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Homocysteine and Cognitive Performance in the Framingham Offspring Study: Age Is Important

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Plasma total homocysteine (tHcy) concentrations are associated with deficits in cognitive performance in persons free from dementia. The extent to which age modifies these associations is in need of further investigation in large, community-based, prospective studies combining the following elements: 1) multiple cognitive tests; 2) statistical adjustment for the role of the vitamin cofactors folate, vitamin B_6, and vitamin B_{12}; and 3) adjustment for the presence of risk factors for cardiovascular disease and stroke. Using data collected between 1991 and 2002, the authors investigated the associations between tHcy and multiple measures of cognitive performance in 2,096 dementia- and stroke-free participants of the Framingham Offspring Study, who were stratified into three age groups (40–49 years, 50–59 years, 60–82 years), after findings of statistically significant tHcy-by-age interactions for multiple cognitive measures. Regardless of statistical adjustment for age, sex, gender, the vitamin cofactors, and cardiovascular risk factors, statistically significant inverse associations between tHcy and multiple cognitive domains were observed for individuals aged 60 or more years; no such associations were observed for participants aged less than 60 years. Early preventive interventions may be important, because the inverse association between tHcy and cognitive performance is observed beyond middle age.

aging; cognition; folic acid; homocysteine; memory disorders; risk factors; vitamin B 6; vitamin B 12

Abbreviations: *APOE*, apolipoprotein E gene; tHcy, plasma total homocysteine.

There is mounting evidence that risk factors for stroke and cardiovascular disease are associated with cognitive decrement, cognitive decline, and dementia (1–4). High plasma total homocysteine (tHcy), an amino acid generated during one-carbon metabolism, has emerged as a new risk factor (5–11). A number of mechanisms have been advanced to explain the inverse associations between tHcy and levels of cognitive performance: 1) higher levels of tHcy are neurotoxic (12, 13); 2) elevation in tHcy is seen in the presence of deficits in the tHcy vitamin cofactors, that is, folate, vitamin B_6, and vitamin B_{12} (14, 15); and 3) higher levels of tHcy are positively associated with carotid artery atherosclerosis (16–18), coronary heart disease (19, 20), silent brain infarction (21, 22), brain atrophy (22), and stroke (23–27).

It is not yet clear when in the adult life span tHcy becomes a risk factor for lowered cognitive performance, although previous work (10, 11, 28) indicates that the magnitude of association between tHcy and cognitive performance increases with advancing age. Adults above 55 years of age have been the focus of most of the investigations relating tHcy to cognitive performance (28). Budge et al. (10), in a small sample of 158 community-dwelling volunteers ranging from 60 to 91 years of age, reported a significant interaction between age and tHcy concentration. Increments in age and tHcy concentrations were

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associated with lower levels of performance on a composite cognitive scale measuring multiple cognitive abilities.

Duthie et al. (11) reported associations between tHcy and cognitive performance for subjects in the seventh, but not the sixth, decade of age. Wright et al. (28) reported an inverse association between tHcy and results from a Dutch version of the Mini-Mental State Examination among individuals who were aged 65 or more years, but they found no significant relations for individuals who ranged in age from 40 to 64 years. Either tHcy does not relate to cognitive performance in middle-aged adults, or the Mini-Mental State Examination is not sufficiently sensitive to detect these relations among younger persons. To help resolve this question and to further examine the role played by age in modifying relations between tHcy and cognitive performance, we used a prospective design to examine age-by-tHcy interactions in a community sample of persons aged 40–82 years for whom data on multiple cognitive abilities were available.

It has been hypothesized that the increased magnitude of association between tHcy and cognitive performance with advanced age can be related to the following phenomena: 1) longer exposure to the neurotoxic effects of tHcy; 2) confounding due to a greater deficiency in one or more of the vitamin cofactors (14, 15); and/or 3) a higher prevalence of cardiovascular disease and risk factors for cardiovascular disease (16–20, 23–27) at older ages. Consequently, we addressed two major hypotheses, with a focus on the latter two possibilities: 1) Associations between tHcy and cognitive performance will not be seen in young and middle-aged adults but will be seen in older individuals; 2) associations between tHcy and cognitive performance at older ages will be diminished in magnitude when adjusted for the vitamin cofactors and when adjusted for risk factors for vascular disease, including risk for stroke. Previous studies (cited above) have addressed these two possibilities, but the design of the Framingham Offspring Study made it possible to combine the following design features into a single study: 1) a cognitive battery with measures of multiple cognitive domains that have been associated with cardiovascular risk factors previously (3); 2) a prospective design; 3) statistical adjustment for the vitamin cofactors; 4) statistical adjustment for cardiovascular risk factors, including estimated risk for future stroke; 5) adjustment for other important covariates of tHcy and cognitive performance; and 6) a large sample of participants spanning a wide age range. Moreover, to our knowledge, our focus on controlling for the risk of future stroke in a stroke-free sample is unique to our investigation.

MATERIALS AND METHODS

Study sample

Informed consent was obtained from all study participants according to the procedures approved by the institutional review board for human research at the Boston University School of Medicine.

Framingham Offspring Study participants, recruited in 1971, have been examined seven times over a 30-year period to identify risk factors for cardiovascular and cerebrovascular disease. The primary criterion for enrollment was that at least one of the participant's biologic parents or one of his/her spouse's parents was a member of the original Framingham Cohort (29).

The 3,587 offspring who were 40 or more years of age and who attended the fifth examination (from January 1991 to December 1994) were eligible to participate by virtue of the availability of data for tHcy concentrations; plasma concentrations of cyanocobalamin (vitamin B_{12}), pyridoxal-5′-phosphate (the enzyme form of vitamin B_6), and folate; and the risk factor covariates used in the present study. Of these subjects, 2,126 had been given the offspring neuropsychological examination (3, 30).

Following the seventh examination, Framingham Offspring Study participants were invited to participate in a comprehensive cognitive assessment as part of a large ancillary study of cognitive functioning (3, 30). Thus, the offspring included in our analyses were those who attended the fifth and seventh offspring examinations, were administered the neuropsychological battery between April 1999 and December 2001, and for whom data on cardiovascular disease, cardiovascular risk factors, and tHcy vitamin cofactors were available ($n = 2,126$). The mean and median intervals between the fifth examination (tHcy surveillance period) and the neuropsychological examination were 7.6 years and 7.5 years, respectively (standard deviation: 1.02 years).

Individuals were excluded from the analyses if they experienced a clinical stroke ($n = 27$) or were diagnosed with dementia ($n = 2$) prior to or at any time during the surveillance, covariate measurement, and cognitive outcome measurement periods, or if they exhibited an extreme tHcy concentration of greater than 90 μmol/liter ($n = 1$). The final sample size totaled 2,096 individuals.

Details of stroke surveillance and diagnosis methods have been published previously (31). Stroke was defined as a focal neurologic deficit of acute onset persisting for greater than 24 hours. Offspring participants suspected of stroke are given a neurologic examination in the hospital, at 3 months, 6 months, and 1 year annually, using computed tomography or magnetic resonance imaging. Scan films are reviewed, and hospital surveillance data are collected to identify all in-hospital strokes. The screening and examination procedures for dementia have been described previously (5). The final clinical diagnosis of dementia was determined by a neurology-neuropsychology review panel using well-established clinical criteria (32, 33).

Plasma homocysteine and vitamin determinations

Levels of tHcy, folate, vitamin B_{12}, and vitamin B_6 (pyridoxal-5′-phosphate) were measured at the fifth examination (34). As part of the fifth examination, blood samples were obtained after a fast of greater than 10 hours. The availability of tHcy values and the vitamin cofactors at examination 5 permitted a prospective design and allowed us to benefit from the fact that the fifth examination began before folic acid fortification (34). The tHcy concentration in plasma was determined by high-performance liquid chromatography with fluorimetric detection. Plasma folate was determined by a microbial (*Lactobacillus* cases) assay in

a 96-well plate. Plasma pyridoxal-5′-phosphate (vitamin B_6) was measured by the tyrosine decarboxylase apoenzyme method, and plasma cyanocobalamin (vitamin B_{12}) was measured by a radioimmunoassay (Quantahase II; Bio-Rad, Hercules, California) (34). The coefficients of variation for these assays were 8 percent for tHcy, 13 percent for folate, 16 percent for pyridoxal-5′-phosphate, and 7 percent for cyanocobalamin (34).

Other covariates

Risk of future stroke was estimated by using the Framingham Stroke Risk Profile. This profile (35, 36) provides an estimate of the 10-year probability (or risk) of stroke for a given subject based on the following stroke-risk factors: age, systolic blood pressure, antihypertensive medication, diabetes, cigarette smoking status, cardiovascular disease, left ventricular hypertrophy, and atrial fibrillation. The probability of stroke in the next 10 years, as estimated by the Framingham Stroke Risk Profile function, has been significantly and inversely related to lowered performance in multiple cognitive domains for persons who had not experienced stroke (3).

The risk factor data necessary to calculate the Framingham Stroke Risk Profile for the present investigation were obtained at the fifth Framingham Offspring Study examination. Systolic blood pressure was recorded as the average of two physician-recorded measurements in the sitting position. Subjects were classified as being ''on medication for hypertension'' or ''not on medication for hypertension'' at this examination. Diabetes mellitus was defined as a fasting blood sugar level of 140 or more mg/dl, a diagnosis of diabetes mellitus on a previous examination, or use of a hypoglycemic agent or insulin. The participants were categorized with respect to their cigarette smoking status as current smokers or nonsmokers. Consistent with the definition used in the construction of the Framingham Stroke Risk Profile, prior cardiovascular disease events were defined as a diagnosis of coronary heart disease, congestive heart failure, or peripheral vascular disease. The diagnoses of atrial fibrillation and left ventricular hypertrophy were based on a 12-lead electrocardiogram.

Additional renal and cardiovascular risk factors (variables not included in the Framingham Stroke Risk Profile) were also used as covariates: serum creatinine, serum total cholesterol, body mass index (weight (kg)/height $(m)^2$), self-reported mean number of drinks per day converted to ounces of alcohol consumed per week, self-reported cups of coffee per day, and apolipoprotein E genotype (*APOE*). Data for these variables (except for *APOE*) were available from examinations 2 through 5. Consequently, data from each examination were used to construct mean risk factor scores using data collected in examinations 2 through 5. For example, for body mass index, the mean of the body mass index levels measured at examinations 2 through 5 was used as a covariate.

The *APOE* genotype, also a covariate, was determined using samples from the fourth examination by the procedure described by Myers et al. (37). Briefly, *APOE* genotypes were determined after DNA amplification and restriction isotyping. The presence of particular alleles was determined by means of isoelectric focusing of the plasma confirmed by DNA genotyping (38, 39). Participants were divided into two cohorts, one including persons with an allele producing the ε4 type of apolipoprotein E (*APOE*E4*) (*E2/*E4, *E3/*E4, *E4/*E4*) and another comprising persons without an *E4* allele. The role of these covariates in the statistical models is defined in the statistics section.

Neuropsychological test battery

The neuropsychological test battery (30) was designed to be sensitive to cognitive impairment of vascular origin and dementia. By use of standardized test instructions, tests were administered and scored blindly by experienced psychometricians. Table 1 describes the individual tests and displays the raw score means and standard deviations for the entire sample. Time scores from Trails A and Trails B tests were positively skewed. Natural log transformations were performed for Trails A and Trails B.

To facilitate comparison of the regression coefficients for the different cognitive measures, the scores on the neuropsychological tests were transformed to z scores for statistical analyses. This linear transformation, based on the pooled sample of participants, had no effect on the distribution of test scores, but it allowed regression coefficients to be expressed in units of standard deviation. A ''global composite score'' was derived by computing the mean of the z scores for each of the individual tests. The raw score means and standard deviations in table 1 provide the raw score means and standard deviations necessary to interpret regression coefficients in z scores in relation to raw scores.

Statistical analysis

Preliminary analyses indicated that the distributions of tHcy, folate, vitamin B_6, and vitamin B_{12} were skewed. We used natural log transformations of these variables to promote normality in their distributions but performed a parallel set of analyses using untransformed values. Findings were the same for the log-transformed and untransformed scores. We present results only for the untransformed scores, because they provide the most direct interpretation of relations between increments in tHcy and decrements in cognitive performance.

Multivariable linear regression analyses were used to relate tHcy to cognitive performance. First, with age, education, and gender adjusted, we performed a test of interactions between age (years) and log tHcy (plasma concentration). In response to significant age-by-tHcy interactions ($p < 0.05$, all cognitive tests), we then stratified participants into three age groups (40–49 years, 50–59 years, 60–82 years) to reflect young-adult, middle-age, and elder periods of the life span and also to achieve balance in the number of subjects per cell.

Separate multivariable regression analyses for each cognitive outcome measure were performed, first with adjustment for age, education, and gender (basic covariate set) and then with more complete sets of covariates. Three vitamin covariate models were constructed by adding one of

TABLE 1. Description of the cognitive tests administered to participants and raw score means and standard deviations for the total sample, Framingham Offspring Study, 1991–2002

Neuropsychological test	Latent cognitive construct measured	Mean	Standard deviation
Wechsler Adult Intelligence Scale Similarities subtest	Abstract reasoning	16.56	3.77
Wechsler Memory Scale subtests			
Paired-Associates Learning	New verbal learning and memory	13.63	3.33
Logical Memory-Immediate Recall	Immediate recall of verbal passages	11.18	3.53
Logical Memory-Delayed Recall	Delayed recall of verbal passages	10.27	3.69
Logical Memory-Delayed Recognition	Delayed recognition of verbal passages	9.47	1.28
Visual Reproductions-Immediate Recall	Immediate memory of visual/spatial stimuli	8.80	3.25
Visual Reproductions-Delayed Recall	Delayed recall of visual/spatial stimuli	7.93	3.43
Visual Reproductions-Delayed Recognition	Delayed recognition of visual/spatial stimuli	2.99	1.04
Halstead-Reitan tests			
Trails A	Concentration, scanning, and motor tracking	34.05	16.77
Trails B	Concentration and tracking, visual motor, and executive functioning	86.05	47.77
Hooper Visual Organization Test	Visual organization ability and some demands on executive functioning	24.78	3.44
Boston Naming Test	Object naming and language	33.02	3.03

the following covariables to the basic set: 1) folic acid, 2) vitamin B_6, or 3) vitamin B_{12}. Reflecting our interest in adjusting for risk of stroke, two additional covariate models were created: 4) the stroke risk covariate model (gender, education, and the Framingham Stroke Risk Profile function score) and 5) a risk factors covariate model (gender, education, Framingham Stroke Risk Profile score, creatinine, alcohol consumption, total cholesterol, body mass index, coffee consumption, and *APOE* genotype). Age was not included as a covariate in models 4 and 5, because it is one of the components of the Framingham Stroke Risk Profile.

Given our research hypotheses with regard to null findings in the two younger groups and positive inverse associations between tHcy and cognitive performance in the older group, we used an alpha level of less than 0.05 (two-tailed p value) for the global composite score and for each of the individual test scores. Our major strategy was to first assess the association between tHcy and the major score of interest, the global composite score, and to then determine which individual tests were important with respect to this relation.

RESULTS

Table 2 summarizes demographic data and other covariates used in various analyses for each of the three age groups. Using univariable categorical regression tests (two tailed), we found positive associations with age ($p < 0.0001$) for the following variables: tHcy, 10-year risk of stroke (Framingham Stroke Risk Profile scores), folate, systolic blood pressure, diastolic blood pressure, and total cholesterol. Educational level, body mass index, cigarette use, and coffee consumption were inversely associated with age ($p < 0.01$). Chi-squared analyses indicated positive associations ($p < 0.0001$) among diabetes, cardiovascular disease, and age. The proportion of persons with folate deficits was inversely associated with advancing age ($p < 0.0001$). Pearson's product-moment correlations between the vitamins and tHcy were as follows for the four age groups: folate ($r = -0.38, -0.33, -0.35, -0.29$); vitamin B_6 ($r = -0.15, -0.15, -0.16, -0.26$); and vitamin B_{12} ($r = -0.22, -0.06, -0.30, -0.19$). All p values were less than 0.001, except for $r = -0.06$.

Preliminary tests

We examined possible nonlinear associations between tHcy and cognitive outcomes by testing for significant quadratic trends after adjusting for linear trends. There were no significant nonlinear associations.

The patterns of findings were highly similar when both the stroke risk covariate and the risk factors covariate models were used. Thus, results for only the risk factors model are reported.

As a check on the appropriateness of stratification by three age groups, we conducted tests of age (continuous)-by-tHcy interactions (age, education, and gender adjusted) within each of the three age groups formed by stratification. Tests of interactions for the global composite score were all nonsignificant (all tests: $p > 0.12$). For the 13 individual test scores, only one interaction was significant. For the Halstead-Reitan Trails A test, the multiplicative combination of age and tHcy concentration was associated with lower levels of performance for the oldest group ($\beta = -0.0072$, $p = 0.0072$). Given this single interaction, we performed our planned linear regression analyses for persons stratified by three age groups.

TABLE 2. Descriptive data for the demographic variables by age group, Framingham Offspring Study, 1991–2002

Demographic variable	Age group								
	40–49 years ($n = 659$)			50–59 years ($n = 732$)			60–82 years ($n = 705$)		
	Mean	Standard deviation	%	Mean	Standard deviation	%	Mean	Standard deviation	%
Total homocysteine (μmol/liter)*	9.46	3.53		9.76	3.74		10.32	3.75	
Framingham Stroke Risk Profile*,†	1.59	1.18		3.30	2.39		7.70	6.56	
Age (years)*	45.30	2.66		54.63	3.25		65.23	4.02	
Education (years)*	15.14	2.29		14.44	2.43		13.85	2.57	
Folate (ng/ml)*	6.56	5.23		7.74	6.27		8.78	7.05	
Vitamin B_6 (nmol/liter)	73.76	62.05		76.67	64.11		74.93	16.13	
Vitamin B_{12} (pg/ml)	457.68	229.64		453.29	231.94		446.92	248.12	
Creatinine (mg/dl)	1.06	0.17		1.04	0.20		1.05	0.21	
Alcohol (ounces/week)‡	2.81	3.28		3.07	3.90		3.08	4.11	
Systolic blood pressure (mmHg)*	115.72	11.43		122.31	13.18		130.48	14.95	
Diastolic blood pressure (mmHg)*	75.54	8.32		78.04	7.96		79.00	7.54	
Cigarettes (per day)*	6.13	10.87		5.67	10.71		3.08	9.04	
Coffee (cups/day)‡	2.53	1.98		2.36	2.02		2.18	1.90	
Total cholesterol (mm/dl)*	194.40	31.70		206.17	32.16		217.77	32.30	
Body mass index (weight (kg)/height $(m)^2$)*	25.64	4.69		26.67	4.48		26.76	4.25	
Women			55.84			52.91			51.35
APOE§ (**E2*, **E3*, or **E4/*E4*)			20.00			21.65			18.42
Diabetic*			1.97			5.20			8.23
History of cardiovascular disease*			1.37			5.95			12.20
Folate deficient*,¶			22.45			16.83			12.12
Vitamin B_6 deficient¶			2.31			2.65			1.84
Vitamin B_{12} deficient¶			2.88			2.96			2.41

* $p < 0.01$.
† Ten-year risk of stroke.
‡ One ounce = 29.6 ml; 1 cup = 236.6 ml.
§ *APOE*, apolipoprotein E gene (alleles).
¶ Vitamin deficiency: folate (<7 nmol/liter of plasma); vitamin B_6 (<20 nmol/liter); vitamin B_{12} (<140 pg/ml).

Analyses within age groups: global score

Table 3 summarizes findings for the global composite scores for the three age groups. There were no significant associations between tHcy and cognitive performance for the groups aged 40–49 and 50–59 years regardless of the covariate models used. In contrast, for all the regression models, significant associations between tHcy and cognitive performance were observed for the participants in the group aged 60–82 years. Despite adjustments for folate, vitamins B_6 and B_{12}, and the risk factor covariates, significant relations between tHcy and cognitive performance were observed.

Consistent with findings for the global composite score, no significant inverse association between tHcy and cognitive performance was observed for any of the individual test scores for the younger two age groups, that is, 40–49 or 50–59 years. When adjusted for the linear function, age, gender, and education, there were no significant quadratic trends. Consequently, the younger two groups were not included in any further analyses.

Analyses of individual tests for participants aged 60–82 years

Table 4 relates 1-μmol/liter increments in tHcy to cognitive performance tests where significant associations were obtained. As may be seen in table 4, eight of the 12 cognitive test scores were significantly associated with tHcy with adjustment for the basic covariate model: the Wechsler Adult Intelligence Scale Similarities subtest, the Wechsler Memory Scale Paired-Associates Learning subtest, the Hooper Visual Organization Test, the Wechsler Memory Scale Visual Reproductions-Delayed Recall subtest, the Wechsler Memory Scale Logical Memory-Immediate Recall subtest, the Halstead-Reitan Trails A and Trails B tests, and the Boston Naming Test. tHcy was also significantly and inversely related to the Logical Memory-Delayed Recall subtest, but only with adjustment for the folate, vitamin B_6, and risk factors covariate set.

For the Similarities subtest, the Paired-Associates Learning subtest, the Hooper Visual Organization Test, and the

TABLE 3. Decrease in the global composite *z* scores (standard deviation units) related to 1-μmol/liter increments in total homocysteine shown as regression coefficients with their 95% confidence intervals, Framingham Offspring Study, 1991–2002

Covariate model and age group	β coefficient	95% confidence interval	*p* value
Basic*			
40–49 years	0.0045	−0.0069, 0.0159	0.4409
50–59 years	0.0060	−0.0042, 0.0163	0.2599
≥60 years	−0.0286	−0.0419, −0.0143	<0.0001
Basic + folate			
40–49 years	0.0113	−0.0016, 0.0243	0.0856
50–59 years	0.0078	−0.0037, 0.0193	0.1810
≥60 years	−0.0358	−0.0510, −0.0205	<0.0001
Basic + vitamin B_6			
40–49 years	0.0078	−0.0037, 0.0193	0.1854
50–59 years	0.0078	−0.0029, 0.0185	0.1529
≥60 years	−0.0295	−0.0433, −0.0157	<0.0001
Basic + vitamin B_{12}			
40–49 years	0.0046	−0.0073, 0.0164	0.4499
50–59 years	0.0032	−0.0086, 0.0151	0.5923
≥60 years	−0.0252	−0.0399, −0.0105	<0.0008
Risk factors†			
40–49 years	0.0046	−0.0105, 0.0196	0.5521
50–59 years	0.0056	−0.0076, 0.0189	0.4051
≥60 years	−0.0365	−0.0515, −0.0215	<0.0001

* Basic covariate model: age, education, and gender.

† Risk factors: education, gender, creatinine, alcohol consumption, total cholesterol, body mass index, coffee consumption, apolipoprotein E genotype (*APOE*), and stroke risk (Framingham Stroke Risk Profile function). Age was not included in the risk factor covariate model because it is included in the Framingham Stroke Risk Profile.

Trails A and Trails B tests, the relations between tHcy and cognitive performance were robust regardless of adjustment for each of the vitamin covariate models. In contrast, for the Visual Reproductions-Delayed Recall subtest, adjustment for the vitamin cofactors rendered relations between tHcy and cognitive performance nonsignificant ($p > 0.05$). For the Logical Memory-Delayed Recall subtest and the Boston Naming Test, the relations between tHcy and cognitive performance were rendered nonsignificant by adjustment for vitamin B_{12} only.

It is clear (table 4) that associations between tHcy and cognitive performance were maintained, and in some cases enhanced, with statistical adjustment for the risk factor covariates models.

Given previous findings that relations between tHcy and cognitive performance were attenuated by statistical adjustment for hormone replacement therapy (40), we performed a secondary analysis in which 225 women treated with hormone replacement therapy were excluded from the sample. The pattern of significant findings (as reported above) was unaltered.

The vitamins

Associations between the vitamins and cognition for persons aged 60 or more years were examined to gain insight into vitamins' attenuation of some relations between tHcy and cognitive performance. Regression coefficients relating vitamins to cognitive performance are multiplied by 100. Only vitamin B_{12} was positively related to cognition. This was true for the global composite ($\beta = 0.0210$, $p < 0.05$), Visual Reproductions-Immediate Recall subtest ($\beta = 0.0356$, $p < 0.02$), Visual Reproductions-Delayed Recall subtest ($\beta = 0.0409$, $p < 0.03$), Visual Reproductions-Delayed Recognition subtest ($\beta < 0.0413$, $p < 0.01$), Logical Memory-Immediate Recall subtest ($\beta = 0.0330$, $p < 0.045$), and Logical Memory-Delayed Recall subtest ($\beta = 0.0430$, $p < 0.01$).

DISCUSSION

Consistent with a previous investigation involving measurement by the Mini-Mental State Examination in a community-based sample (28), the current investigation found significant inverse associations between tHcy and cognitive performance in older, but not in younger and middle-aged, adults. Our investigation extends this finding to multiple measures of cognitive performance. For nondemented and stroke-free individuals aged 60 or more years, tHcy was inversely related to the global composite score and to nine of the 12 cognitive outcome measures. Higher concentrations of tHcy are associated with deficits in multiple cognitive abilities. Although three of the nine tests were unrelated to tHcy, that is, the Logical Memory-Delayed Recognition subtest, the Visual Reproductions-Immediate Recall subtest, and the Visual Reproductions-Delayed Recognition subtest, variants of these three test measures were significantly associated with cognitive performance. Notably, both of the "delayed recall" variants of the visual and verbal memory tests were associated with tHcy, and these are very likely the most difficult variants of the memory tests. Referring back to table 1, it may be seen that tHcy was related to tests indexing the following abilities (table 1): abstract reasoning; new verbal learning and memory; verbal and visual memory; concentration, scanning, tracking, and executive performance; visual organization; and object naming and language. Thus, tHcy was related to a broad range of cognitive abilities.

There are a number of possible reasons why cognitive deficits associated with elevated tHcy may manifest only beyond middle age. Elderly individuals may be more vulnerable to the causal mechanisms intervening between tHcy and performance, and older persons may be exposed to higher tHcy levels for longer periods of time. Clearly, longitudinal studies are needed. Two longitudinal studies (13, 41), one over 6 years and one over a period of 2.7 years from baseline, failed to find that tHcy was related to change in cognitive ability. Given the long period of the life span where we did not see relations between tHcy and cognition,

TABLE 4. Decrease in cognitive test *z* scores (standard deviation units) related to 1-μmol/liter increments in total homocysteine shown as regression coefficients with their 95% confidence intervals for the study participants aged 60–82 years, Framingham Offspring Study, 1991–2002

Cognitive test and model	β coefficient	95% confidence interval	*p* value
Similarities*			
Basic†	−0.0393	−0.0594, −0.0192	0.0001
Basic + folate	−0.0469	−0.0699, −0.0240	<0.0001
Basic + vitamin B_6	−0.0431	−0.0639, −0.0224	<0.0001
Basic + vitamin B_{12}	−0.0409	−0.0629, −0.0190	0.0003
Risk factors‡	−0.0449	−0.0674, −0.0224	0.0001
Paired-Associates Learning§			
Basic	−0.0318	−0.0510, −0.0126	0.0012
Basic + folate	−0.0416	−0.0636, −0.0197	0.0002
Basic + vitamin B_6	−0.0344	−0.0543, −0.0145	0.0007
Basic + vitamin B_{12}	−0.0314	−0.0525, −0.0104	0.0035
Risk factors	−0.0425	−0.0640, −0.0211	0.0001
Hooper Visual Organization Test			
Basic	−0.0392	−0.0632, −0.0153	0.0014
Basic + folate	−0.0378	−0.0653, −0.0103	0.0072
Basic + vitamin B_6	−0.0384	−0.0632, −0.0136	0.0024
Basic + vitamin B_{12}	−0.0368	−0.0635, −0.0102	0.0068
Risk factors	−0.0540	−0.0812, −0.0268	0.0001
Visual Reproductions-Delayed Recall§			
Basic	−0.0211	−0.0407, −0.0016	0.0341
Basic + folate	−0.0200	−0.0425, 0.0024	0.0805
Basic + vitamin B_6	−0.0187	−0.0389, 0.0015	0.0701
Basic + vitamin B_{12}	−0.0117	−0.0329, 0.0096	0.2814
Risk factors	−0.0217	−0.0438, 0.0005	0.0557
Logical Memory-Immediate Recall§			
Basic	−0.0293	−0.0508, −0.0078	0.0077
Basic + folate	−0.0437	−0.0683, −0.0191	0.0005
Basic + vitamin B_6	−0.0324	−0.0546, −0.0101	0.0045
Basic + vitamin B_{12}	−0.0211	−0.0445, −0.0024	0.0779
Risk factors	−0.0399	−0.0636, −0.0161	0.0010
Logical Memory-Delayed Recall§			
Basic	−0.0210	−0.0429, 0.0009	0.0598
Basic + folate	−0.0293	−0.0544, −0.0042	0.0224
Basic + vitamin B_6	−0.0226	−0.0453, 0.0000	0.0505
Basic + vitamin B_{12}	−0.0104	−0.0341, 0.0132	0.3852
Risk factors	−0.0341	−0.0585, −0.0096	0.0064
Trails A¶			
Basic	−0.0397	−0.0612, −0.0182	0.0003
Basic + folate	−0.0472	−0.0717, −0.0228	0.0002
Basic + vitamin B_6	−0.0396	−0.0618, −0.0174	0.0003
Basic + vitamin B_{12}	−0.0422	−0.0658, −0.0187	0.0005
Risk factors	−0.0453	−0.0701, −0.0206	0.0004
Trails B¶			
Basic	−0.0349	−0.0551, −0.0147	0.0007
Basic + folate	−0.0389	−0.0621, −0.0157	0.0010
Basic + vitamin B_6	−0.0282	−0.0490, −0.0074	0.0079
Basic + vitamin B_{12}	−0.0316	−0.0533, −0.0100	0.0043
Risk factors	−0.0456	−0.0692, −0.0228	<0.0001
Boston Naming Test			
Basic	−0.0268	−0.0505, −0.0031	0.0270
Basic + folate	−0.0317	−0.0590, −0.0044	0.0227
Basic + vitamin B_6	−0.0271	−0.0517, −0.0026	0.0306
Basic + vitamin B_{12}	−0.0210	−0.0472, 0.0051	0.1145
Risk factors	−0.0311	−0.0572, −0.0049	0.0199

* A subtest of the Weschler Adult Intelligence Scale.

† Basic covariate model: age, education, and gender.

‡ Risk factors: education, gender, creatinine, alcohol consumption, total cholesterol, body mass index, coffee consumption, apolipoprotein E genotype (*APOE*), and stroke risk (Framingham Stroke Risk Profile function). Age was not included in the risk factor covariate model because it is included in the Framingham Stroke Risk Profile.

§ A subtest of the Wechsler Memory Scale.

¶ A Halstead-Reitan test.

it is possible that these time periods may have been too short to see a significant change. One study (42) did report a positive association between tHcy concentrations and cognitive decline over a 5-year period.

It appears that tHcy is not simply a marker for vitamin deficiency or for cardiovascular risk factors. For the global composite score and four of the cognitive measures, adjustment for the vitamins had little effect on relations between tHcy and cognitive performance. Nevertheless, it cannot be concluded that vitamins do not play a role in attenuating relations between tHcy and cognitive performance. Rather, the attenuation was selective. It was observed for the Logical Memory-Immediate Recall subtest, the Logical Memory-Delayed Recall subtest, and the Boston Naming Test. In this context, we note that only vitamin B_{12} was positively related to multiple measures of cognitive performance for the oldest group.

Associations between tHcy and cognitive performance were rendered neither nonsignificant nor appreciably attenuated by statistical adjustment for the risk factors covariate set, which included the stroke risk function variable (Framingham Stroke Risk Profile). In fact, depending on the cognitive outcome variable used, tHcy–cognitive performance associations were enhanced modestly by adjustment for the vascular risk factors and cardiovascular disease. These findings weaken the argument that tHcy is simply a marker for cardiovascular disease. However, they do not justify the conclusion that cardiovascular disease plays no role in the relation between tHcy and cognitive performance. The possibility remains that subclinical vascular disease operates as one of a number of mechanisms intervening between tHcy and cognitive performance. Neuroimaging studies indicate that tHcy may relate to cognitive performance decrements via silent infarcts, white matter lesions, and brain atrophy including hippocampal atrophy (43–45).

There are other mechanisms that might possibly intervene between tHcy and cognitive performance (46). For example, studies with cultured neurons indicate that high tHcy concentrations can lead to neurotoxicity in the absence of any contribution from vascular disease (46–49). Teunissen et al. (41), among others, argue that high concentrations of tHcy in the central nervous system inhibit the vasodilating action of nitric oxide (50) and thus induce excitotoxicity (51, 52) and/or decreased availability of methionine with consequent effects on the synthesis and degradation of neurotransmission (53).

There were limitations associated with our study. Most of the participants were Caucasian individuals. We did not have longitudinal data for our cognitive measures, nor did we relate subclinical vascular disease to cognition. Nevertheless, our cross-sectional cognitive data provide practical information for health-care providers who treat different age cohorts. In this context, our findings have potentially important implications for intervention strategies with respect to tHcy-related cognitive deficits: 1) There appears to be a long interval of the adult life span during which intervention can occur prior to the earliest signs of tHcy-related cognitive decrement; 2) deficits in global cognitive ability, accompanied by deficits in multiple cognitive domains, may forecast cognitive deficit; and 3) for some cognitive abilities, intervention with vitamin treatment and management or prevention of cardiovascular risks may not be completely successful in preventing or reducing cognitive deficits associated with elevated tHcy. Therefore, it is important to continue to explore other possible mechanisms that may intervene between tHcy and lowered cognitive performance.

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