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High-Dose B Vitamin Supplementation and Cognitive Decline in Alzheimer Disease

A Randomized Controlled Trial

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Cooperative Study

ALZHEIMER DISEASE (AD) IS among the most important health problems worldwide, and although advances in symptomatic treatments targeting cognitive function provide meaningful benefits, effective disease modifying treatment is needed. Toward this goal, active drug development strategies aim to reduce amyloid accumulation and toxicity,¹ slow tau phosphorylation and tangle formation,² and/or promote neuronal survival and synaptic function.³ Evidence of homocysteine elevation in AD and the involvement of homocysteine in neuropathological mechanisms suggest that reduction of homocysteine may offer an approach to disease modification.^{4,5} Indeed, B vitamins that influence homocysteine metabolism have been considered as a therapeutic option to reduce risk of AD or slow its progression.⁶

For editorial comment see p 1819.

Context Blood levels of homocysteine may be increased in Alzheimer disease (AD) and hyperhomocysteinemia may contribute to disease pathophysiology by vascular and direct neurotoxic mechanisms. Even in the absence of vitamin deficiency, homocysteine levels can be reduced by administration of high-dose supplements of folic acid and vitamins B₆ and B₁₂. Prior studies of B vitamins to reduce homocysteine in AD have not had sufficient size or duration to assess their effect on cognitive decline.

Objective To determine the efficacy and safety of B vitamin supplementation in the treatment of AD.

Design, Setting, and Patients A multicenter, randomized, double-blind controlled clinical trial of high-dose folate, vitamin B₆, and vitamin B₁₂ supplementation in 409 (of 601 screened) individuals with mild to moderate AD (Mini-Mental State Examination scores between 14 and 26, inclusive) and normal folic acid, vitamin B₁₂, and homocysteine levels. The study was conducted between February 20, 2003, and December 15, 2006, at clinical research sites of the Alzheimer Disease Cooperative Study located throughout the United States.

Intervention Participants were randomly assigned to 2 groups of unequal size to increase enrollment (60% treated with high-dose supplements [5 mg/d of folate, 25 mg/d of vitamin B₆, 1 mg/d of vitamin B₁₂] and 40% treated with identical placebo); duration of treatment was 18 months.

Main Outcome Measure Change in the cognitive subscale of the Alzheimer Disease Assessment Scale (ADAS-cog).

Results A total of 340 participants (202 in active treatment group and 138 in placebo group) completed the trial while taking study medication. Although the vitamin supplement regimen was effective in reducing homocysteine levels (mean [SD], -2.42 [3.35] in active treatment group vs -0.86 [2.59] in placebo group; $P < .001$), it had no beneficial effect on the primary cognitive measure, rate of change in ADAS-cog score during 18 months (0.372 points per month for placebo group vs 0.401 points per month for active treatment group, $P = .52$; 95% confidence interval of rate difference, -0.06 to 0.12; based on the intention-to-treat generalized estimating equations model), or on any secondary measures. A higher quantity of adverse events involving depression was observed in the group treated with vitamin supplements.

Conclusion This regimen of high-dose B vitamin supplements does not slow cognitive decline in individuals with mild to moderate AD.

Trial Registration clinicaltrials.gov Identifier: NCT00056225

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Homocysteine is a sulfur amino acid involved in essential metabolic pathways, including methylation reactions. Elevation of homocysteine in blood is a marker of genetic disorders and deficiencies of vitamins B₁₂ and fo-

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late (cofactors in homocysteine pathways); homocysteine elevation is associated with endothelial dysfunction and vascular disease, as well as neuropsychiatric disorders.⁷ Homocysteine is associated with neurovascular ischemic disease, including stroke, silent infarctions, and white matter disease.^{8,9} Studies have linked homocysteine to amyloid and glutamate neurotoxicity, and to cognitive dysfunction and AD. For example, homocysteine elevation induces hippocampal neuron loss in transgenic mice with brain amyloid deposition⁵; the mechanisms are incompletely understood but may involve impaired DNA repair and induction of apoptotic cell death.^{4,5}

Studies have demonstrated a relationship between plasma homocysteine level and AD (including neuropathologically confirmed AD), and cognitive function in individuals without dementia^{10,11}; this relationship spans the normal range of homocysteine levels.¹¹ There is intriguing evidence that homocysteine levels may be related to plasma levels of amyloid peptides in individuals with AD,^{12,13} and that reduction of homocysteine levels may lower amyloid levels.¹⁴ Reduction of homocysteine levels can be readily achieved with high doses of folic acid, vitamin B₁₂, and vitamin B₆ in the absence of vitamin B deficiency in the general population¹⁵ and in individuals with AD,¹⁶ and could plausibly represent a disease modifying intervention in AD.

Randomized controlled trials of homocysteine reduction by B vitamin supplementation have yielded conflicting results. A systematic review¹⁷ of 14 trials concluded that there is insufficient evidence of a beneficial effect on cognition of such supplementation in individuals with normal or impaired cognition. A study of 3 years of supplementation in older individuals without dementia and with a plasma homocysteine concentration of at least 13 $\mu\text{mol/L}$ indicated a favorable influence on cognitive function,¹⁸ and a 2-year study in older individuals not selected on the basis of homocysteine levels did not.¹⁹ Short-term (2 to 6 months) B vitamin supplementation did not influ-

ence cognition in AD,^{16,20,21} except in 1 study in individuals with increased homocysteine levels.²² Long-term treatment studies in AD have not previously been reported.

We conducted a multicenter, randomized, placebo-controlled clinical trial to determine if reduction of homocysteine levels with high-dose folic acid, vitamin B₆, and vitamin B₁₂ supplementation for 18 months would slow the rate of cognitive decline in individuals with mild to moderate AD.

METHODS

Study Design

Our study, which was conducted by the Alzheimer Disease Cooperative Study (ADCS), a consortium of US centers funded by the National Institute on Aging to conduct therapeutic trials, used a randomized, double-blind, 2-group parallel design comparing high-dose vitamin supplements with placebo. The treatment period was 18 months. Forty sites participated in this trial after obtaining approval from their local institutional review boards. Written informed consent was obtained from study participants, legally authorized representatives, or both, according to local guidelines.

Individuals with probable AD,²³ recruited primarily from the sites' clinic populations, were eligible if they were medically stable. Inclusion criteria included age older than 50 years and a Mini-Mental State Examination (MMSE)²⁴ score within the range of 14 to 26. Individuals were excluded if they had levels of vitamin B₁₂ or folate below normal (vitamin B₁₂ <175 pg/mL; folate <4.2 ng/mL), or renal insufficiency (serum creatinine \geq 2.0 mg/dL). Conversion factors for vitamin B₁₂ to pmol/L is 0.7378, for folate to nmol/L is 2.266, and for serum creatinine to $\mu\text{mol/L}$ is 88.4. Individuals were also excluded if within the prior 2 months they had regularly used drugs with significant central anticholinergic effects, sedatives, anti-Parkinsonian medications, or any investigational treatment for AD. Stable use (for at least 3 months) of cholinesterase inhibitors and memantine was allowed.

The randomization process used a permuted block design with a block size of

5 (3 in the active treatment group and 2 in the placebo group). Unequal group assignment, with a greater likelihood of assignment to active treatment, was intended to improve the recruitment rate.

Because individuals using multivitamin tablets, which typically contain 400 μg of folic acid, show a smaller homocysteine reduction in response to high-dose supplements compared with nonvitamin users,¹⁶ we restricted enrollment of multivitamin users to no more than 2 individuals in each block of 5. Individuals taking daily vitamin supplements containing more than 400 μg of folic acid were excluded.

Study Medication, Assignment, and Masking

The active study medication consisted of 5 mg/d of folic acid, 1 mg/d of vitamin B₁₂ (cyanocobalamin), and 25 mg/d of vitamin B₆ (pyridoxine hydrochloride). The placebo tablet was identical in appearance. The active regimen was selected to maximize reduction of fasting and postprandial homocysteine levels with minimal risk. Although the regimen assessed in a pilot AD study included 50 mg of vitamin B₆,¹⁶ this was reduced to 25 mg to reduce risk of neuropathy.²⁵ Each individual received a bottle of study medication with a coded label at baseline and at the 3-, 6-, 9-, 12-, and 15-month visits. The randomization sequence was generated by the ADCS data center. "Scratch-off" codebreakers were used so that instances of unblinding would be documented; all codebreakers were collected at the end of the trial. Adequacy of masking was assessed by questionnaires completed by participants, caregivers, psychometrists, and site investigators.

Safety assessments, including vital signs, physical examination, urinalysis, and hematology and chemistry blood tests, were performed at each visit. Cognitive and behavioral assessments were performed at baseline and at months 3, 6, 9, 12, 15, and 18.

Outcome Measures

The primary outcome measure was the 18-month change score on the cognitive subscale of the Alzheimer Disease Assess-

ment Scale (ADAS-cog),²⁶ an instrument that evaluates memory, attention, language, orientation, and praxis. The ADAS-cog is a 70-point scale, with higher scores indicating greater impairment. Considering the expected safety and tolerability of the intervention, we considered a significant benefit to be a 25% reduction (in comparison with the placebo group) in cognitive decline as indicated by change in ADAS-cog score.

Secondary outcome measures included the MMSE,²⁴ Clinical Dementia Rating (CDR) sum of boxes,²⁷ Alzheimer Disease Cooperative Study activities of daily living (ADCS-ADL) scale,²⁸ Neuropsychiatric Inventory,²⁹ Quality of

Life-AD,³⁰ and the time to attainment of significant end points (4-point decline from baseline ADAS-cog score, death, institutionalization, 1 stage worsening on the global CDR scale, and 15-point decline on the ADCS-ADL scale).³¹

Assays

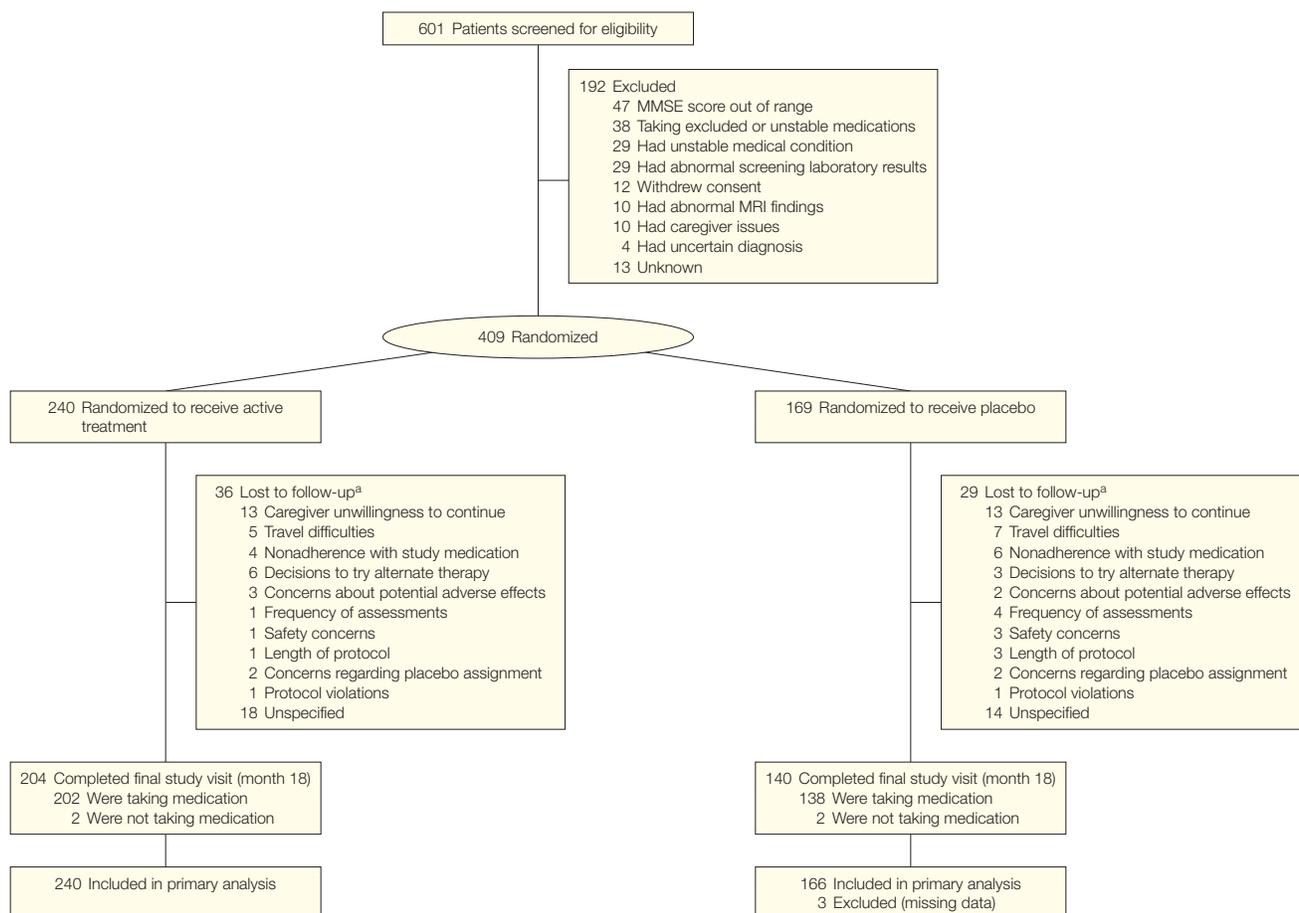
Plasma Total Homocysteine. Total homocysteine in plasma was measured by using a high-performance liquid chromatography method with fluorescence detection.³² Briefly, the method consists of reduction of the sample with tri-n-butylphosphine, precipitation of proteins with perchloric acid, and derivitization with 7-fluorobenzo-2-oxa-1,3-diazole-4-

sulphonate. The derivatized samples were injected directly into the high-performance liquid chromatography system.

Plasma Pyridoxal Phosphate. Plasma pyridoxal phosphate (vitamin B₆) was determined by high-performance liquid chromatography with fluorescence detection as previously described.³³ Plasma was deproteinized with 2 volumes of perchloric acid, and 20 μ L of the clear extract obtained after centrifugation was injected directly into the high-performance liquid chromatography–fluorescence detection system.

Genotyping of MTHFR C677T. Genomic DNA was extracted from periph-

Figure 1. Participant Flow



MMSE indicates Mini-Mental State Examination; MRI, magnetic resonance imaging. In the 38 patients excluded after screening for eligibility, those taking unstable medication meant that in the clinical judgment of the site investigator, the project director, or both, concomitant medication use was not stable at the time of screening for enrollment. In general, a change in medication use within 1 month of screening raised concern, resulting in discussion among the investigators regarding suitability.^a Patients were allowed to specify multiple reasons for dropping out of the study.

eral blood by using QIAamp DNA blood minikit (Qiagen, Valencia, California) and stored at -20°C . The extracted DNA was then assayed for the mutation in the *methylene tetrahydrofolate reductase* (*MTHFR*) gene (accession number U09806) by using the polymerase chain reaction for DNA amplification and restriction digestion of polymerase chain reaction products with *HinfI* for the $677\text{C}\rightarrow\text{T}$ as previously described.³⁴

Statistical Analysis

The goal of the primary analysis was to determine if the rate of cognitive decline differed between the group assigned to high-dose supplement treatment and the group assigned to receive placebo. The method of generalized estimating equations (GEE)³⁵ was used for the primary analysis.

Power calculations were based on GEE analysis of repeated ADAS-cog score data from participants in the ADCS Prednisone Study³⁶ (visit to visit correlation, 0.853; mean ADAS-cog score standard deviation, 11.5). A sample size of 240 in the active treatment group and 160 in the placebo group, assuming a 20% attrition rate and a 10% drop-in rate (the rate among those participants assigned to placebo of starting high-dose B vitamins in violation of the protocol) evenly dispersed along the 18 months of treatment and $\alpha = .05$, would provide more than 80% power to detect a 25% reduction in the rate of ADAS-cog decline. We considered this effect size appropriate, considering the safety and minimal expense associated with the treatment.

The primary analysis was an intention-to-treat analysis and included all randomized participants. Individuals were analyzed in the group to which they were randomized regardless of medication adherence. Additionally, all available ADAS-cog assessments were used in the analysis for individuals who discontinued medication but continued to be followed up.

A list of covariates anticipated to be associated with rate of change in homocysteine level, ADAS-cog score, or both at 18 months in this study population was compiled before study initiation. This list con-

sisted of apolipoprotein $\epsilon 4$ allele count, *MTHFR* $\text{C}677\text{T}$ allele count, baseline plasma homocysteine, age, sex, baseline serum creatinine level, smoking status, and baseline ADAS-cog score. The assessment of covariates consisted of a univariate 2-sample test comparing baseline values between treatment groups, and a bivariate measure of association between the baseline variable value and the rate of change measure. If for any particular variable, the first test, assessing the equivalence of the baseline distributions, was significant at the $\alpha = .10$ level and the second test, measuring association with response, was significant at the $\alpha = .15$ level, the variable would be included as a covariate in the GEE model. For the primary analysis, age was found to be unbalanced and

correlated with the rate of change in the ADAS-cog score; therefore, age was included in the model as a confounding variable for the primary analysis.

A planned secondary analysis of time to reach any 1 of 5 end points considered to be clinically significant (death, institutionalization, change in global CDR score, loss of 4 points on the ADAS-cog score, loss of 15 points on the ADCS-ADL score) used a Cox proportional hazards regression model; this analysis has been used previously.¹⁶ Analysis of secondary outcome measures followed the method of the primary analysis. No interim analysis was performed.

The statistical software R version 2.7.1 (R Foundation for Statistical Computing, Vienna, Austria) was used for

Table 1. Baseline Characteristics of the Participants^a

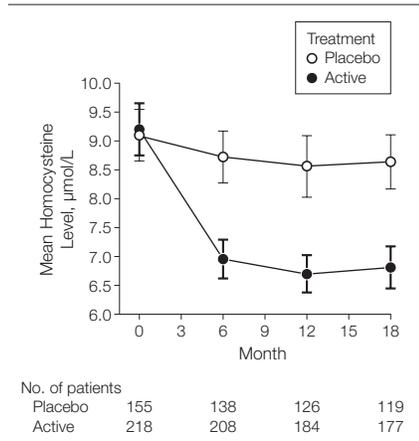
Characteristics	Active Treatment Group (n = 240)	Placebo Group (n = 169)	All Participants (N = 409)
Demographics, No. (%)			
Age, mean (SD), y	75.7 (8.0)	77.3 (7.9)	76.3 (8.0)
Women	138 (57.5)	91 (53.9)	229 (56.0)
Years of education, mean (SD)	14.0 (2.9)	13.9 (3.2)	13.9 (3.1)
Smoker	79 (32.9)	49 (29.0)	128 (31.3)
Multivitamin usage	89 (37.1)	77 (45.6)	166 (40.6)
Carrier of alleles, No. (%)			
Apolipoprotein $\epsilon 4$	141 (64.7)	105 (70.0)	246 (66.9)
<i>MTHFR</i> genotype			
CC	80 (41.5)	54 (40.6)	134 (41.1)
CT	86 (44.6)	56 (42.1)	142 (43.6)
TT	27 (14.0)	23 (17.3)	50 (15.3)
Plasma and vitamin levels, mean (SD)			
Serum creatinine, mg/dL	0.92 (0.24)	0.93 (0.22)	0.93 (0.23)
Total plasma homocysteine, $\mu\text{mol/L}$	9.20 (3.4)	9.10 (2.8)	9.16 (3.2)
Folate	25.74 (17.65)	23.59 (14.35)	24.85 (16.38)
Vitamin B ₁₂	605.68 (304.10)	577.46 (281.08)	593.99 (294.76)
Vitamin B ₆ , median (IQR)	5.00 (5.00-67.65)	6.55 (5.00-81.85)	5.00 (5.00-71.95)
Cognitive and functional measure scores, mean (SD) ^b			
ADAS-cog	22.43 (9.0)	22.63 (8.6)	22.51 (8.8)
MMSE	20.98 (3.4)	20.91 (3.7)	20.95 (3.5)
CDR sum of boxes	5.61 (2.7)	5.85 (2.9)	5.71 (2.8)
NPI, median (IQR)	6 (0-13)	5 (1-11)	6 (0-12)
ADCS-ADL scale	61.31 (11.58)	59.66 (12.9)	60.63 (12.15)

Abbreviations: ADAS-cog, Alzheimer Disease Assessment Scale; ADCS-ADL, Alzheimer Disease Cooperative Study activities of daily living; CDR, Clinical Dementia Rating; IQR, interquartile range; MMSE, Mini-Mental State Examination; *MTHFR*, *methylene tetrahydrofolate reductase*; NPI, Neuropsychiatric Inventory.

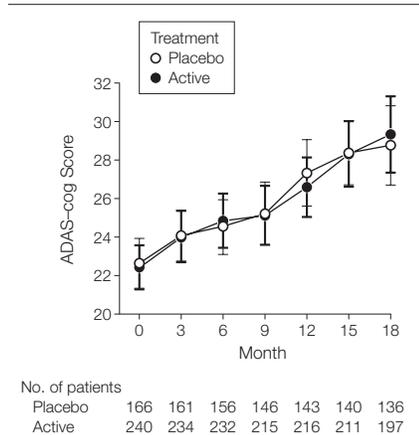
SI conversions: To convert serum creatinine to $\mu\text{mol/L}$, multiply by 76.25.

^aActive treatment group included high-dose supplements of 5 mg/d of folate, 25 mg/d of vitamin B₆, and 1 mg/d of vitamin B₁₂. Years of education was the number of years of formal schooling, with a range of 0 to 20. The investigator made the judgment as to whether a patient was a current smoker.

^bThe ADAS-cog (total score range, 0-70, where 70 is the most impaired) is a performance instrument that evaluates memory, attention, reasoning, language, orientation, and praxis. The MMSE (total score range, 0-30, where 30 is the least impaired) is a performance instrument that evaluates orientation, memory, attention, concentration, naming, repetition, comprehension, and ability to create a sentence and to copy 2 intersecting polygons. The CDR sum of boxes (range, 0-18, where 18 is the most impaired) is a clinical staging assessment using structured interviews of the participant and a study partner to assess memory, orientation, judgment and problem solving, community affairs, home and hobbies, and personal care. The NPI (range, 0-144, where 144 is the most impaired) assesses the frequency and severity of 10 neuropsychiatric disturbances based on an interview with the study partner. The ADCS-ADL scale (range, 0-78, where 78 is the most impaired) uses a structured interview of the study partner to assess functional performance (grooming, dressing, walking, bathing, feeding, toileting, shopping, preparing meals, using household appliances, keeping appointments, reading).

Figure 2. Mean Homocysteine Level by Treatment and Visit

Error bars indicate standard deviation of the mean. Some patients had missing values; therefore, 155 patients in the placebo group and 218 patients in the active treatment group were used for the analysis.

Figure 3. Mean ADAS-cog Score by Treatment and Visit

ADAS-cog indicates Alzheimer Disease Assessment Scale. Error bars indicate standard deviation of the mean. Three patients in the placebo group had missing values and were not included in the analysis.

all statistical analyses. For the primary hypothesis, analysis was duplicated by using SAS version 9.1 (SAS Institute Inc, Cary, North Carolina) for verification purposes. The a priori level of significance was set at $P < .05$.

RESULTS

Study Participants and Follow-up

The flow of participants through the study protocol is shown in FIGURE 1. Parti-

pants were recruited between February 20, 2003, and May 19, 2005. From a total of 601 participants screened, 409 met the study criteria and were randomized to 1 of the 2 treatment groups (active treatment or placebo). The median (interquartile) length of follow-up was 17.9 (17.7-18.1) months.

Protocol Alteration in Response to Data and Safety Monitoring Board Concern

During the course of the trial, the data and safety monitoring board indicated that there was an excess of adverse events related to depression in the active treatment group. With data and safety monitoring board concurrence, the protocol was altered to increase surveillance of depressive symptoms and to include Neuropsychiatric Inventory testing at each study visit. In addition, the informed consent documents were modified to indicate a possible association of depressive symptoms with study medication. The randomization code was not broken during the trial in any instance.

The final study visit was completed for 204 participants (85%) in the active treatment group and 140 participants (82%) in the placebo group. The predominant reasons for early discontinuation were caregiver issues (eg, caregiver unwilling or unable to continue participation or caregiver-perceived lack of efficacy).

There were no statistically significant differences in baseline characteristics between participants who discontinued early and study completers (data available by request from authors). Dropout rates in the active treatment group and placebo group were similar ($P = .42$, by Fisher exact test).

Using the $P < .15$ cutpoint for inclusion in the GEE model, baseline plasma homocysteine levels were correlated with age (Spearman correlation coefficient analysis, 0.2; $P < .001$), baseline serum creatinine (Spearman correlation coefficient analysis, 0.4; $P < .001$), Neuropsychiatric Inventory total score (Spearman correlation coefficient analysis, 0.1; $P = .14$), Neuropsychiatric Inventory depression subscore (Spearman correlation coefficient

analysis, 0.1; $P = .03$), plasma levels of vitamin B₁₂ (Spearman correlation coefficient analysis, -0.3; $P < .001$), plasma levels of folate (Spearman correlation coefficient analysis, -0.3; $P < .001$), and multivitamin use (linear regression model, coefficient -1.34; $P < .001$). Mean (SD) baseline homocysteine levels did not vary by *MTHFR* genotype (CC: 8.92 [2.75], $n = 133$; CT: 9.18 [3.29], $n = 142$; TT: 9.71 [3.33], $n = 50$; $P = .30$, by analysis of variance). Homocysteine levels were not related to ADAS-cog or CDR sum of boxes scores. A total of 371 participants (91.2%) were taking cholinesterase inhibitors at the time of enrollment. Seven participants (5 in the active treatment group and 2 in the placebo group) started treatment with cholinesterase inhibitors before the month 18 visit. These participants were included in the primary intention-to-treat analyses; in a secondary analysis excluding these participants, the results were similar. A total of 166 of 409 participants (40.6%) were taking multivitamins at enrollment; the proportion of multivitamin users did not differ across treatment groups ($P = .10$, by Fisher exact test).

The results of questionnaires administered at the month 18 visit indicated that the percentage of participants who believed that they were taking active study medication did not differ across the treatment group (68.0% in active treatment group vs 71.4% in placebo group; $P = .53$, by Fisher exact test), indicating that blinding was adequately maintained. Similarly, survey results indicated that the blinding was maintained among informants, study coordinators, and study physicians at the participating sites.

The demographic and clinical characteristics of the 2 treatment groups at baseline are shown in TABLE 1. There were no significant differences between the groups on any of the demographic or baseline characteristics.

Analysis of Outcomes

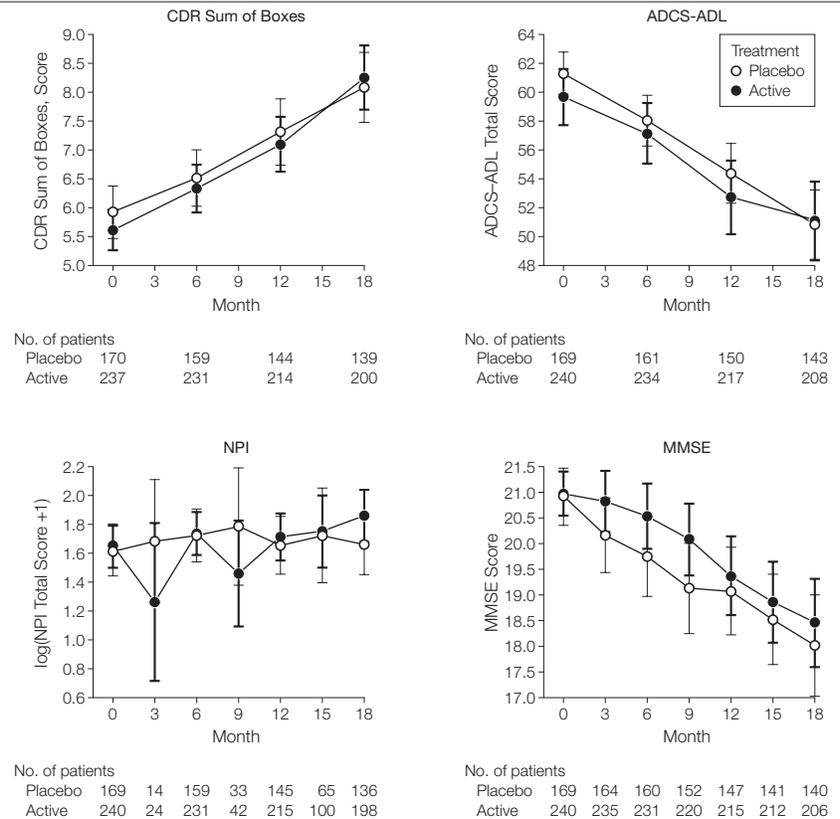
As expected, levels of each vitamin increased over baseline at 18 months in the active treatment group, but not in the placebo group (mean [SD]: vitamin B₆, 230.45 [176.53] in active treatment group vs -2.52 [87.62] in placebo group;

$P < .001$ by Kruskal-Wallis rank sum test; vitamin B₁₂, 789.15 [770.68] in active treatment group vs -110.50 [202.64] in placebo group; $P < .001$ by Kruskal-Wallis rank sum test; and folate, 176.68 [139.43] in active treatment group vs -5.11 [13.67] in placebo group; $P < .001$ by Kruskal-Wallis rank sum test). Homocysteine levels decreased during the trial in the active treatment group (mean [SD], -2.42 [3.35] in active treatment group vs -0.86 [2.59] in placebo group; $P < .001$) (FIGURE 2). In the placebo group, homocysteine levels at 12 months decreased by 7% overall, 9% among individuals taking multivitamins and 1% in individuals not taking multivitamins.

Primary Outcome Measure

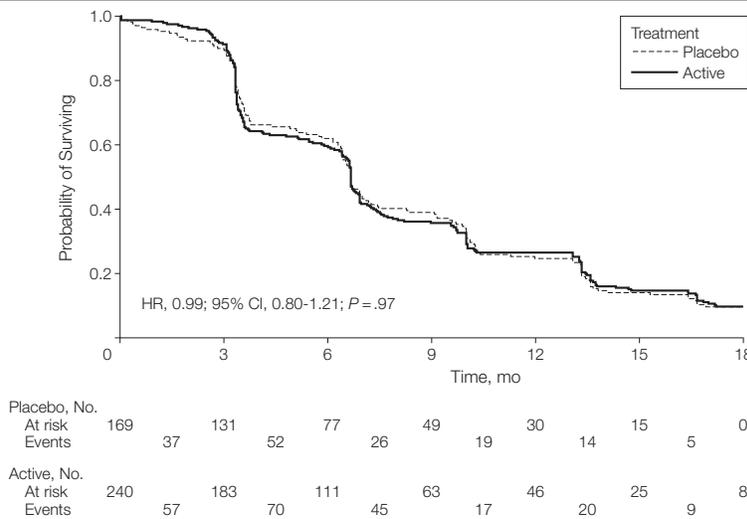
The effect of treatment on the primary and secondary outcome measures is shown in FIGURE 3, FIGURE 4, FIGURE 5, and TABLE 2. For the primary GEE analysis of the effect of supplementation on change in ADAS-cog score, the covariates included in the model were treatment, month, age, and treatment and month interaction. The rate of change in ADAS-cog score did not differ between treatment groups (0.372 points per month in placebo group vs

Figure 4. Mean CDR Sum of Boxes, ADCS-ADL, NPI, and MMSE Scores by Treatment and Visit



CDR indicates Clinical Dementia Rating; ADCS-ADL, Alzheimer Disease Cooperative Study activities of daily living; NPI, Neuropsychiatric Inventory; MMSE, Mini-Mental State Examination. Error bars indicate standard deviation of the mean. Some patients had missing CDR sum of boxes values; therefore, 170 patients in the placebo group and 237 patients in the active treatment group were used for the analysis.

Figure 5. Survival Rate by Treatment



The probability of surviving to any 1 of 5 clinically relevant end points (loss of 4 points on the ADAS-cog [Alzheimer Disease Assessment Scale], increase in global CDR [Clinical Dementia Rating] score, loss of 15 points on the ADCS-ADL [Alzheimer Disease Cooperative Study activities of daily living] scale, institutionalization, or death). P value was determined by Cox proportional hazards regression model.

Table 2. Changes From Baseline in Cognitive and Functional Measures^a

Cognitive and Functional Measures Test	Change in Score From Baseline, Mean (SD)						P Value ^b
	3 Month	6 Month	9 Month	12 Month	15 Month	18 Month	
ADAS-cog							.52
Placebo	1.51 (4.68)	1.72 (4.74)	2.71 (6.63)	4.46 (6.32)	5.80 (6.34)	6.54 (8.17)	
Active treatment	1.58 (5.61)	2.44 (6.04)	2.97 (6.57)	4.42 (6.61)	6.31 (7.71)	7.38 (9.72)	
MMSE							.69
Placebo	-0.67 (2.89)	-1.13 (3.13)	-1.70 (3.83)	-1.80 (3.56)	-2.51 (3.59)	-3.08 (4.46)	
Active treatment	-0.17 (3.02)	-0.44 (3.19)	-0.89 (3.26)	-1.64 (3.84)	-2.15 (4.21)	-2.65 (4.56)	
CDR sum of boxes							.57
Placebo	NA	0.79 (1.65)	NA	1.60 (2.12)	NA	2.51 (2.57)	
Active treatment	NA	0.69 (1.67)	NA	1.50 (1.92)	NA	2.58 (2.45)	
Neuropsychiatric Inventory							.28
Placebo	0.64 (4.83)	1.03 (8.82)	0.76 (8.90)	1.19 (9.31)	3.83 (13.74)	2.20 (11.12)	
Active treatment	0.71 (6.31)	0.97 (10.82)	0.12 (11.27)	1.03 (10.53)	2.97 (11.20)	3.31 (12.84)	
ADCS-ADL scale							.42
Placebo	NA	-2.86 (7.80)	NA	-7.82 (10.00)	NA	-10.00 (11.09)	
Active treatment	NA	-3.28 (7.99)	NA	-7.38 (9.97)	NA	-10.96 (12.36)	

Abbreviations: ADAS-cog, Alzheimer Disease Assessment Scale; ADCS-ADL, Alzheimer Disease Cooperative Study activities of daily living; CDR, Clinical Dementia Rating; MMSE, Mini-Mental State Examination; NA, not applicable.

^aActive treatment group included high-dose supplements of 5 mg/d of folate, 25 mg/d of vitamin B₆, and 1 mg/d of vitamin B₁₂. See footnote of Table 1 for descriptions of all cognitive and functional measures tests.

^bBy generalized estimating equations model.

Table 3. Adverse Events^a

Adverse Event	No. (%) of Participants		P Value ^b
	Active Treatment Group	Placebo Group	
Arthralgia	16 (6.7)	7 (4.1)	.38
Depression	67 (27.9)	30 (17.8)	.02
Dyspnea	18 (7.5)	9 (5.3)	.43
Headache	20 (8.3)	9 (5.3)	.33
Hyperhidrosis	23 (9.6)	7 (4.1)	.053
Joint swelling	17 (7.1)	6 (3.6)	.19
Restlessness	29 (12.1)	14 (8.3)	.25
Upper respiratory tract infection	16 (6.7)	6 (3.6)	.19
Urinary tract infection	16 (6.7)	8 (4.7)	.52
Vision blurred	13 (5.4)	3 (1.8)	.07

^aAdverse events that occurred in at least 5% of participants in either treatment group. Active treatment group included high-dose supplements of 5 mg/d of folate, 25 mg/d of vitamin B₆, and 1 mg/d of vitamin B₁₂.

^bBy Fisher exact test.

0.401 points per month in active treatment group, *P* = .52; 95% confidence interval [CI] of rate difference, -0.06 to 0.12; based on GEE model).

A confirmatory analysis of covariance yielded similar results. With baseline ADAS-cog scores and age included as covariates, the treatment had no effect on change in ADAS-cog score at 18 months (*P* = .56).

Secondary Outcome Measures

The GEE analysis revealed no difference in rate of decline on the CDR sum

of boxes in the 2 treatment groups (*P* = .57). The planned survival analysis considered the time interval from the baseline visit to the first among 5 possible end points: death, institutionalization, increase in global CDR score, 15-point decrease on the ADCS-ADL scale, or 4-point decrease on ADAS-cog score. A total of 372 participants (91.0%) reached at least 1 end point (219 [91.3%] in active treatment group and 153 [91.0%] in placebo group). Time to first end point did not differ between treatment groups (hazard ratio,

0.99; 95% CI, 0.80-1.21; *P* = .97, by Cox proportional hazards regression model). When time to individual end points was examined, there was no significant difference between groups (*P* > .50 for each end point).

Adverse Events

Numbers of adverse events (224/240 [93.3%] in active treatment group and 161/169 [95.3%] in placebo group; *P* = .52, by Fisher exact test), serious adverse events (123/240 [51.3%] in active treatment group and 95/169 [56.2%] in placebo group; *P* = .37), hospitalizations (111/240 [46.3%] in active treatment group and 87/169 [51.5%] in placebo group; *P* = .30), and deaths (3/240 [1.3%] in active treatment group and 4/169 [2.4%] in placebo group; *P* = .39) were similar in the active treatment and placebo groups. Treatment emergent adverse events were grouped into categories for analysis (TABLE 3).

There was an excess number of adverse events involving depression (defined as all adverse events mentioning depression, including depressed mood, depression, and depressive symptom) in the high-dose supplement group (67/240 [28%] vs 30/169 [18%]; *P* = .02, by Fisher exact test, unadjusted for mul-

multiple comparisons). However, change in depression subscore of the Neuropsychiatric Inventory did not differ (mean [SD], 0.15 [1.54] in placebo group vs 0.24 [2.12] in active treatment group; $P = .97$, by Wilcoxon rank sum test) and the percentage of participants starting antidepressants during the trial was similar in the 2 groups (21% in placebo group and 26% in active treatment group; $P = .24$, by Fisher exact test).

The differences in adverse events across groups (total adverse events and each category individually) did not reach statistical significance ($P > .10$ for each comparison, by Fisher exact test), except for hyperhidrosis ($P = .053$) and blurred vision ($P = .07$).

Subgroup Analyses

A planned analysis assessed the effect of treatment on change in ADAS-cog score within quartiles of baseline homocysteine levels; treatment had no effect in the highest quartile (mean [SD] 18-month change in ADAS-cog score, 6.55 [8.75] in active treatment group vs 5.13 [8.45] in placebo group; $P = .65$, by GEE model) or the lowest quartile (mean [SD] 18-month change in ADAS-cog score, 7.49 [10.92] in active treatment group vs 10.64 [9.82] in placebo group; $P = .15$, by GEE model). Additional planned subgroup analyses among multivitamin nonusers and groups defined by apolipoprotein $\epsilon 4$ genotype showed no treatment effect (data available from authors by request).

Planned analyses of change in ADAS-cog score in participants above and below the median MMSE score at baseline numerically favored placebo in the lower MMSE group and favored active treatment in the higher MMSE group at each time point. These treatment effects were only significant at the 15-month time point (mean [SD] change for lower MMSE score group, 5.18 [6.57] in placebo group vs 8.63 [8.19] in active treatment group; $P = .004$; and for higher MMSE score group, 6.43 [6.08] in placebo group vs 3.93 [6.40] in active treatment group; $P = .001$, by

Wilcoxon rank sum test, unadjusted for multiple comparisons). The GEE analysis showed a significant interaction between baseline MMSE stratum and treatment effect on ADAS-cog change ($P = .02$, unadjusted for multiple analyses).

COMMENT

This study was designed to determine if use of high-dose supplements to maximally reduce homocysteine levels in individuals with mild to moderate AD would slow the decline in cognition, clinical status, function, and behavior. The intervention was successful in reducing homocysteine levels but, in the study population as a whole, there was no evidence of benefit on any outcome measure. The general recommendation for B vitamin supplementation cannot be supported in patients with mild to moderate AD in the absence of B vitamin deficiency, at least in environments with folate enrichment of grains such as in the United States.

Our study included multivitamin users and nonusers, because high-dose supplementation reduces homocysteine in both groups.¹⁶ Because homocysteine reduction is greater in nonusers of multivitamins, this subgroup was examined separately in a planned secondary analysis. Although homocysteine levels decreased by 31%, supplementation had no effect on any outcome measure in this subgroup. Similarly, among participants with homocysteine levels in the highest quartile at baseline, high-dose B vitamin supplementation had no apparent benefit.

Apart from replacement therapy in individuals with low vitamin levels, the value of high-dose B vitamin supplementation to reduce normal homocysteine levels has not been demonstrated unequivocally in any clinical setting. Randomized studies in individuals without dementia have yielded conflicting results; supplementation may be useful in older individuals with relatively high homocysteine levels. The identification of groups that may benefit from such treatment remains an im-

portant goal. The trend toward opposite effects in milder vs more moderately impaired individuals, while possibly due to chance, suggests that studies in more narrowly defined groups of individuals with AD, or perhaps amnesic mild cognitive impairment, may be warranted.

In addition to the stage of dementia of participants, other issues that may have influenced the negative outcome of this trial should be considered. Although the goal of the active intervention was a 25% reduction in homocysteine level, this target was selected on the basis of feasibility in a US population. Populations in countries that do not supplement grain products with folic acid are expected to have higher homocysteine levels and show a greater reduction with supplementation.³⁷ Thus, supplementation might be clinically useful in other countries, or in participants selected on the basis of relatively high homocysteine levels. However, the absence of any evidence of benefit in our study participants with the highest baseline homocysteine levels is not encouraging in this regard. It is also plausible that homocysteine reduction might be effective in patients with AD with significant concomitant cerebrovascular disease; such individuals were excluded from our trial.

Aside from the issue of lack of cognitive or clinical benefit, these results raise a potential safety issue regarding the B vitamin supplementation. Adverse events involving depression were more common in the active treatment group. This is particularly surprising in view of the association noted between increased homocysteine levels and depression in patients with Parkinson disease,³⁸ and the observation that folate augmentation may increase the efficacy of antidepressant medications.^{39,40} However, the adverse event finding reached marginal significance without adjustment for multiple categorical adverse event analyses, and analysis of change on the depression item of the Neuropsychiatric Inventory scale yielded only a trend toward support of the finding. Furthermore, no

difference was evident in the number of antidepressant medications prescribed to individuals in the 2 groups. Attention to this possible adverse effect in other trials of such treatment may be appropriate.

Many studies suggest that relative elevation of homocysteine is characteristic of AD, and laboratory research implicates homocysteine in neurodegenerative mechanisms. High-dose B vitamin supplementation in individuals with normal levels of B vitamins was effective in reducing homocysteine levels. However, our study does not support the treatment of individuals with mild to moderate AD and normal vitamin levels with B vitamin supplements.

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