of the neuropsychologic tests, $P > 0.05$ (mixed models with Tukey's post hoc test).

[2] Higher scores indicate more time needed to complete a task and thus poorer performance.

[3] Treatment effects (changes from baseline within groups) are significantly different between the 3 treatment groups, as indicated by a significant time × treatment interaction, $P < 0.05$ (mixed models).

[4] Higher scores indicate poorer interference abilities.

The American Journal of Clinical Nutrition

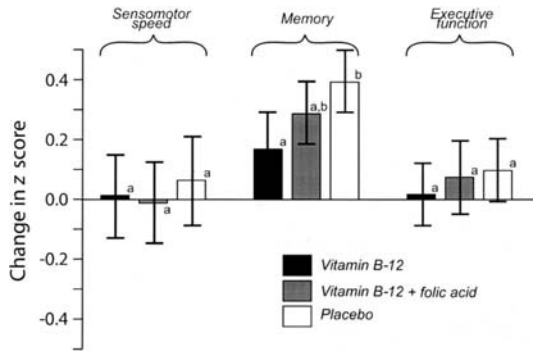

FIGURE 2. Mean changes (95% CI) in cognitive function after 24 wk of B vitamin supplementation in older persons with mild vitamin B-12 deficiency. In the vitamin B-12 group ($n = 54$), data for 47 (sensomotor speed), 53 (memory), and 51 (executive function) participants were available for analysis. In the vitamin B-12 + folic acid group ($n = 51$), data for 44 (sensomotor speed), 50 (memory), and 46 (executive function) participants were available for analysis. In the placebo group ($n = 57$), data for 50 (sensomotor speed), 55 (memory), and 54 (executive function) participants were available for analysis. Bars with different superscript letters are significantly different (mixed models with Tukey's post hoc test). The mean difference in change in z score between the vitamin B-12 and placebo groups in the domain of memory was 0.22 (95% CI: 0.07, 0.37).

vitamin B-12 deficiency and more rapid progression of neurologic symptoms in persons with vitamin B-12 deficiency who were treated with high-dose folic acid alone (53). Therefore, a compromise was made by including an intervention arm with coadministration of vitamin B-12 and folic acid. Because folate acts as a cosubstrate and methyl group donor in the methionine synthase reaction, which is vitamin B-12 dependent, additional folic acid supplementation would ensure improved remethylation of homocysteine to methionine (7). Indeed, we observed an additional homocysteine-lowering effect of combined treatment with both folic acid and vitamin B-12.

The present study was performed to confirm or refute the effects of vitamin B-12 supplementation on cognitive function observed in previous smaller, non-randomized placebo controlled trials (9, 19–22). We did this by using a larger sample size with a longer study duration and more rigorous methods to assess cognitive function. The results are consistent with previous randomized placebo controlled trials that reported null findings (23–25). The latter trials included older people with mild to moderate vitamin B-12 deficiency, and differed in criteria to identify vitamin B-12 deficiency. The durations of treatment varied from 1 to 5 mo, and treatment was administered either by injections (23, 25) or daily oral capsules with 10 to 50 μg vitamin B-12 (24), which is considered to be an insufficient oral dose to normalize vitamin B-12 deficiency (28). One trial included older people with severe cognitive impairment (23). All trials used some global neuropsychological tests to assess cognitive function, such as the MMSE, the Alzheimer Disease Assessment Scale—Cognitive Subscale (ADAS-cog), or the Cambridge Cognitive (CAMCOG) Examination. The present study adopted an extensive battery of sensitive neuropsychologic tests as the main outcome measure to assess the effects of supplementation; however, the comparison of study results was hampered by differences in the test batteries used in the different studies. The study by Hvas et al (25) showed that test scores on cognitive function improved slightly overall in both the treatment and the placebo groups, and improvement was greater in the placebo group. This finding could be explained by a placebo effect and a learning effect of repeated cognitive testing. The learning effect is considered to be small when parallel versions of tests are used, which was the case in the present study. Interestingly, we observed significantly less improvement in the domain of memory, on the basis of the compound z score, in the vitamin B-12 group than in the placebo group. However, the relevance of this finding is questionable because it was significant in only 2 of the 6 tests that measured memory function, which suggests that this was likely a chance finding. High-dose vitamin B-12 supplements are considered to be safe, and no tolerable upper intake level has been set for vitamin B-12 supplements in the United States (54) or Europe (55). Moreover, no adverse effects from high-dose cyanocobalamin supplements have been reported in previous trials or in the present trial. We therefore assumed it to be unlikely that the supplements might have had short-term deleterious effects on memory.

The strengths of this trial were its double-blind, randomized, placebo-controlled design; its relatively large number of carefully selected participants; and its extensive assessment of cognitive function relative to most previous trials with similar objectives. To detect any potential beneficial effects of vitamin supplementation on cognitive function, the dose of vitamins, duration of treatment, characteristics of the study population, and methods of cognitive assessment are important. The oral doses given were effective in correcting mild vitamin B-12 deficiency in peripheral blood, but it is still uncertain whether normalization of impaired plasma vitamin B-12 status reflects vitamin B-12 status in the cerebrospinal fluid and cells in the central nervous system.

Magnetic resonance imaging studies indicate that the repair of signs of demyelination may require treatment periods of >1 y with high-dose vitamin B-12 supplementation administered by injection, and resolution of clinical symptoms may require a longer period (56). However, a pilot trial had shown beneficial effects of vitamin B-12 injections on cognitive function after 5 mo of vitamin B-12 supplementation administered by injection in apparently healthy older persons (21). Hence, an intervention period of 24 wk was chosen for the present trial, but these findings cannot exclude beneficial effects of vitamin B-12 supplementation on cognitive function from longer-term treatment.

The participants were selected on the basis of mild vitamin B-12 deficiency, which may be associated with subtle cognitive impairment (11, 12). The selection criteria were based on the literature (28) and laboratory experience, but there is no consensus on the diagnostic criteria for vitamin B-12 deficiency. Older persons with severe vitamin B-12 deficiency, as indicated by a serum vitamin B-12 concentration <100 pmol/L, were excluded because of an assumed higher risk of progression to neurologic damage (53). When these persons were identified during a screening visit ($n = 22$), they were referred to their general practitioners for treatment and further follow-up. Participants were not selected on the basis of mild cognitive or memory impairment. It is possible that subtle cognitive dysfunction may have been present for several years before the onset of clinically overt severe cognitive impairment (57). It has been hypothesized that persons who have had mild cognitive impairment for <6 mo are more likely to respond to vitamin B-12 therapy (9). If the duration of cognitive problems is >6 mo, there may be widespread neurologic damage and loss of the ability to repair neurons

(27). A limitation of all studies conducted thus far is the lack of information on the duration of cognitive impairment.

Vitamin B-12 deficiency is common in older persons, mainly because of malabsorption (3). The high prevalence of impaired vitamin B-12 status is associated with cognitive impairment. However, such associations may not be causal. Furthermore, even though the expected proportion of true reversible dementia in patients with vitamin B-12 deficiency is low (58), impaired vitamin B-12 metabolism may contribute as one of many factors in the development of cognitive impairment and dementia and may modulate the course of the disease. The mechanisms by which this occurs are not completely understood and may include elevated concentrations of MMA (5), homocysteine (59), or B vitamins in the maintenance of the integrity of the blood-brain barrier (60) and reduced methylation capacity (61).

Despite the strengths of the present study, 24 wk of supplementation with vitamin B-12 administered alone or in combination with folic acid showed no improvements in cognitive function in older persons with mild vitamin B-12 deficiency.

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SJE, LCdG, RC, WHH and WAvS contributed to the study design. LWJ designed the neuropsychologic test battery. RC conducted the randomization procedure. SJE and RJB supervised the data collection. PMU, JS, and HJB analyzed the biochemical data. SJE drafted the manuscript. LCdG, LWJ, RJB, RC, PMU, JS, HJB, WHH, and WAvS contributed to the data analysis and critically revised the manuscript. None of the authors had any financial or personal conflict of interest.

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