ORIGINAL ARTICLE

A Controlled Trial of Homocysteine Lowering and Cognitive Performance

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ABSTRACT

BACKGROUND

The results of observational studies suggest that plasma homocysteine concentrations are inversely related to cognitive function in older people. Our objective was to test the hypothesis that lowering the plasma homocysteine concentration improves cognitive function in healthy older people.

METHODS

We conducted a two-year, double-blind, placebo-controlled, randomized clinical trial involving 276 healthy participants, 65 years of age or older, with plasma homocysteine concentrations of at least 13 μmol per liter. Homocysteine-lowering treatment was a daily supplement containing folate (1000 μg) and vitamins B_{12} (500 μg) and B_6 (10 mg). Tests of cognition were conducted at baseline and after one and two years of treatment. Treatment effects were adjusted for baseline values, sex, and education.

RESULTS

On average, during the course of the study, the plasma homocysteine concentration was 4.36 μmol per liter (95 percent confidence interval, 3.81 to 4.91 μmol per liter) lower in the vitamin group than in the placebo group (P<0.001). Overall, there were no significant differences between the vitamin and placebo groups in the scores on tests of cognition.

CONCLUSIONS

The results of this trial do not support the hypothesis that homocysteine lowering with B vitamins improves cognitive performance. (Australian Clinical Trials registry number, ACTR NO 12605000030673.)

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The prevalence of cognitive impairment increases with age and represents a major public health concern in aging populations. There is evidence that circulating homocysteine concentrations may be a modifiable risk factor for cognitive decline.[1] Homocysteine concentrations have been reported to be higher in persons with suspected or confirmed Alzheimer's disease than in age-matched controls.[2-4] Alzheimer's disease was more likely to develop over an eight-year period in persons with plasma homocysteine concentrations above 14 μmol per liter than among those with lower concentrations.[5] Furthermore, the results of several cross-sectional and prospective studies of community-based older adults indicate that homocysteine is inversely associated with performance on some tests of cognition.[6-15]

The inverse relation between homocysteine concentrations and blood concentrations of folate and vitamin B_{12} makes it difficult to disentangle the independent effects of each on cognitive function.[16] Severe deficiency of folate or vitamin B_{12} leads to cognitive impairment.[17,18] At concentrations of these vitamins not normally associated with deficiency, associations with lower performance on various tests of cognition have been reported in several observational studies involving healthy older people.[10,12-15,19,20]

Supplements containing folate, with or without vitamins B_{12} and B_6, decrease homocysteine concentrations.[21] In two randomized, controlled trials of less than four months' duration, lowering homocysteine with B vitamins had no effect on scores of cognition in people at high risk for dementia[22] or in community-dwelling older people.[23] Longer-term trials are required to determine whether lowering homocysteine concentrations influences cognitive function in healthy older people. We conducted a two-year clinical trial to test the hypothesis that in older people with elevated plasma homocysteine concentrations, decreasing homocysteine with B vitamins would result in better cognitive performance.

METHODS

PARTICIPANTS

This double-blind, placebo-controlled, randomized clinical trial of two years' duration was conducted in Dunedin, New Zealand, between August 2002 and December 2004. The University of Otago Human Ethics Committee approved the trial, and written informed consent was obtained from all participants before screening. Volunteers 65 years of age or older were recruited from service clubs (e.g., Rotary International), through advertisements in newspapers, and by direct mail. Participants were ineligible if they had suspected dementia; were taking medications known to interfere with folate metabolism (e.g., oral hypoglycemic agents or antiepileptic agents); were taking vitamin supplements containing folic acid, vitamin B_{12}, or vitamin B_6; were being treated for depression; had diabetes; or had a history of stroke or transient ischemic attacks.

Eligible participants attended a screening clinic at which a blood sample was collected after an overnight fast for measurement of plasma homocysteine and creatinine. Tests of cognition were not administered at the screening visit. Those with a fasting homocysteine concentration of at least 13 μmol per liter and a normal plasma creatinine concentration (≤133 μmol per liter [1.5 mg per deciliter] in men and ≤115 μmol per liter [1.3 mg per deciliter] in women) were invited to participate in the trial.

Before randomization, all eligible participants were stratified according to the median values for age and homocysteine concentration in the screening population. Random decimals between 0 and 1 were generated for each person in each of the four strata. Those below the median of the random numbers in each stratum were assigned to the vitamin group, and the remainder were assigned to the placebo group. Participants were asked to consume one capsule daily for two years. The placebo capsules contained a blend of magnesium stearate and microcrystalline cellulose as a filler, whereas the treatment capsules contained the filler plus 1000 μg of folate (the calcium salt of l-5-methyltetrahydrofolate), 500 μg of vitamin B_{12} (cobalamin), and 10 mg of vitamin B_6 (pyridoxine) (Merck Eprova). All capsules were gelatin-coated, identical in color and shape, and packaged in blister packs. Compliance was assessed by counting returned capsules. The primary end points were the scores on tests of cognition, and the secondary end points were the biochemical measurements.

END POINTS

Neuropsychological tests were administered at baseline and at one and two years to assess a broad range of the participants' cognitive func-

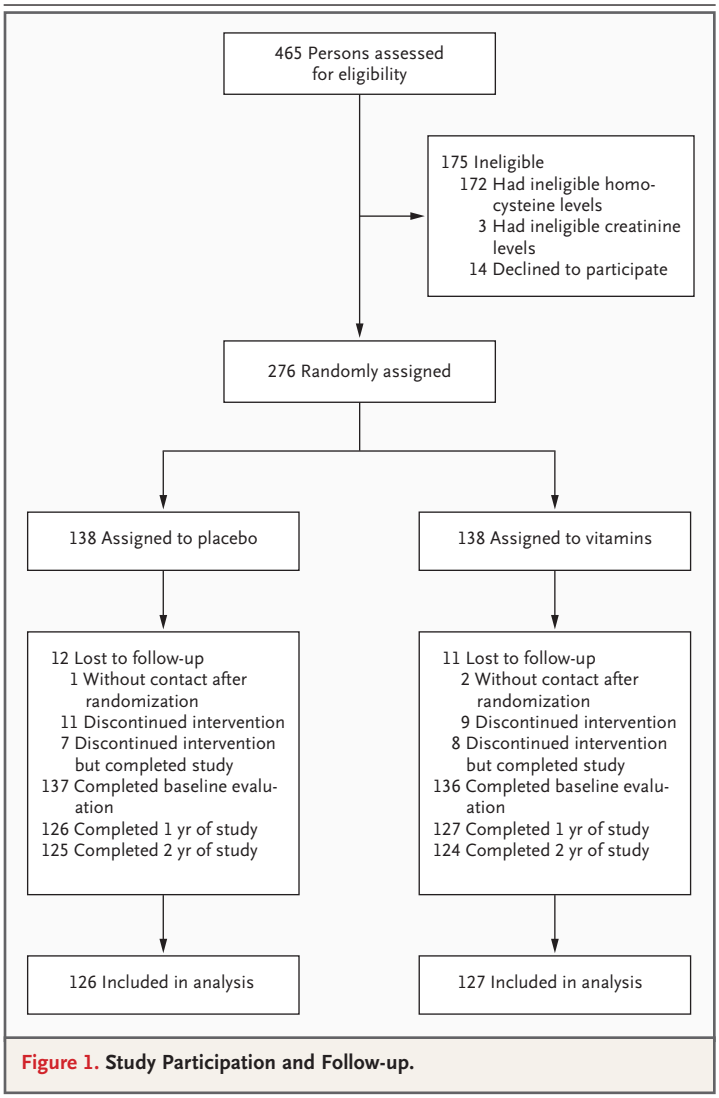

Figure 1. Study Participation and Follow-up.

tions. To minimize the effect of learning and practice, alternate forms of the tests (e.g., various paragraphs, letters, word categories, or matrices) were used where possible.

The Mini–Mental State Examination (MMSE) was included to provide a global measure of cognitive function and to assess participants for dementia.[24,25] Memory and learning capacity were assessed with the Rey Auditory Verbal Learning Test[24,26] and paragraph-recall tests from the Wechsler Memory Scales.[24,27] The 15-item parallel-word lists used for the administration of the Rey Auditory Verbal Learning Test are described by Lezak,[24] and the total score on the first five trials (trials I through V) and the score on the delayed trial (trial VII) were used as indicators of learning and recall ability, respectively. Verbal fluency was assessed with use of the Controlled Oral Word Association Test of the Multilingual Aphasia Examination,[28] which requires participants to generate as many words as possible with a specified initial letter in 60 seconds. Three letters were used on each occasion: C, F, and L at baseline; P, R, and W at one year; and F, A, and S at two years. Semantic fluency was assessed with use of the Category Word Fluency test as described by Lezak,[24] with the score being the total number of words generated in a specific category in 60 seconds. On each occasion, participants were instructed to generate words from three specified categories (e.g., animals, fruit and vegetables, and means of transportation) in 60 seconds.

As a measure of information-processing speed, the Reitan Trail Making Test was administered, and the time taken to complete Part B, which measures visual search and attentional processes, was used in the subsequent analysis. At baseline and the two-year follow-up, the original version of the Trail Making Test was administered; at the one-year follow-up, the order of the letter and digit stimulus elements was reversed.[24,26,29] Finally, as a measure of reasoning ability, a different set of 20 items from the Raven's Progressive Matrices was administered on each occasion. At baseline, the set comprised item 1 and every third item thereafter; the other two sets were constructed in the same way, starting with item 2 and item 3. The National Adult Reading Test,[24,30] which assesses the ability to read aloud words of irregular pronunciation, was used to estimate the IQ at baseline. One of the authors administered all the cognitive tests. The tests were conducted in the same order during each session, and whenever possible, the one-year and two-year tests for a given participant were carried out at the same time on the same day of the week as the baseline tests.

Secondary end points were plasma concentrations of homocysteine, folate, and vitamin B_{12}. Blood samples were collected at baseline and every six months by venipuncture after a 10-to-12-hour overnight fast. Plasma was obtained by centrifuging whole blood at 1650×*g* for 15 minutes at 4°C within 2 hours after collection. Blood samples were stored at −80°C until analyzed. Total l-homocysteine in plasma was measured with the IMx fluorescence polarization immunoassay (Abbott). The five samples (obtained at baseline and at 6, 12, 18, and 24 months) from each subject were analyzed in the same batch to reduce varia-

Table 1. Baseline Characteristics of the Participants.*

Characteristic	Placebo Group (N=126)	Vitamin Group (N=127)	P Value
Demographic characteristics			
Age — yr	73.4±5.7	73.6±5.8	0.79
Female sex — no. (%)	65 (52)	47 (37)	0.02
Current smoker — no. (%)	1 (1)	6 (5)	0.12
Education attained — no. (%)			
<3 yr secondary	41 (33)	48 (38)	
≥3 yr secondary	11 (9)	16 (13)	0.31
Tertiary	74 (59)	63 (50)	
History of hypertension — no. (%)†	51 (40)	56 (44)	0.56
Body-mass index‡	26.6±3.7	26.9±4.3	0.49
Apolipoprotein E *ε4* carrier — no. (%)§	26 (24)	36 (31)	0.21
Reading ability — no. of words pronounced correctly/50 words on the National Adult Reading Test	36.8±6.8	35.6±6.5	0.13
Biochemical measures			
Plasma total homocysteine — μmol/liter	16.3±4.4	16.8±5.4	0.43
Plasma folate — ng/ml	10±5	10±5	0.78
Plasma vitamin B_{12} — pg/ml	385±138	380±136	0.74
Plasma cholesterol — mg/dl	232±50	247±46	0.03

* Plus–minus values are means ±SD. Because of rounding, not all percentages total 100. To convert the values for folate to nanomoles per liter, multiply by 2.266. To convert the values for vitamin B_{12} to picomoles per liter, multiply by 0.738. To convert the values for cholesterol to millimoles per liter, multiply by 0.0259.
† Participants were considered to have a history of hypertension if they were taking a prescribed antihypertensive medication.
‡ Body-mass index was calculated as the weight in kilograms divided by the square of the height in meters.
§ Apolipoprotein E genotyping results were available for 110 participants in the placebo group and 116 in the vitamin group.

tion. Plasma folate concentrations were determined by a microtiter technique, exactly as described by O'Broin and Kelleher,[31] with chloramphenicol-resistant *Lactobacillus casei* as the test microorganism. Plasma vitamin B_{12} was measured with the Advia Centaur vitamin B_{12} assay, a competitive immunoassay based on direct chemiluminescent technology. Plasma creatinine was measured colorimetrically with kits on a Cobas Mira analyzer (Roche Diagnostics). Coefficients of variation for these assays were 6.7 percent for plasma homocysteine, 7.7 percent for plasma folate, 5.6 percent for plasma vitamin B_{12}, and 7.2 percent for plasma creatinine.

STATISTICAL ANALYSIS

We estimated that 100 participants in each of the two study groups would be required for us to detect a minimum treatment effect size of 0.4 for the Controlled Oral Word Association Test (standard deviation, 10.5) and Rey Auditory Verbal Learning Test, trials I through V (standard deviation, 7.5), with a power of 80 percent and a two-sided alpha level of 0.05.[32] Allowing for a dropout rate of 10 percent and death rate of 13 percent, we recruited 130 participants per group. Differences in the characteristics of the participants in the vitamin and placebo groups at baseline were determined with use of Student's t-test for continuous variables and the chi-square test or Fisher's exact test for categorical variables. Generalized estimating equations with an exchangeable correlation matrix and robust standard errors were used to analyze the data (Stata 8.0).

The results are presented as estimates (with 95 percent confidence intervals) of the difference between the two treatments, for both time periods combined, for each test of cognition. The difference between the treatments for each variable was estimated after adjusting for its baseline values in the first model and its baseline values, sex, and education in the second model. Because of

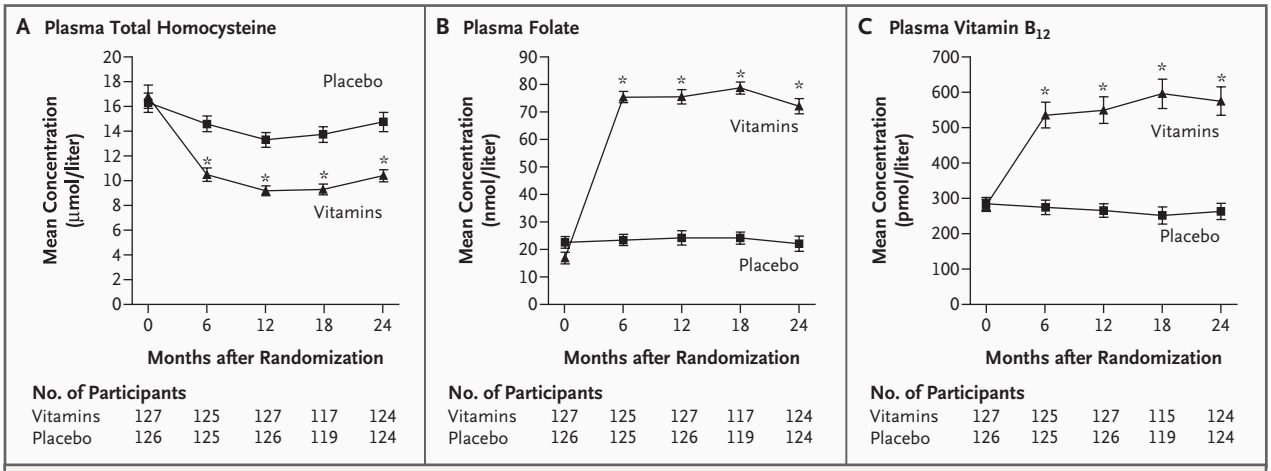

Figure 2. Effect of Vitamins on Biochemical Measurements.

I bars represent 95 percent confidence intervals. Asterisks indicate a significant difference from the placebo group (P<0.001). To convert the values for folate to nanograms per milliliter, divide by 2.266. To convert the values for vitamin B_{12} to picograms per milliliter, divide by 0.738.

their positive skew, scores for Part B of the Reitan Trail Making Test were log-transformed before analysis, and the results were presented as a ratio. To make the results easier to compare, an effect size for each test was calculated by dividing the difference between the treatments by the standard deviation of the test result for the whole sample at baseline. A generalized estimating equation was used to estimate a combined effect for the difference between treatments. The scores for each test were converted to standard deviation scores by dividing each score by the standard deviation at baseline; the result was then analyzed in a model that included a term for each test and that adjusted for its baseline value, sex, and education. Interim analyses were not conducted during the course of the study. The primary end points, including the combined score, and statistical comparisons were prespecified. All P values were two-sided and were not adjusted for multiple testing.

The authors designed the study, gathered and analyzed the data, and wrote the manuscript. Dr. Skeaff is the guarantor of the data and the analysis.

RESULTS

PARTICIPANTS

Of the 465 people who were screened, 175 were excluded because they had a fasting plasma homocysteine level of less than 13 μmol per liter (172 people) or an abnormal plasma creatinine level (3 people) (Fig. 1). An additional 14 people declined to participate in the intervention after screening. The remaining 276 people were randomly assigned — 138 to the vitamin group and 138 to the placebo group. Three participants withdrew before baseline values were collected. Twelve participants in the placebo group and 11 in the vitamin group were lost to follow-up. Fifteen participants discontinued taking the supplements but completed the tests of cognition and were included in the final analysis. The statistical analysis of the end points included 253 participants. The characteristics of the two groups at baseline are shown in Table 1. Five participants (three in the placebo group and two in the vitamin group) had a baseline MMSE score of less than 26. Overall, 215 of the participants (85 percent) took at least 95 percent of their study capsules. Equal numbers in both groups (2.6 percent) reported side effects associated with taking the capsules; effects included swallowing difficulties and increased thirst.

END POINTS

The plasma total homocysteine concentration was lower and the folate and vitamin B_{12} concentrations were higher in the vitamin group than in the placebo group at every time point (Fig. 2). At six months, the homocysteine concentration was 4.23 μmol per liter (95 percent confidence interval, 3.56 to 4.90 μmol per liter) lower in the vitamin group than in the placebo group (P<0.001).

Table 2. Cognition-Test Scores and Differences in Scores between the Two Groups during the Study Period.*

Test of Cognition	Baseline	Year 1	Year 2	Difference Adjusted for Baseline Value (95% CI)†	P Value	Fully Adjusted Difference (95% CI)‡	P Value
Mini–Mental State Examination (total score; maximum possible, 30)				−0.06 (−0.27 to 0.15)	0.58	−0.09 (−0.30 to 0.13)	0.42
Placebo	29.17±1.06	29.34±0.98	29.32±1.10				
Vitamin	29.19±0.97	29.29±1.02	29.29±1.41				
Wechsler Paragraph Recall test (total score on two 25-item tests; maximum possible, 50)				−1.19 (−2.30 to −0.04)	0.03	−0.88 (−1.98 to 0.21)	0.12
Placebo	23.41±7.63	23.87±6.78	20.76±7.21				
Vitamin	22.49±7.05	22.65±6.13	18.67±6.55				
Category Word Fluency test (total no. of words generated in three 1-min tests)				−1.62 (−4.08 to 0.84)	0.20	−0.86 (−3.25 to 1.53)	0.48
Placebo	55.78±10.61	63.70±14.77	68.78±13.71				
Vitamin	54.17±10.58	61.09±15.04	65.72±14.96				
Rey Auditory Verbal Learning Test, trials I–V (sum of five trials with the same list; maximum possible, 75 words)				−0.81 (−2.11 to 0.48)	0.22	−0.98 (−2.29 to 0.34)	0.14
Placebo	42.60±9.38	42.09±9.58	44.22±9.90				
Vitamin	42.08±10.33	40.31±10.03	43.90±9.70				
Rey Auditory Verbal Learning Test, trial VII (30-min delayed recall; maximum possible, 15 words)				−0.28 (−0.78 to 0.22)	0.27	−0.35 (−0.85 to 0.14)	0.16
Placebo	7.71±3.59	7.13±3.35	7.50±3.65				
Vitamin	7.75±3.64	6.97±3.34	7.29±3.35				
Raven's Progressive Matrices (20-item test; maximum possible score, 20)				−0.35 (−0.85 to 0.14)	0.16	−0.31 (−0.81 to 0.19)	0.22
Placebo	14.23±2.80	13.53±2.98	11.90±3.05				
Vitamin	14.21±2.39	13.15±2.85	11.60±2.92				
Controlled Oral Word Association Test (total no. of words generated in three 1-min tests)				0.30 (−1.53 to 2.13)	0.75	0.31 (−1.55 to 2.18)	0.74
Placebo	39.31±11.41	39.78±13.45	41.00±12.44				
Vitamin	37.55±12.22	39.54±12.37	40.11±14.08				
Part B of the Reitan Trail Making Test (sec to completion)				1.07 (1.02 to 1.13)§	0.009	1.08 (1.02 to 1.14)§	0.007
Placebo	100.74±43.00	108.03±46.81	98.96±40.75				
Vitamin	104.83±48.13	123.49±66.10	114.40±84.23				

* Plus–minus values are means ±SD. In all tests except Part B of the Reitan Trail Making Test, higher scores indicate better function. Data at baseline reflect test results from 126 participants in the placebo group and 127 in the vitamin group; data at year 1, results from 126 and 127 participants, respectively; and data at year 2, results from 125 and 124 participants, respectively.
† The difference is based on an overall estimate of one- and two-year scores on cognitive tests, adjusted for baseline values.
‡ The difference is adjusted for sex, education, and baseline values.
§ The exponent of the difference between log-transformed values is the ratio of the result in the vitamin group to the result in the placebo group.

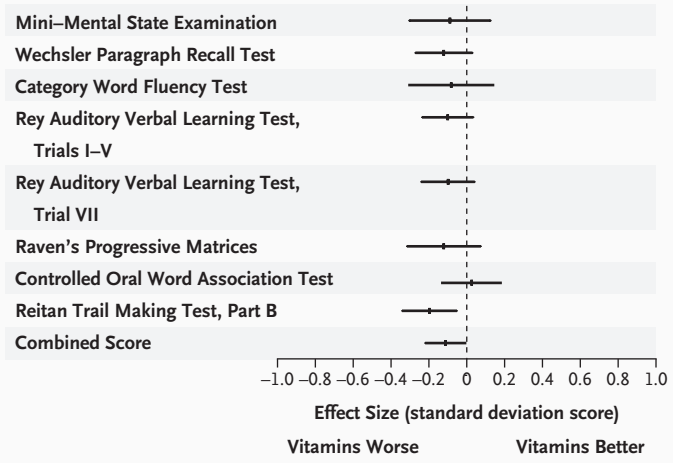

Figure 3. Effect of Vitamins on Cognitive Function.

For the combined score, a generalized estimating equation was used to estimate a combined effect for the difference between treatments. The scores for each test were converted to standard deviation scores by dividing the score by the standard deviation at baseline; the result was then analyzed in a model that included a term for each test and that adjusted for its baseline value, sex, and education. Interim analyses were not conducted during the course of the study. Horizontal bars represent 95 percent confidence intervals. The result of Part B of the Trail Making Test was worse with vitamins than with placebo (P=0.007). The combined score was worse with vitamins than with placebo (P=0.05).

The mean difference between the two groups in the plasma homocysteine concentration, averaged for months 6, 12, 18, and 24, was 4.36 μmol per liter (95 percent confidence interval, 3.81 to 4.91 μmol per liter) (P<0.001). Plasma folate and vitamin B_{12} concentrations were higher by 22.8 ng per milliliter (51.7 nmol per liter) (95 percent confidence interval, 21.1 to 24.5 ng per milliliter [47.9 to 55.5 nmol per liter]) and 355 pg per milliliter (262 pmol per liter) (95 percent confidence interval, 309 to 401 pg per milliliter [228 to 296 pmol per liter]), respectively, in the vitamin group at six months (P<0.001 for both comparisons).

The mean score on the Wechsler Paragraph Recall test was lower in the vitamin group than in the placebo group (difference, –1.19; 95 percent confidence interval, –2.30 to –0.04; P=0.03), but the difference did not remain significant after adjustment for sex and education (–0.88; 95 percent confidence interval, –1.98 to 0.21; P=0.12) (Table 2). On Part B of the Reitan Trail Making Test, the mean time to completion of the test was 7 percent longer in the vitamin group than in the placebo group (95 percent confidence interval, 2 to 13 percent; P=0.009). Further adjustment for sex and education did not alter the difference (8 percent; 95 percent confidence interval, 2 to 14 percent; P=0.007). The differences in cognitive scores between the vitamin and placebo groups are shown in Figure 3 as standard deviation scores. Part B of the Reitan Trail Making Test was the only individual test with an effect size different from zero, at –0.20 (95 percent confidence interval, –0.34 to –0.05; P=0.007). The combined treatment score for the eight tests of cognition was –0.11 standard deviation scores poorer in the vitamin group than in the placebo group (95 percent confidence interval, –0.22 to 0; P=0.05).

DISCUSSION

In this two-year, double-blind, placebo-controlled, randomized clinical trial, daily use of a supplement containing folate and vitamins B_{12} and B_6 reduced plasma homocysteine concentrations in healthy older people by 4.36 μmol per liter. There were no significant differences between scores on the tests of cognition in the vitamin and placebo groups, with the exception of Part B of the Reitan Trail Making Test, with participants in the vitamin group taking longer than those in the placebo group to complete the test. We chose a range of tests to assess cognition; thus, it is conceivable that, with the large number of statistical comparisons, the results relating to this test are a chance finding.

Many observational studies suggest an inverse association between homocysteine concentrations and cognitive performance.[6-15] Morris et al. reported an inverse association between homocysteine concentration and cognition on two tests of short delayed recall in 1200 healthy older Americans.[8] In a cross-sectional analysis of 2096 dementia- and stroke-free participants in the Framingham Offspring Study, plasma homocysteine was inversely associated with performance on a range of cognitive tests in persons 60 years of age or older.[33]

Several elements of our study lend weight to the veracity of the findings. The trial had sufficient power to detect a clinically meaningful difference in cognitive function between the treatments, few participants were lost to follow-up, compliance with supplement use was high, and there was a marked increase in plasma concentrations of folate and B_{12} in the vitamin group, causing a large decrease in homocysteine concentra-

tions. Furthermore, the participants were selected on the basis of an elevated homocysteine concentration and did not have dementia or a history of stroke. Finally, we used a variety of tests to assess a range of cognitive abilities.

On the other hand, several factors may limit the extent to which the results may be generalized. The trial may not have been long enough to establish the true effect of decreasing plasma homocysteine on cognitive performance, and thus we cannot rule out the possibility of a benefit with long-term treatment; in addition, the trial did not address the issue of whether homocysteine lowering reduces the risk of dementia. Moreover, we cannot rule out the possibility that lower doses of vitamins would have led to a different result. The participants were a healthy group of older people with high MMSE scores and a high level of education but were selected on the basis of a plasma homocysteine concentration of at least 13 μmol per liter. It is possible that a group with dementia or mild cognitive impairment, lower MMSE scores, a different level of education, or a higher initial homocysteine concentration might have had a different response.

Neuropsychological tests are highly intercorrelated, so a composite score will be a valid and reliable measure of global cognitive capacity. Nonetheless, since each test measures a specific aspect of cognition that may be differentially responsive to treatment, the aggregation of test scores may obscure any such effect. For this reason, we analyzed the test scores both singly and in combination. The combined score of the cognitive tests was lower in the vitamin group than in the placebo group. This finding is consistent with a report by Morris et al., who observed an association between high folate intake and accelerated cognitive decline over a six-year period among 3718 older people living in Chicago.[34] However, the magnitude of the difference in the combined score between the vitamin and placebo groups was small, with an effect size of −0.11 (P=0.05). Furthermore, the effect was in the opposite direction to that suggested by the weight of a substantial body of observational evidence. In summary, the results of our trial do not support the hypothesis that homocysteine lowering with folate, vitamins B_{12}, and B_6 improves cognitive performance in healthy older people.

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No potential conflict of interest relevant to this article was reported.

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