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Significant correlations of plasma homocysteine and serum methylmalonic acid with movement and cognitive performance in elderly subjects but no improvement from short-term vitamin therapy: a placebo-controlled randomized study[1–3]

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ABSTRACT

Background: Deficiencies of vitamin B-12, folic acid, and vitamin B-6—as defined by laboratory measures—occur in 10–20% of elderly subjects. The clinical significance remains unresolved.

Objective: The objective was to explore any association between vitamin status and vitamin treatment and movement and cognitive performance in elderly subjects.

Design: Community-dwelling subjects ($n = 209$) with a median age of 76 y were randomly assigned to daily oral treatment with 0.5 mg cyanocobalamin, 0.8 mg folic acid, and 3 mg vitamin B-6 or placebo (double blind) for 4 mo. Movement and cognitive performance tests were performed before and after treatment.

Results: A high plasma total homocysteine (tHcy) concentration ($\geq$16 μmol/L) was found in 64% of men and in 45% of women, and a high serum methylmalonic acid (MMA) concentration ($\geq$0.34 μmol/L) was found in 11% of both sexes. Movement time, digit symbol, and block design (adjusted for age, sex, smoking, and creatinine) correlated independently with plasma tHcy ($P < 0.01$, < 0.05, and < 0.01, respectively); the simultaneity index and block design correlated with serum MMA ($P < 0.05$ for both). Vitamin therapy significantly decreased plasma tHcy (32%) and serum MMA (14%). No improvements were found in the movement or cognitive tests compared with placebo. Neither vitamin therapy nor changes in plasma tHcy, serum MMA, serum vitamin B-12, plasma folate, or whole-blood folate correlated with changes in movement or cognitive performance.

Conclusions: High plasma tHcy and serum MMA were prevalent and correlated inversely with movement and cognitive performance. Oral B vitamin treatment normalized plasma tHcy and serum MMA concentrations but did not affect movement or cognitive performance. This might have been due to irreversible or vitamin-independent neurocognitive decline or to an insufficient dose or duration of vitamins. *Am J Clin Nutr* 2005;81:1155–62.

KEY WORDS Elderly, homocysteine, methylmalonic acid, cognition, movement, controlled trial

INTRODUCTION

The clinical significance of vitamin B-12, folic acid, and vitamin B-6 deficiency, as defined by laboratory measures, is unresolved (1–3). Correlations between low vitamin status and poor cognitive function have been found in community-dwelling elderly subjects (4–9), prospective community-based studies (10–12), and neuropsychiatric patients (13–17). Some open studies have shown correlations between vitamin supplementation and cognitive improvement (18–21), whereas double-blind placebo-controlled studies diverge in outcome (22, 23).

Total plasma homocysteine (tHcy) concentrations are elevated in Parkinson disease (24), a disorder that also carries an increased risk of depression (25) and other mental disturbances (26). L-Dopa can cause hyperhomocysteinemia in Parkinson disease patients and the extent is influenced by B vitamin status (27). Gait abnormalities in elderly nondemented subjects have been found to be a significant predictor of non-Alzheimer dementia (28). Plasma tHcy correlated with a subsequent decline in physical functioning during 3 y in subjects aged 70–79 y (29).

Reasons for the neurocognitive impairment from vitamin B-12, folic acid, and vitamin B-6 deficiencies are an inadequate supply of methyl groups, DNA damage, and premature apoptosis caused by disturbed methionine and folate metabolism (30, 31). Furthermore, homocysteine is toxic to neurons (32–34), vascular endothelial cells (35), and connective tissue (36).

Normalization of laboratory vitamin status in the elderly may be accomplished with oral vitamins but the clinical significance remains unclear. The relations between vitamin status (including related metabolites) and cognitive and movement performance in the elderly have, to our knowledge, not yet been investigated. Furthermore, the diagnosis of clinical vitamin deficiency is controversial.

The aims of the present study were to investigate, in an elderly population, any association between movement and cognitive

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TABLE 1
Observations in the vitamin and placebo groups at the start of the study

	Vitamin group (n = 126)	Placebo group (n = 69)	P^1
Age (y)	75.7 ± 4.7[2]	75.6 ± 4.0	NS
Men (%)	38	44	NS
BMI (kg/m^2)	25.8 ± 3.2	25.1 ± 3.8	0.04
Smokers (%)	14	9	NS
Dropouts (%)	13	16	NS
Use of cardiovascular medication (%)	47	49	NS
Use of antiepileptics, neuroleptics, or antidepressants (%)	22	23	NS
PLM test (s)			
Movement time	2.1 ± 0.6	2.1 ± 0.5	NS
Postural phase	0.9 ± 0.2	0.9 ± 0.1	NS
Locomotor phase	1.6 ± 0.4	1.5 ± 0.3	NS
Manual phase	1.4 ± 0.4	1.4 ± 0.4	NS
Simultaneity index	1.8 ± 0.2	1.9 ± 0.1	NS
Cognitive test (score)			
Digit span forward	5.8 ± 1.1	5.9 ± 1.2	NS
Digit span backward	4.5 ± 1.2	4.6 ± 1.0	0.11
Identical forms	23.2 ± 7.7	24.6 ± 7.6	NS
Visual reproduction	6.9 ± 3.0	7.0 ± 2.9	NS
Synonyms	22.4 ± 4.8	22.6 ± 4.8	NS
Block design	18.3 ± 6.3	19.8 ± 7.2	0.18
Digit symbol	35.4 ± 9.9	37.8 ± 11.4	0.18
Thurstone's picture memory test	20.5 ± 4.5	21.0 ± 3.9	NS
Figure classification	15.8 ± 4.6	16.6 ± 4.6	NS
Serum measurements			
Vitamin B-12 (pmol/L)	305 ± 130	359 ± 198	0.06
Methylmalonic acid (μmol/L)	0.22 ± 0.1	0.22 ± 0.1	NS
Creatinine (μmol/L)	101 ± 15.9	101 ± 18.8	NS
Iron (μmol/L)	15.8 ± 4.5	15.8 ± 4.3	NS
Iron-binding capacity (μmol/L)	56.3 ± 6.5	55.0 ± 7.1	NS
Plasma measurements			
Folate (nmol/L)	15.7 ± 6.1	16.4 ± 5.1	NS
Total homocysteine (μmol/L)	17.8 ± 5.5	16.1 ± 4.5	0.07

[1] Age and sex-adjusted P values (n = 195). Comparisons between groups made according to the method of O'Brien (48).
[2] Unadjusted $\bar{x}$ ± SD (all such values).

performance and vitamin status and whether treatment with therapeutic doses of vitamins B-12, folic acid, and vitamin B-6 improved this clinical performance.

SUBJECTS AND METHODS

Subjects

The total study group comprised 209 community-dwelling men and women with a mean age of 76 y and 5 mo (37). At baseline, all subjects underwent cognitive testing. One hundred ninety-five persons (117 women and 78 men) were also investigated with the Postural-Locomotor-Manual (PLM) test (**Table 1**). Of these 195 subjects, 126 were randomly assigned to receive vitamin therapy and 69 to receive placebo. The vitamin and placebo groups were well balanced with respect to baseline laboratory tests, movement and cognitive performance, and medication use for neurologic and cardiovascular disorders (Table 1).

Subjects in the vitamin group received a daily tablet containing 500 μg cyanocobalamin, 800 μg folic acid, and 3 mg vitamin B-6 hydrochloride (manufactured and supplied by Recip AB, Årsta, Sweden), and all subjects in the placebo group received an identical (other than the vitamin content) placebo tablet. The duration of the intervention was 4 mo. To ensure compliance, all subjects received a specified blinded number of tablets, and at the end of the study, the number of remaining tablets was compared with the initial number and planned intake during the study period. Informed consent was obtained from all probands, and the Research Ethics Committee of the Medical Faculty of Göteborg University approved the protocol.

Postural-Locomotor-Manual test

Movement performance (n = 195) was measured with a Postural-Locomotor-Manual (PLM) test, a noninvasive optoelectronic technique using infrared light (Qualisys AB, Göteborg, Sweden). The PLM test (38) consists of a complex motion during which the patient moves an object from the floor 1.5 m forward and positions it on a stand at the height of their chin (**Figure 1**). Six reflective markers are placed on the right side of the head, shoulder, elbow, hip, ankle, and left foot of each subject. The seventh marker is placed on the test object, a metal handle fastened to a cylindrical horizontal plate weighing 550 g. A camera system registers the infrared light pulses reflected from the markers. The position of the markers is calculated 50 times/s as 2-dimensional (x, y) Cartesian room coordinates and is stored in a computer. The coordinate data are processed by using commercially available software, the PLM program. The time taken *1*) to move the object from the floor to the shelf (movement time; MT), *2*) to raise the body after the object is picked up (postural phase; P phase), *3*) to move the feet from start until stop in front of the stand (locomotor phase, L phase), and *4*) for the goal-directed active arm movement to lift up and place the object on the stand (manual phase; M phase) were calculated. The overlap

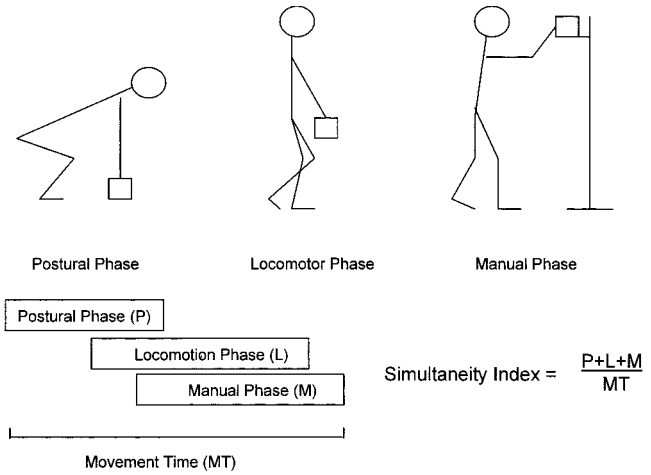

FIGURE 1. Schematic of the Postural-Locomotor-Manual test.

of the different phases is illustrated by using the simultaneity index (SI), as calculated from the sum of the P, L, and M phase durations divided by the MT: SI = (P + L + M)/MT. A high SI value (near 2.0) indicated good coordination of the P, L, and M phases into a smooth and efficient body movement, whereas a value approaching 1.0 represented poor motor coordination with more sequential performance of the phases. Each subject performed 5 PLM trials; the last 2 were analyzed, and the fastest was used for further analyses. Data were selected from a representative file according to a previously described method (38–41). The variables chosen to represent the PLM test in statistical analyses were MT, which is an indicator of the overall movement performance in this test, and the SI, which measures the coordination of different parts (ie, phases) of the motor act.

Cognitive tests

Cognitive testing was conducted by the same psychologist (GS) at baseline and after 4 mo. A comprehensive battery of cognitive tests was administered to characterize the overall level of cognitive abilities among the probands. Testing took ≈1 h. All tests except the memory test had time limits. Test scores were equal to the number of correct responses, except for figure grouping and identical forms, where corrections were made for guesses. In the analysis directed toward potential relations across markers of vitamin status and movement performance we focused on tests measuring psychomotor ability and mental speed, eg, the digit symbol test and block design. The following tests were used on the 2 occasions:

digit span forward/backward, which measures short-term memory. The subjects have to reproduce a series of digits, which increase gradually. In the backward version, the subjects have to repeat the digits backward. The maximum (best) score is 9 in the forward and 8 in the backward subtests (42, 43).

identical forms, which measures perceptual speed. This test contains 60 items of identification. For each item, a complex figure is compared with 5 other figures, and the one that is identical is marked. The maximum (best) score is 60 (44).

visual reproduction, which is a measure of visual memory. In this test 4 drawings are shown to the tested subject to be remembered and reproduced. The function is dependent on the memory for visuospatial relations but also to some extent to motor functions. The scoring followed the Wechsler Memory Scale (42). The maximum (best) score is 14.

synonyms, which measures verbal ability. The subjects have to select from among 5 words a synonym for a given word. The maximum (best) score is 30 (44).

block design, which measures spatial ability. This test consists of 7 designs that have to be made out of red, white, and red and white blocks. The maximum (best) score is 42. Bonuses are given for rapid performances (43, 44).

digit symbol, which is a test of perceptual speed with a time limit of 90 s. The subjects are asked to replace digits with symbols according to an existing code. This presumes concentration, sustained attention, learning, visual-motor coordination, and cognitive flexibility. This test has also been used as a biomarker of aging in many studies. The maximum (best) score is 90 (43).

Thurstone's Picture Memory Test, which measures long-term memory. The subjects look at 28 pictures consecutively, which are presented at a rate of every 5 s; they are later asked to identify the picture among 4 similar pictures. The pictures were enlarged to minimize problems due to vision impairments in the subjects. The maximum (best) score is 28 (44, 45).

figure classification, which measures inductive reasoning. In each item, 5 figures are given. The figure that is different from the others is to be marked. The maximum (best) score is 30 (45).

Blood sampling and laboratory methods

Blood samples were collected at the start of the study and after 1 and 4 mo. Samples were obtained with the subjects in a recumbent position, after an overnight fast. Laboratory methods are described in detail elsewhere (37). Serum methylmalonic acid (MMA) was measured by using capillary gas chromatography and mass spectrometry (46). Plasma tHcy was measured by using HPLC with fluorescence detection (47). The current health-related upper reference limits for routine clinical use by the laboratory were 16 μmol/L for plasma tHcy and 0.34 μmol/L for serum MMA, defined as the 97.5% percentile of values of blood donors and healthy persons aged 20–60 y (no age or sex difference).

Statistical analysis

In testing for differences between 2 groups, a method according to O'Brien was used (48), which provided a means of analyzing differences in not only the mean values (location) but also in distribution, ie, location as well as shape and SD. Partial correlation coefficients, adjusted for age and sex, were calculated between vitamin status and PLM and between vitamin status and cognitive performance, respectively. Multiple regression analyses, adjusted for age, sex, smoking habits, and serum creatinine were conducted for selected PLM and cognitive variables. In addition, stepwise multiple regression analysis with both demographic and laboratory variables as possible explanatory variables was performed. Possible effects of vitamin treatment were analyzed with a pairwise test of change within each group, which was followed by a test of differences in mean change between both groups. Two-tailed tests were used throughout, and a significance level of $P < 0.05$ was considered statistically significant. Non-Gaussian distributions were log transformed. The software used was part of a statistical program system developed at the Department of Geriatric Medicine, Göteborg University.

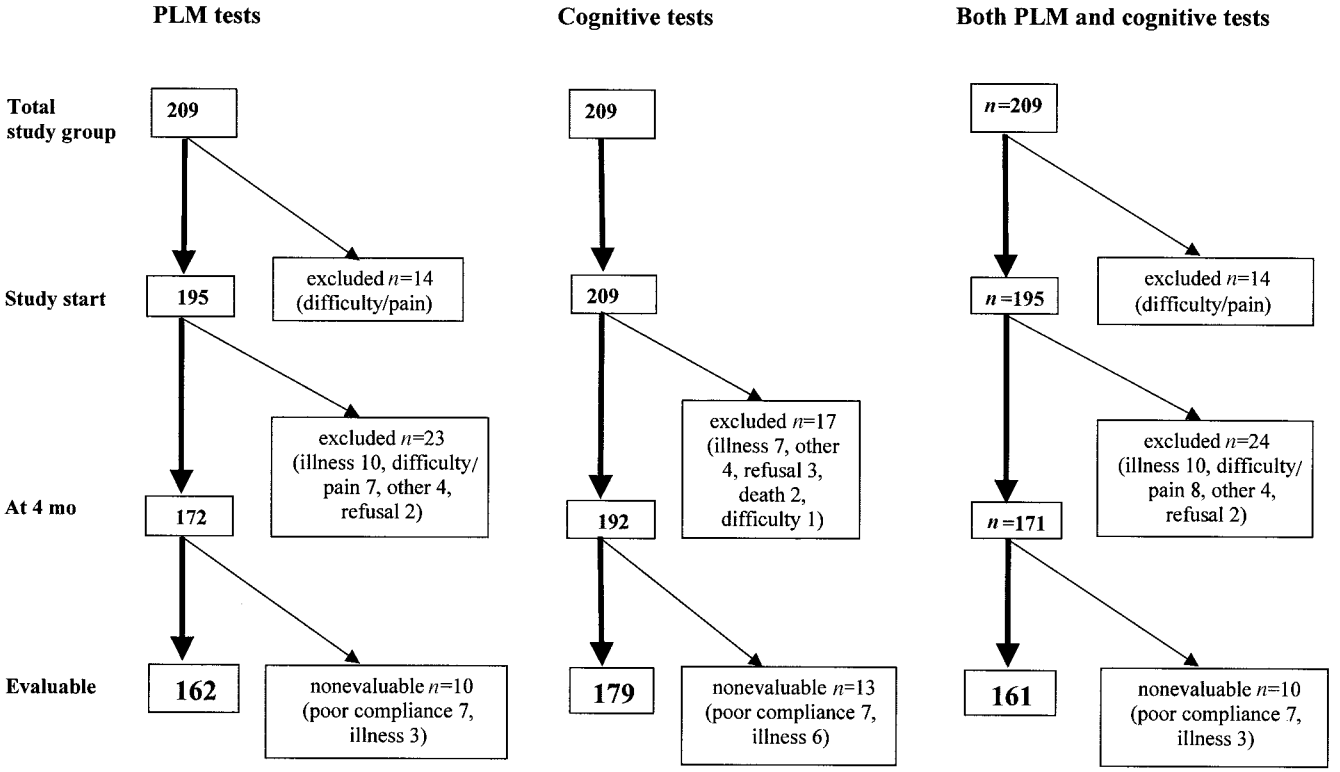

FIGURE 2. The number of subjects undergoing the Postural-Locomotor-Manual (PLM) test, the cognitive test, and both tests at different times of the study.

RESULTS

The study population was described in terms of the numbers in each test group and the reduction in numbers of the total study group (**Figure 2**). The number of participants in both the PLM and cognitive tests was 195 at start of the study and 171 after 4 mo. At the end of the study, 162 participants could be evaluated with the PLM tests, 179 with the cognitive tests, and 161 with both tests. Dropouts and excluded subjects ($n = 48$) were slightly older than and had longer MTs and L phases than did the remaining participants ($n = 161$). There were no significant differences in cognitive performance and laboratory values between the groups (data not shown).

Univariate analysis at baseline

High plasma tHcy concentrations, as defined by laboratory reference intervals (≥16 μmol/L), were found in 64% of the men and in 45% of the women; high serum MMA (≥0.34 μmol/L) concentrations were found in 11% of both sexes (37). Serum MMA, plasma folate, whole-blood folate, and serum vitamin B-12 correlated significantly with plasma tHcy, but serum vitamin B-12 did not correlate with serum MMA (**Table 2**). Four of 5 PLM components correlated with plasma tHcy and 2 with serum MMA. Seven of 9 components of the cognitive performance tests correlated with plasma tHcy and 3 with serum MMA (Table 2). There were no significant correlations across plasma and blood folate concentrations and PLM and cognitive variables. Serum vitamin B-12 correlated only with the digit span backward test ($r = 0.22$, $P < 0.01$) and with none of the PLM variables (data not shown). MT, L phase, M phase, and SI correlated significantly with all cognitive variables, except SI, which did not correlate with digit span forward or synonyms. The P phase correlated with digit span forward ($r = -0.15$, $P = 0.04$) and synonyms ($r = -0.18$, $P < 0.05$). Subjects defined as being vitamin B-12 deficient (7.2%) (37) showed inferior MT, SI, visual reproduction, and block design compared with nondeficient subjects, whereas subjects defined as folate deficient (11%) did not show such differences. However, in the total study group, the vitamin concentrations were not related to movement or cognitive performance, except for the correlation between serum vitamin B-12 and digit span backward.

Multivariate analysis at baseline

Multivariate analyses, adjusted for age, sex, smoking habits, and serum creatinine, were performed with 2 movement and 2 cognitive variables as dependent variables (**Table 3**). MT correlated with plasma tHcy, SI with serum MMA, digit symbol with plasma tHcy, and block design with plasma tHcy and serum MMA. Further multivariate regression models, including sex, smoking habits, blood hemoglobin, erythrocyte mean cell volume, whole-blood folate, serum creatinine, serum vitamin B-12, plasma folate, transferrin saturation, and anamnestic and laboratory exclusion criteria indicating a nonhealthy state (37), were also performed (data not shown). Significant correlations were found for the same variables, except for block design versus serum MMA. In this extensive multivariate analysis, inferior performance in all 4 dependent variables correlated independently with age and in 3 (digit symbol, block design, and MT) with plasma tHcy. Male sex correlated with superior performance in MT and block design.

TABLE 2
Laboratory measures, Postural-Locomotor-Manual (PLM) and cognitive tests, and correlations at the start of the study[1]

			Correlations[2] with			
			Plasma tHcy		Serum MMA	
	n	$\bar{x} \pm$ SD	*r*	*P*	*r*	*P*
Serum MMA (μmol/L)	208	0.22 ± 0.10	0.35	<0.001	—	—
Plasma tHcy (μmol/L)	209	17.2 ± 5.41	—	—	—	—
Plasma folate (nmol/L)	209	16.0 ± 6.61	−0.33	<0.001	−0.04	NS
Whole-blood folate (nmol/L)	209	351 ± 134.9	−0.16	0.020	−0.05	NS
Serum vitamin B-12 (pmol/L)	209	325 ± 159.3	−0.15	0.029	−0.11	0.10
PLM test (s)						
Movement time	195	2.10 ± 0.58	0.27	<0.001	0.16	0.023
Postural phase	195	0.91 ± 0.15	0.11	0.13	0.002	NS
Locomotor phase	195	1.54 ± 0.36	0.21	<0.01	0.08	NS
Manual phase	195	1.37 ± 0.41	0.23	<0.01	0.11	0.12
Simultaneity index	195	1.85 ± 0.16	−0.16	0.026	−0.20	<0.01
Cognitive test (score)						
Digit span forward	209	5.80 ± 1.12	−0.14	0.039	−0.05	NS
Digit span backward	209	4.46 ± 1.14	−0.16	0.018	−0.12	0.1
Identical forms	206	23.49 ± 8.05	−0.21	<0.01	−0.08	NS
Visual reproduction	205	6.88 ± 2.99	−0.18	<0.01	−0.21	<0.01
Synonyms	202	22.42 ± 4.89	−0.14	0.045	−0.01	NS
Block design	207	18.74 ± 6.89	−0.28	<0.001	−0.20	<0.01
Digit symbol	204	35.83 ± 10.78	−0.20	<0.01	−0.11	0.12
Thurstone's picture memory test	205	20.57 ± 4.54	−0.06	>0.2	−0.08	NS
Figure classification	205	15.94 ± 4.78	−0.10	0.15	−0.15	0.03

[1] MMA, methylmalonic acid; tHcy, total homocysteine.
[2] Partial (adjusted for age and sex) correlation coefficients.

Intervention study

Plasma tHcy and serum MMA

Mean plasma tHcy values decreased by 32% and mean serum MMA by 14% in vitamin-treated subjects. These significant changes resulted in a distribution of these values similar to those of younger healthy subjects (37).

Movement and cognitive function

The mean time span for the PLM tests became somewhat shorter in both groups after 4 mo, but the differences between the vitamin and the placebo groups were not significant (**Table 4**). Improvements in the cognitive tests were observed in both groups. For identical forms and synonyms, the mean scores of the

TABLE 3
Multiple regression model (in the total study group) of the correlation between movement time, simultaneity index, digit symbol, block design, and laboratory variables at the start of the study[1]

Dependent variable	*n*	Explanatory variable	Regression coefficient (β)	R^2	*P*
Movement time	194	Serum MMA	0.68	0.13	NS
	195	Plasma tHcy	0.024	0.15	<0.01
	195	Plasma folate	−0.0054	0.12	NS
	195	Whole-blood folate	−0.000022	0.12	NS
	195	Serum vitamin B-12	−0.00032	0.12	NS
Simultaneity index	194	Serum MMA	−0.29	0.11	<0.05
	195	Plasma tHcy	−0.0033	0.09	NS
	195	Plasma-folate	−0.000056	0.08	NS
	195	Whole-blood folate	−0.000085	0.08	NS
	195	Serum vitamin B-12	0.000070	0.08	NS
Block design	206	Serum MMA	−12.21	0.16	<0.05
	207	Plasma tHcy	−0.31	0.17	<0.01
	207	Plasma folate	0.029	0.13	NS
	207	Whole-blood folate	−0.0031	0.13	NS
	207	Serum vitamin B-12	0.0028	0.13	NS
Digit symbol	203	Serum MMA	−11.08	0.10	NS
	204	Plasma tHcy	−0.38	0.12	<0.05
	204	Plasma folate	0.083	0.10	NS
	204	Whole-blood folate	−0.004	0.10	NS
	204	Serum vitamin B-12	0.0038	0.10	NS

[1] Values were adjusted for age, sex, smoking habits, and serum creatinine. *See* Table 1 for units. MMA, methylmalonic acid; tHcy, total homocysteine.

TABLE 4
Movement and cognitive performance at baseline, mean changes after treatment, and *P* values for differences in mean changes between the vitamin and placebo groups[1]

	Vitamin group			Placebo group			
	n	Before treatment[2]	After treatment (change)[3]	*n*	Before treatment[2]	After treatment (change)[3]	*P*[4]
PLM test (s)							
Movement time	105	2.08 ± 0.6	−0.06 ± 0.04	57	2.00 ± 0.4	−0.07 ± 0.05	NS
Postural phase	105	0.91 ± 0.2	−0.02 ± 0.01	57	0.91 ± 0.1	−0.03 ± 0.01	NS
Locomotor phase	105	1.53 ± 0.4	−0.05 ± 0.02	57	1.49 ± 0.3	−0.07 ± 0.03	NS
Manual phase	105	1.37 ± 0.4	−0.06 ± 0.03	57	1.32 ± 0.3	−0.07 ± 0.04	NS
Simultaneity index	105	1.86 ± 0.2	−0.001 ± 0.01	57	1.87 ± 0.1	−0.01 ± 0.02	NS
Cognitive test (score)							
Digit span forward	115	5.8 ± 1.1	0.24 ± 0.09	64	5.9 ± 1.2	0.33 ± 0.14	NS
Digit span backward	115	4.4 ± 1.2	0.25 ± 0.09	64	4.6 ± 1.0	0.22 ± 0.16	NS
Identical forms	115	23.3 ± 7.6	0.13 ± 0.37	61	24.8 ± 8.1	1.5 ± 0.56	0.039
Visual reproduction	113	6.9 ± 3.1	0.61 ± 0.23	62	7.0 ± 3.0	0.6 ± 0.28	NS
Synonyms	110	22.5 ± 4.7	0.31 ± 0.25	61	22.4 ± 5.0	1.3 ± 0.3	0.017
Block design	114	18.5 ± 6.3	0.99 ± 0.37	61	20.0 ± 7.7	0.80 ± 0.50	NS
Digit symbol	113	35.1 ± 10.0	0.95 ± 0.52	62	38.0 ± 12.1	2.31 ± 0.51	0.093
Thurstone's picture memory test	115	20.3 ± 4.8	1.75 ± 0.30	63	21.1 ± 3.9	2.41 ± 0.42	0.197
Figure classification	113	15.8 ± 4.8	1.45 ± 0.33	62	16.8 ± 4.9	0.60 ± 0.55	0.164

[1] PLM, Postural-Locomotor-Manual. There were no significant differences in baseline values between the vitamin and placebo groups.
[2] All values are $\bar{x}$ ± SD.
[3] All values are $\bar{x}$ ± SEM.
[4] Two-sample *t* test of the differences in mean change between the vitamin and placebo groups.

placebo group increased more than did those of the vitamin group, and these differences were significant. The univariate correlations at baseline between serum MMA and plasma tHcy and movement and cognitive performance remained significant after treatment in both the placebo- and vitamin-treated groups (data not shown). Neither basal plasma tHcy, serum MMA, serum vitamin B-12, plasma and whole-blood folate nor changes in these components during the treatment period or vitamin therapy per se showed any associations with change in movement or cognitive performance.

DISCUSSION

In this population of community-dwelling elderly subject, deficiency of vitamin B-12 was observed in 7.2% and of folic acid in 11%. High plasma tHcy concentrations were common (64% in men, 45% in women), and high serum MMA concentrations were present in 11% of the population (37). Almost all cognitive tests correlated inversely with plasma tHcy before treatment. These results are consistent with previous findings (4, 9, 12, 13). In addition, we found correlations between both movement and cognitive performance on the one hand and serum MMA on the other. However, the influence of vitamin concentrations on performance in the total study population was limited. This is consistent with some reports (4, 12), but conflicts with others (5, 49). These discrepancies are presumably due to differences between populations with regard to health status, sex, and age and to methodologic differences.

Multivariate analyses showed significant and independent correlations between movement and cognitive performance and plasma tHcy and serum MMA concentrations. A novel finding in this study was the indication of different significances of metabolite levels for different aspects of movement and cognitive performance (Table 3).

MT and decreased mobility, in both legs and arms (L and M phases), correlated with plasma tHcy. Slow MT and poor coordination of the motor act (SI) correlated with serum MMA. MT in the PLM test was previously shown to be associated with vascular disease, brain atrophy, and cerebral white matter lesions in the elderly (40, 41, 50). SI deteriorates with age (40), in Parkinson disease (51), and in normal-pressure hydrocephalus (52). The speed (MT) and coordination (SI) of the motor function in many Parkinson disease patients have been shown to indicate deficient functioning of certain brain regions, eg, basal ganglia (51, 52). It is noteworthy that the SI was able to measure the coordinative motor capacity in the brain in these patients. Whether movement deterioration, which resembles early Parkinsonism (53, 54) and was seen in this study, is preventable with vitamin supplementation needs to be confirmed in controlled trials. The associations between plasma tHcy and movement performance have not been extensively investigated. In a longitudinal study, a decline in physical function over a 3-y interval correlated with baseline plasma tHcy (29).

The findings in this study support the assumption of a possible connection between high blood concentrations of plasma tHcy and serum MMA and impaired function in brain regions that execute and coordinate movements in the elderly. Furthermore, the correlations of the 2 metabolites with functional abilities in elderly persons were not parallel, which suggests different pathophysiologic mechanisms.

No significant clinical improvement could be ascribed to vitamin therapy. The PLM test and most cognitive tests improved both in the vitamin- and placebo-treated groups. However, the changes were numerically very small and probably attributable to a practice effect and general familiarity with the testing. The univariate correlations at baseline between the metabolites and movement and cognitive performance remained at follow-up and were essentially unchanged after the intervention. This finding

indicates a lack of significant clinical response to vitamin therapy in the present study.

The vitamin doses were chosen to treat pronounced deficiencies of these vitamins. The vitamin B-12 dose of 0.5 mg is 250 times the current US recommended daily allowance (55) and, as for the dose of folic acid, is considered adequate (56–59). Correlations were found between duration of cognitive symptoms and neurologic symptoms and response to therapy (20, 60). In a placebo-controlled study, the vitamin doses used were lower than those in the present study but were given for 12 mo instead of 4 mo and led to significant improvements in almost all cognitive tests (23). The duration of both symptoms and therapy might also be important in this community-dwelling, essentially nondemented, and mobile population. Instead of analyzing the group in terms of men and women, we adjusted for sex in the analyses. A possible differential role of vitamin status in women and men needs to be studied in larger sample sizes.

The short duration of the intervention and the relatively small sample size were limitations of the study. Its strengths were the use of an optoelectronic method, which enabled an objective and precise measurement of the subject's mobility of the upper and lower extremities and of movement coordination, and the comprehensive cognitive test battery. Further studies are needed to investigate the importance of the different B vitamins for various neurocognitive functions.

In summary, high concentrations of plasma tHcy and serum MMA were more common than were actual vitamin B deficiencies in this population of community-dwelling elderly subjects. Plasma tHcy and serum MMA correlated independently and differently with movement and cognitive performance, which suggests different pathophysiologic mechanisms. However, 4 mo of oral vitamin treatment normalized plasma tHcy and serum MMA but failed to improve movement and cognitive performance. This result might be attributable to irreversible or vitamin-independent neurocognitive decline or to an insufficient dosage of vitamins or duration of vitamin treatment.

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BS and HN-E were the principal designers of the study. MM performed and analyzed the PLM test. GS performed and analyzed the cognitive tests. CL gathered data and was responsible for the statistical calculations and for the preliminary preparation of the manuscript. All authors contributed to the scientific workup and the revision of the manuscript. None of the authors had a personal or financial conflict of interest with respect to this study.

REFERENCES

1. Hvas AM, Ellegaard J, Nexø E. Increased plasma methylmalonic acid level does not predict clinical manifestations of vitamin B12 deficiency. Arch Intern Med 2001;161:1534–41.
2. Björkegren K, Svärdsudd K. Reported symptoms and clinical findings in relation to serum cobalamin, folate, methylmalonic acid and total homocysteine among elderly Swedes: a population-based study. J Intern Med 2003;254:343–52.
3. Hvas AM, Ellegaard J, Nexø E. Vitamin B12 treatment normalizes metabolic markers but has limited clinical effect: a randomized placebo-controlled study. Clin Chem 2001;47:1396–404.
4. Ravaglia G, Forti P, Maioli F, et al. Homocysteine and cognitive function in healthy elderly community dwellers in Italy. Am J Clin Nutr 2003; 77:668–73.
5. Duthie SJ, Whalley LJ, Collins AR, Leaper S, Berger K, Deary IJ. Homocysteine, B vitamin status, and cognitive function in the elderly. Am J Clin Nutr 2002;75:908–13.
6. Riggs KM, Spiro A III, Tucker K, Rush D. Relations of vitamin B-12, vitamin B-6, folate, and homocysteine to cognitive performance in the Normative Aging Study. Am J Clin Nutr 1996;63:306–14.
7. Miller JW, Green R, Ramos MI, et al. Homocysteine and cognitive function in the Sacramento Area Latino Study on Aging. Am J Clin Nutr 2003;78:441–7.
8. Prins ND, Den Heijer T, Hofman A, et al. Homocysteine and cognitive function in the elderly: the Rotterdam Scan Study. Neurology 2002;59: 1375–80.
9. Budge M, Johnston C, Hogervorst E, et al. Plasma total homocysteine and cognitive performance in a volunteer elderly population. Ann N Y Acad Sci 2000;903:407–10.
10. McCaddon A, Hudson P, Davies G, Hughes A, Williams JH, Wilkinson C. Homocysteine and cognitive decline in healthy elderly. Dement Geriatr Cogn Disord 2001;12:309–13.
11. Seshadri S, Beiser A, Selhub J, et al. Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. N Engl J Med 2002;346:476–83.
12. Teunissen CE, Blom AH, Van Boxtel MP, et al. Homocysteine: a marker for cognitive performance? A longitudinal follow-up study. J Biol Chem 2003;7:153–9.
13. Lehmann M, Gottfries CG, Regland B. Identification of cognitive impairment in the elderly: homocysteine is an early marker. Dement Geriatr Cogn Disord 1999;10:12–20.
14. McCaddon A, Davies G, Hudson P, Tandy S, Cattell H. Total serum homocysteine in senile dementia of Alzheimer type. Int J Geriatr Psychiatry 1998;13:235–9.
15. Joosten E, Lesaffre E, Riezler R, et al. Is metabolic evidence for vitamin B-12 and folate deficiency more frequent in elderly patients with Alzheimer's disease? J Gerontol [A] Biol Sci Med Sci 1997;52:M76–9.
16. Clarke R, Smith AD, Jobst KA, Refsum H, Sutton L, Ueland PM. Folate, vitamin B12, and serum total homocysteine levels in confirmed Alzheimer disease. Arch Neurol 1998;55:1449–55.
17. Refsum H, Smith AD. Low vitamin B-12 status in confirmed Alzheimer's disease as revealed by serum holotranscobalamin. J Neurol Neurosurg Psychiatry 2003;74:959–61.
18. Nilsson K, Gustafson L, Hultberg B. Improvement of cognitive functions after cobalamin/folate supplementation in elderly patients with dementia and elevated plasma homocysteine. Int J Geriatr Psychiatry 2001;16:609–14.
19. Lehmann M, Regland B, Blennow K, Gottfries CG. Vitamin B12–B6-folate treatment improves blood-brain barrier function in patients with hyperhomocysteinaemia and mild cognitive impairment. Dement Geriatr Cogn Disord 2003;16:145–50.
20. Martin DC, Francis J, Protetch J, Huff FJ. Time dependency of cognitive recovery with cobalamin replacement: report of a pilot study. J Am Geriatr Soc 1992;40:168–72.
21. van Asselt DZ, Pasman JW, van Lier HJ, et al. Cobalamin supplementation improves cognitive and cerebral function in older, cobalamin-deficient persons. J Gerontol [A] Biol Sci Med Sci 2001;56:M775–9.
22. Clarke R, Harrison G, Richards S. Effect of vitamins and aspirin on markers of platelet activation, oxidative stress and homocysteine in people at high risk of dementia. J Intern Med 2003;254:67–75.
23. Chandra RK. Effect of vitamin and trace-element supplementation on cognitive function in elderly subjects. Nutrition 2001;17:709–12.
24. Kuhn W, Roebroek R, Blom H, et al. Elevated plasma levels of homocysteine in Parkinson's disease. Eur Neurol 1998;40:225–7.
25. McDonald WM, Richard IH, DeLong MR. Prevalence, etiology, and treatment of depression in Parkinson's disease. Biol Psychiatry 2003; 54:363–75.
26. Fahn S. Description of Parkinson's disease as a clinical syndrome. Ann N Y Acad Sci 2003;991:1–14.
27. Miller JW, Selhub J, Nadeau MR, Thomas CA, Feldman RG, Wolf PA. Effect of L-dopa on plasma homocysteine in PD patients: relationship to B-vitamin status. Neurology 2003;60:1125–9.
28. Verghese J, Lipton RB, Hall CB, Kuslansky G, Katz MJ, Buschke H. Abnormality of gait as a predictor of non-Alzheimer's dementia. N Engl J Med 2002;347:1761–8.
29. Kado DM, Bucur A, Selhub J, Rowe JW, Seeman T. Homocysteine levels and decline in physical function: MacArthur Studies of Successful Aging. Am J Med 2002;113:537–42.
30. Huang RF, Huang SM, Lin BS, Wei JS, Liu TZ. Homocysteine thiolactone induces apoptotic DNA damage mediated by increased intracellular hydrogen peroxide and caspase 3 activation in HL-60 cells. Life Sci 2001;68:2799–811.
31. Kruman II, Culmsee C, Chan SL, et al. Homocysteine elicits a DNA damage response in neurons that promotes apoptosis and hypersensitivity to excitotoxicity. J Neurosci 2000;20:6920–6.

32. White AR, Huang X, Jobling MF, et al. Homocysteine potentiates copper- and amyloid beta peptide-mediated toxicity in primary neuronal cultures: possible risk factors in the Alzheimer's-type neurodegenerative pathways. J Neurochem 2001;76:1509–20.
33. Baig SM, Qureshi GA, Minami M. The interrelation between the deficiency of vitamin B12 and neurotoxicity of homocysteine with nitrite in some of neurologic disorders. Biogenic Amines 1998;14:1–14.
34. Lipton SA, Kim WK, Choi YB, et al. Neurotoxicity associated with dual actions of homocysteine at the *N*-methyl-D-aspartate receptor. Proc Natl Acad Sci U S A 1997;94:5923–8.
35. McDowell IF, Lang D. Homocysteine and endothelial dysfunction: a link with cardiovascular disease. J Nutr 2000;130:369S–72S.
36. Krumdieck CL, Prince CW. Mechanisms of homocysteine toxicity on connective tissues: implications for the morbidity of aging. J Nutr 2000; 130:365S–8S.
37. Lewerin C, Nilsson-Ehle H, Matousek M, Lindstedt G, Steen B. Reduction of plasma homocysteine and serum methylmalonate concentrations in apparently healthy elderly subjects after treatment with folic acid, vitamin B12 and vitamin B6: a randomised trial. Eur J Clin Nutr 2003; 57:1426–36.
38. Matousek M, Baba S, Sonn U, Johnels B, Steg G, Steen B. Motor function in 90-year olds measured by optoelectronic kinesiology and activities of daily living. Aging (Milano) 1994;6:444–50.
39. Guo X, Matousek M, Sonn U, Sundh V, Steen B. Self-reported and performance-based mobility related to instrumental activities of daily living in women aged 62 years and older. A population study. Aging (Milano) 2000;12:295–300.
40. Guo X, Matousek M, Sundh V, Steen B. Motor performance in relation to age, anthropometric characteristics, and serum lipids in women. J Gerontol [A] Biol Sci Med Sci 2002;57:M37–44.
41. Guo X, Steen B, Matousek M, et al. A population-based study on brain atrophy and motor performance in elderly women. J Gerontol [A] Biol Sci Med Sci 2001;56:M633–7.
42. Wechsler D. A standardized memory scale for clinical use. J Psychol 1945;19:87–95.
43. Wechsler D. The measurement and appraisal of adult intelligence. Baltimore, MD: Williams & Wilkins; 1958.
44. Dureman I, Sälde H. Psykometriska och experimentalpsykologiska metoder för klinisk tillämpning. (Psychometric and experimental psychological methods for clinical application.) Stockholm, Sweden: Almqvist & Wiksell, 1959 (in Swedish).
45. Thurstone LL. Primary mental abilities. Psychometric monographs. Chicago, IL: University of Chicago Press,1938:5.
46. Rasmussen K. Solid-phase sample extraction for rapid determination of methylmalonic acid in serum and urine by a stable-isotope-dilution method. Clin Chem 1989;35:260–4.
47. Bald E, Sypniewski S. Ion-pair high-performance liquid chromatography of cysteine and metabolically related compounds in the form of their *S*-pyridinium derivates. J Chromatogr A 1994;676:321–30.
48. O'Brien P. Comparing two samples: extensions of the t, rank-sum, and log-rank tests. J Am Stat Assoc 1988;83:52–61.
49. Goodwin JS, Goodwin JM, Garry PJ. Association between nutritional status and cognitive functioning in a healthy elderly population. JAMA 1983;249:2917–21.
50. Guo X, Skoog I, Matousek M, et al. A population-based study on motor performance and white matter lesions in older women. J Am Geriatr Soc 2000;48:967–70.
51. Johnels B, Ingvarsson PE, Thorselius M, Valls M, Steg G. Disability profiles and objective quantitative assessment in Parkinson's disease. Acta Neurol Scand 1989;79:227–38.
52. Matousek M, Wikkelso C, Blomsterwall E, Johnels B, Steg G. Motor performance in normal pressure hydrocephalus assessed with an optoelectronic measurement technique. Acta Neurol Scand 1995;91:500–5.
53. Matousek M. Movement performance in the elderly. Investigations with an optoelectronic technique. Academical thesis. Göteborg, Sweden: Vasastadens Bokbinderi AB, 1995.
54. Guo X. Movement performance in elderly women. Relation to neuroimaging findings, medical conditions and activities of daily living. Academic thesis. Göteborg, Sweden: Vasastadens Bokbinderi AB, 2002.
55. Russell RM, Suter PM. Vitamin requirements of elderly people: an update. Am J Clin Nutr 1993;58:4–14.
56. Hathcock JN, Troendle GJ. Oral cobalamin for treatment of pernicious anemia? JAMA 1991;265:96–7.
57. de Bree A, van Dusseldorp M, Brouwer IA, van het Hof KH, Steegers-Theunissen RP. Folate intake in Europe: recommended, actual and desired intake. Eur J Clin Nutr 1997;51:643–60.
58. Clarke R. Lowering blood homocysteine with folic acid based supplements: meta-analysis of randomised trials. Homocysteine Lowering Trialists' Collaboration. Br Med J 1998;316:894–8.
59. Wald NJ, Law MR, Morris JK, Wald DS. Quantifying the effect of folic acid. Lancet 2001;358:2069–73.
60. Abyad A. Prevalence of vitamin B12 deficiency among demented patients and cognitive recovery with cobalamin replacement. J Nutr Health and Aging 2002;6:254–60.