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Homocysteine, Folate, and Vitamins B_6 and B_{12} Blood Levels in Relation to Cognitive Performance: The Maine-Syracuse Study

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Homocysteine, Folate, and Vitamins B_6 and B_{12} Blood Levels in Relation to Cognitive Performance: The Maine-Syracuse Study

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Objective: Our objective was to examine associations among plasma homocysteine concentrations (tHcy), the tHcy-cofactors (folate, vitamins B_6 and B_{12}), and multiple domains of cognitive performance, with statistical adjustment for possible confounds, including cardiovascular disease risk factors (CVD-RF) and cardiovascular disease (CVD). **Methods:** Subjects were 812 participants (58% women) of the Maine-Syracuse study who were free of dementia and stroke. Employing a cross-sectional design and multiple regression analyses, fasting concentrations of tHcy and its vitamin cofactors (folate, B_6, and B_{12}) were related to multiple domains of cognitive performance. **Results:** With adjustment for age, education, gender, ethnicity, and the vitamins, tHcy was inversely associated with visual-spatial organization, working memory, scanning-tracking, and abstract reasoning. The same results were found with adjustment for age, education, gender, ethnicity, CVD-RF, and CVD. Vitamin cofactors were positively related to cognitive performance, but with adjustment for CVD-RF and CVD, only vitamin B_6 was related to multiple cognitive domains. **Conclusions:** The inverse association of tHcy with multiple domains of cognitive functioning is not necessarily dependent on vitamin levels, vitamin deficiency, prevalent CVD risk factors, and manifest CVD. Serum folate, serum B_{12}, and plasma B_6 vitamin concentrations are positively associated with cognitive performance. Investigation of other possible mechanisms (e.g., tHcy neurotoxicity) mediating tHcy associations with cognitive performance is important, as are clinical trials examining the efficacy of folate, vitamin B_6, and vitamin B_{12} for maintenance of cognitive functioning. **Key words:** cardiovascular risk factors, cognitive performance, folate, homocysteine, vitamin B_6, vitamin B_{12}.

CVD-RF = cardiovascular disease risk factor; **CVD** = cardiovascular disease; **tHcy** = total plasma homocysteine; **MSLS** = Maine-Syracuse Longitudinal Study; **CES-D** = Center for Epidemiological Studies Depression Scale; **SST** = serum separator tube; **hs-CRP** = high-sensitivity C-reactive protein; **HPLC** = high-performance liquid chromatography; **PCR** = polymerase chain reaction; **BP** = blood pressure; **BMI** = body mass index; **ApoE** = apolipoprotein E; **nl-tHcy** = natural log total homocysteine; **nl-folate** = natural log folate; **nl-B_6** = natural log vitamin B_6.

INTRODUCTION

Homocysteine (tHcy), an amino acid produced during 1-carbon metabolism (1), has emerged as a new risk factor for cognitive deficit (2–9), cognitive decline (10), and dementia (11–13). A number of mechanisms have been proposed as mediators of this association. The most direct hypothesis is that higher levels of plasma concentrations of tHcy have an adverse influence on cognition because they are neurotoxic (14–17).

Rivaling this explanation are two additional independent hypotheses. The first relates to the finding that strong associations between tHcy and vascular disease are observed (18–21). More specifically, tHcy may be associated with cognitive performance via its relationship to cardiovascular and cerebrovascular disease (CVD) (18,19) and cardiovascular risk factors (CVD-RF) such as blood pressure (BP) (19), cigarette smoking (20), and serum cholesterol level (19–21). The second relates to the fact that the metabolism of tHcy is closely associated with that of folic acid and the vitamins B_6 and B_{12} (1). Homocysteine is a sensitive marker of vitamin B_{12} and folate status (1). Higher folate, vitamin B_6, and vitamin B_{12} concentrations have been positively associated with cognitive performance in a number of studies (6,9,22–27). Thus, on one hand, variation in folate, vitamin B_6, and vitamin B_{12} may explain relations between tHcy and cognitive performance. On the other hand, CVD-RF or CVD may intervene between tHcy and cognition. This raises two questions: 1) are associations between tHcy and cognitive performance attenuated or negated with adjustment for the vitamin cofactors (folate, vitamin B_6, vitamin B_{12}) either individually or simultaneously; 2) are associations between tHcy and cognitive performance attenuated or negated with adjustment for CVD and CVD-RF?

Homocysteine has been related to cognitive performance previously (2–13,22–25) but, with a few exceptions (9,23), the approach has been to adjust for vitamins or CVD and CVD-RF in separate studies. In the MacArthur study (23), both tHcy data and baseline cognitive data were collected before folic acid fortification of grain products in the United States, which has lowered levels of tHcy in the US population (28). In the Framingham Offspring investigation, tHcy data were obtained before folic acid food fortification and cognitive performance measures were obtained after fortification (9). In the present study, tHcy and measures of cognition were obtained after vitamin fortification.

Our hypotheses were as follows: 1) tHcy will be inversely related to global performance measures and to multiple cognitive domains; 2) associations between tHcy and cognitive performance will be attenuated but remain significant when

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adjusted for each of the vitamin cofactors and all three vitamin cofactors simultaneously; 3) relations between tHcy and cognitive performance will be attenuated but remain statistically significant with adjustment for CVD and CVD-RF; 4) the vitamin cofactors, folic acid, vitamin B_{12}, and vitamin B_6 will be positively associated with a majority of cognitive measures, and these relations will be modestly attenuated but remain statistically significant with adjustment for CVD and CVD-RF.

METHODS

Participants

The University of Maine and the State University of New York Upstate Medical University institutional review boards have approved the investigation. Informed consent for data collection was obtained from all participants.

The sample (Table 1) was provided by the Maine-Syracuse Longitudinal Study (MSLS) (29,30). This community-based study, initiated in 1975, involved a time-lagged sample of men and women (five cohorts defined by time of entry) who participated between 1975 and 2000 in studies designed to characterize the effects of hypertension and hypertension-related diseases on cognitive functioning (29,30). The MSLS provides data on BP, presence of CVD-RF, history of CVD, stroke, prescription drugs, and cognitive functioning, based on medical examination, an in-depth diagnostic interview, and hospital and physician records. At the sixth study examination (April 2001 to January 2005), additional data on tHcy, plasma concentrations of vitamin B_6, and serum concentrations of folate and vitamin B_{12} were obtained.

Of the 854 participants at the sixth examination, participants were excluded for the following reasons: 1) dementia ($n = 8$); 2) confirmed clinical stroke history ($n = 29$); 3) active dialysis treatment ($n = 5$). The clinical diagnosis of dementia was determined by the investigators using the National Institute of Neurological and Communicative Diseases and Stroke/Alzheimer's Disease and Related Disorders (NINCDS-ADRDA) criteria (31). History of stroke was based on self-report and record review (with permission), confirmed by hospitalization, treatment for stroke, or both. After exclusions, the sample consisted of 812 (58% women) community-dwelling adults, ranging in age from 26 to 98 years. Compared with the sample from an MSLS study ($n = 1679$) of baseline cognitive performance (30), the current sample was older (mean difference = 12.0 years), exhibited lower systolic BP (mean difference = 6.2 mm Hg) and diastolic BP (mean difference = 10.0 mm Hg), and had a higher prevalence of CVD (15.8% versus 3.2%).

Procedure

Participants completed psychological inventories, including the Center for Epidemiological Studies Depression Scale (CES-D), just before attending the study center. They were admitted to the study center following a fast from midnight. A blood sample was drawn by a licensed phlebotomist, and a light breakfast, including decaffeinated coffee or tea, was served. Breakfast was followed by a physical examination, including a detailed medical history. This was followed by administration of the test battery by a psychological examiner.

Assays

Fasting blood samples were collected in standard ethylenediaminetetraacetic acid tubes for plasma tHcy and plasma B_6 determinations and in serum separator tube (SST) (gel and clot activator) tubes for serum determinations. All blood samples were immediately sent on ice to Centrex Clinical Laboratories, Syracuse, NY, for immediate processing and determination of serum folate, serum vitamin B_{12}, lipid profile, triglycerides, glucose, and creatinine. Serum folate and serum vitamin B_{12} concentrations were determined using a paramagnetic particle, chemiluminescent immunoassay on a Beckman Coulter Immunoassay System. Serum creatinine was determined using a 2-point rate test type on a Johnson and Johnson Vitros Instrument. Coefficients of variation for these procedures were less than 5.0%.

Plasma samples used for determination of tHcy, vitamin B_6 (plasma pyridoxal 5′-phosphate), and high-sensitivity C-reactive protein (hs-CRP) were stored at −40°C. Batches of 100 to 150 samples were shipped on dry ice to Oxford, UK, and Cambridge, UK, for analysis. Plasma tHcy concentrations were determined at the University of Oxford (Pharmacology Department) using a fluorescence polarization immunoassay (Axis-Shield, Dundee, UK) on an Abbott IMx auto-analyzer (Abbott Laboratories, Chicago, IL,USA) (32). The coefficient of variation for the tHcy assays was less than 3.5%.

TABLE 1. Demographic and Health Characteristics of the Study Participants ($N = 812$)

Variable	Mean	SD	Variable	Percent
tHcy (μmol/l)	10.0	3.6	Gender (female)	58.1
Age (yrs)	61.8	12.6	Ethnicity (Caucasian)[a]	92.9
Education (yr)	14.6	2.7	Obesity (BMI $\geq$29.9 kg/m^2)	45.3
Folate (ng/ml)	17.0	5.2	CVD[b]	15.8
Vitamin B_6 (PLP,[c] nmol/l)	95.6	93.2	Mild renal dysfunction[d]	16.3
Vitamin B_{12} (pg/ml)	526.0	283.7	Depressed mood[e]	10.1
Systolic BP (mm Hg)	131.2	21.7	Diabetes mellitus[f]	12.4
Diastolic BP (mm Hg)	70.7	10.0	ApoE ϵ4 genotype	26.8
Alcohol (oz/week)	1.5	2.8	Folate deficiency[g]	0.0
Cigarettes/week	9.0	36.8	Vitamin B_{12} deficiency[h]	3.8
Total cholesterol (mg/dl)	203.9	39.5	Vitamin B_6 deficiency[i]	13.7
Body mass index (BMI, kg/m^2)	29.4	6.1		
Coffee (cups/day)	1.8	1.9		
Creatinine clearance[j] (ml/min)	91.8	34.6		

[a] Non-Hispanic Caucasian.
[b] CVD includes the following diagnostic categories: (1) myocardial infarction, $n = 35$ (4.3%); (2) coronary artery disease, $n = 66$ (8.1%); (3) congestive heart failure, $n = 20$ (2.5%); (4) angina pectoris, $n = 49$ (6.0%); (5) transient ischemic attack, $n = 30$ (3.7%).
[c] Pyridoxal 5′-phosphate.
[d] Estimated creatinine clearance <60 ml/min.
[e] CES-Depression scores >16.
[f] Treatment with antidiabetic drugs or fasting glucose levels of 126 mg/dl or greater.
[g] Folate deficiency defined as value <3 ng/ml.
[h] Vitamin B_{12} deficiency defined as value <200 pg/ml.
[i] Vitamin B_6 PLP deficiency defined as value <30 nmol/l.
[j] Estimated using the Cockcroft-Gault Formula (Cockcroft and Gault, 1976 (37)).

Vitamin B_6 (plasma pyridoxal 5′-phosphate) and hs-CRP concentrations were determined at the Medical Research Council Human Nutrition Research Laboratory, Cambridge, UK. For vitamin B_6, a Waters Empower 2010-controlled high-performance liquid chromatography (HPLC) system (Watford, UK) and a Waters 474 Scanning Florescence Detector was used. The HPLC system is a Waters 2695 Alliance separation module (Waters Symmetry Shield RP8, 5 μm, 4.6 $\times$ 250 mm). Assay control is via a dual-level lyophilized standard from ChromSystems diagnostics by HPLC. Coefficients of variation for the plasma pyridoxal 5′-phosphate assays were 3.75% or less (33). Plasma hs-CRP was determined by a latex particle enhanced turbidimetric immunoassay technique (34). The Flex reagent cartridge was used on the Dimension clinical chemistry system.

Standard apolipoprotein E (ApoE) genotyping, using polymerase chain reaction (PCR) and restriction enzyme digest with Hhal (35) was done at the Neuroscience Division, Medical School, University of Birmingham, UK. DNA was extracted from buffy coats using a chemical purification method (DNA isolation kit, Roche). Extracted DNA was used in the PCR, using ABgene Mastermix containing 1.5 mmol/l $MgCl_2$, 10% dimethyl sulfoxide (DMSO), and 1 μM of primers F4 and F6 each (Metabion) for the amplification of exon 4. Amplified products were digested with Hha1 (Roche) following the protocol from the supplier. Digested products were separated on agarose gel containing 0.83% multipurpose agarose and 2.67% Metaphore agarose and 1:10000 Gelstar (Cambrex). Results were viewed under ultraviolet light.

Neuropsychological Battery

The test battery is described in Table 2. It was designed to measure a wide range of cognitive abilities (36) and has been employed in studies relating hypertension and other risk factors to cognitive performance (e.g., 29,30). The factor loadings shown are discussed in the factor analytic section below.

Predictors and Covariates

The independent variables were tHcy (μmol/l), serum folate (ng/ml), plasma vitamin B_6 (nmol/l), and serum vitamin B_{12} (pg/ml). Additional covariables were age (years), gender, education (years), ethnicity, diabetes mellitus, systolic BP (mm Hg), presence of CVD, self-reported number of cigarettes smoked per day, total cholesterol (mg/dl), self-reported alcohol consumption (ounces per week), body mass index (kg/m^2, BMI), self-reported coffee consumption (number of 8-oz cups per day), presence of mild renal dysfunction, ApoE genotype, depressed mood, and hs-CRP. Diabetes mellitus was defined as follows: treatment with insulin, oral antidiabetic agents, or fasting glucose level of 126 mg/dl or above. Mild renal dysfunction was defined as an estimated creatinine clearance using the Cockcroft-Gault formula (37) of less than 60 ml/min (38). ApoE genotype was defined as one or two e4 alleles versus no e4 alleles. Using the Framingham Study criteria (39), CVD was defined by the presence of any of the following: myocardial infarction (4.3%), coronary artery disease (8.1%), congestive heart failure (2.5%), angina pectoris (6.0%), transient ischemic attack (3.7%). We employed CES-D as a categorical variable ($\leq$16 versus $>$16 points) for two reasons: 1) there was a significant skew in CES-D scores; 2) we wished to use a widely employed clinically relevant index of depressed mood (CES-D $>$16) (40). The average number of prior exposures to the tests in each test composite (see factor analysis below) was determined and used as a covariate. All covariates used in our models were identified as important based on the literature on tHcy, and all were related significantly to tHcy or cognitive performance in the present study.

Statistical Analysis Plan

Distributions of tHcy, folate, vitamin B_6, and vitamin B_{12} concentrations were all skewed, and thus were subjected to a natural log transformation to improve normality. Scores (maximum 5 minutes) on Trail Making A and B

TABLE 2. Descriptions of the Cognitive Tests With Factor Loadings From the Factor Analysis Performed in This Study

Test Composite/Tests Included in the Composite	Latent Cognitive Variable Tested	Factor Loadings
Global composite (*z* score)	Global composite of cognitive performance	
Visual-spatial organization		
Visual reproductions, immediate recall[a]	Immediate recall, visual memory, and visual-spatial problem solving	0.78
Visual reproductions, delayed recall[a]	Delayed recall, visual memory and visual-spatial problem solving	0.76
Matrix reasoning[b]	Abstract reasoning and pattern recognition	0.75
Block design[c]	Visual-spatial perception, organization and construction	0.68
Object assembly[c]	Speed of visual-spatial organization	0.66
Hooper visual organization	Visual-spatial organization; some demands on executive function	0.63
Scanning and tracking		
Trail Making A[d]	Visual scanning and tracking	0.81
Trail Making B[d]	Complex visual scanning, tracking and executive function	0.73
Digit symbol substitution[c]	Psychomotor performance	0.71
Symbol search[b]	Visual processing speed	0.67
Verbal memory		
Logical memory, immediate recall[a]	Immediate memory, verbal	0.91
Logical memory, delayed recall[a]	Delayed memory, verbal	0.91
Hopkins Verbal Learning Test	Verbal learning and memory	0.60
Working memory		
Digit span forward[c]	Attention and concentration	0.82
Digit span backward[c]	Attention, concentration, and working memory	0.80
Letter-number sequence[b]	Working memory	0.68
Controlled oral word associations	Verbal fluency and executive functioning	0.46
Similarities[a]	Abstract reasoning	NA[e]

[a] Origin WMS-R.
[b] Origin WAIS III.
[c] Origin WAIS.
[d] Origin Halstead Reitan NP Test Battery.
[e] Excluded from the factor analysis.

were skewed and thus were transformed to natural log values. The natural log values for Trails A and B and the raw scores on each of the other cognitive tests were converted to *z* scores, a linear transformation, in order that regression coefficients could be expressed in terms of SD units.

Following preliminary analyses, multivariable regression analyses were performed. In individual analyses, cognitive test scores were regressed on natural log tHcy (nl-tHcy) using one of the following covariate models in separate analyses: 1) Basic (age, gender, education, prior test exposures, ethnicity/race); 2) Basic+Folate; 3) Basic+vitamin B_6; 4) Basic+vitamin B_{12}; 5) Basic+Folate+vitamin B_6+vitamin B_{12}; 6) Basic+CVD-RF+CVD. The sixth model, Basic+CVD-RF+CVD, included the following covariates in addition to the basic covariate set: systolic BP, diabetes mellitus, cigarette smoking, coffee consumption, total cholesterol, mild renal dysfunction, BMI, alcohol use, ApoE genotype, depressed mood, hs-CRP, and CVD. For the ethnicity/race variable, participants were classified either as non-Hispanic Caucasian ($n = 754$) or Other Ethnicity ($n = 58$), i.e., 47 African-Americans, 2 Hispanic Caucasian-Americans, 3 Asian Americans, and 6 American Indians.

Each of the vitamins also served as an independent variable that, in separate analyses for each vitamin, was related to the measures of cognitive performance. Relations between the vitamins and cognition were not adjusted for tHcy but were adjusted for the Basic and the Basic+CVD-RF+CVD covariate models.

Employing the following criteria, cognitive domains, expressed as composite scores, were identified by principal components analysis and orthogonal rotation (41): 1) each composite score was constructed from variables indexing a common cognitive domain; 2) the same individual test score could not be included in more than one composite; and 3) the domain reflected by the composite score was theoretically plausible or meaningful. The Similarities Test is highly correlated with general intellectual ability (42) and loaded on multiple factors. Similarities was thus excluded from the final factor analysis on an a priori basis but was included as cognitive outcome measure.

RESULTS

Factor Analysis

Principal components and factor analyses yielded four factors with eigenvalues of 7.31, 1.58, 1.52, and 0.96 extracted. The remaining eigenvalues were lower than 0.75. Using a criterion of orthogonally rotated (Varimax) factor pattern scores greater than 0.45, we found that all cognitive outcome variables loaded on one of these four factors. Table 2 summarizes the four factors, briefly describes the tests assigned to each factor, and presents their factor loadings.

In order to allow a straightforward reconstruction of our composite scores by other investigators using different samples, we employed the "averaging method" (43) to construct them. Four composite scores were created by adding the *z* scores for each of the individual test scores loading on each factor and then dividing by the number of *z* scores. These four composite variables and the Similarities Test represented the five individual cognitive domains investigated in this study. In addition, a Global Composite score was employed as a global measure of cognitive ability. The Global Composite score was formed by adding the *z* scores for all of the variables listed above and dividing by the number of variables. Once values for the composite scores were determined, they were again standardized such that the mean for each composite score was 0.00 with a SD = 1.00.

Preliminary Tests

Visual examination of regression and residual (Jacknife) plots confirmed the appropriateness of the straight-line fit (44) for each independent variable. Plasma tHcy concentrations are higher in men than women (18) and tHcy by age interactions have been reported (2,9). Preliminary tests of interactions, conducted by injecting tHcy by gender or tHcy by age (years) interaction terms into the main effects models, were statistically nonsignificant ($p > .20$). Inspection indicated a trend for some regression coefficients relating tHcy to cognition to be larger in an over-60 age group but also clearly indicated tHcy main effects. The differences between regression coefficients for the younger and older groups were nonsignificant ($p > .15$).

For every model employed, R^2 values were statistically significant ($p < .001$). The range of R^2 values, adjusted for shrinkage, was $R^2 = 0.17$ to 0.47 across models and cognitive performance variables. With adjustment for age, education, gender, and race/ethnicity, tHcy was significantly and inversely correlated (Pearson's *r*) with folate ($r = -0.23$, $p < .01$), vitamin B_6 ($r = -0.17$, $p < .01$), and vitamin B_{12} ($r = -0.31$, $p < .01$). Pearson's *r* correlations among the vitamins ranged from 0.31 to 0.39.

Plasma THcy

Table 3 displays regression coefficients and standard errors expressing the magnitude of the associations between nl-tHcy

TABLE 3. Regression Coefficients (β) and Standard Errors (SEβ) Relating Natural Log tHcy to the Cognitive Domain Composite Scores in SD Units (*z* Scores)

Cognitive Test	Covariate Model	($N = 812$)	
		β	SEβ
Global Composite	Basic	−0.3472***	0.0949
	Basic+Folate+B_6+B_{12}	−0.2856**	0.1001
	Basic+CVD-RF+CVD	−0.2492*	0.1004
Visual-Spatial Organization Composite	Basic	−0.3414***	0.1026
	Basic+Folate+B_6+B_{12}	−0.3053**	0.1083
	Basic+CVD-RF+CVD	−0.2135*	0.1082
Verbal Memory Composite	Basic	−0.0616	0.1150
	Basic+Folate+B_6+B_{12}	0.0346	0.1220
	Basic+CVD-RF+CVD	−0.0041	0.1234
Working Memory Composite	Basic	−0.3326**	0.1164
	Basic+Folate+B_6+B_{12}	−0.3051**	0.1236
	Basic+CVD-RF+CVD	−0.3253**	0.1261
Scanning Tracking Composite	Basic	−0.2876**	0.0960
	Basic+Folate+B_6+B_{12}	−0.2683**	0.1014
	Basic+CVD-RF+CVD	−0.2179*	0.1024
Similarities (Abstract Reasoning)	Basic	−0.3653***	0.1111
	Basic+Folate+B_6+B_{12}	−0.2517*	0.1173
	Basic+CVD-RF+CVD	−0.2638*	0.1191

* $p < .05$, ** $p < .01$, *** $p < .001$.

and cognitive performance. As noted previously, associations between tHcy and cognitive performance were adjusted for folate, vitamin B_6, and vitamin B_{12} in separate models. Results for these separate models are not shown in Table 3, because the magnitude of the regression coefficients and the *p* values for nl-tHcy were nearly identical to the values shown for the model in which the vitamin covariates were entered simultaneously.

Statistically significant inverse associations between nl-tHcy and cognitive performance were observed for the Global, Visual-Spatial Organization, Working Memory, and Scanning-Tracking composites and the Similarities test. This was true with adjustment for all of the covariate models employed. Regardless of the models employed, there were no statistically significant associations between nl-tHcy and the Verbal Memory composite.

An a posteriori analysis was conducted in which the associations of nl-tHcy and cognitive performance were adjusted for all of the covariates simultaneously (Basic+Folate+B_6+B_{12}+CVD-RF+CVD). The same pattern of results was observed as was observed for the models driven by the study hypotheses, except that nl-tHcy was no longer related to Similarities.

Magnitude of Association

Comparisons of the regression coefficients in Table 3 provide an indication of the relative magnitude of effect of nl-tHcy on each of the cognitive domains as each cognitive measure is expressed in *z* scores. To further illustrate magnitude of the effects, we compared persons with "high" and "low" tHcy defined in terms of quintiles of the distribution of tHcy. Using the Global Composite score and the highest quintile of tHcy as an example, we defined a high tHcy value as a tHcy concentration >11.8 μmol/l (lowest value of the fifth quintile) and compared individuals with high and low tHcy using a categorical regression analysis. Decrements in cognitive performance associated with tHcy >11.8 μmol/l were as follows: (1) Basic ($\beta = -0.2578$, SE$\beta = 0.0688$, $p < .001$); (2) Basic+Folate+B_6+B_{12} ($\beta = -0.2160$, SE$\beta = 0.0710$, $p < .01$); and Basic+CVD-RF+CVD ($\beta = -0.1447$, SE$\beta = 0.0715$, $p < .05$). Thus, decrements in performance in relation to high versus low tHcy ranged from −0.26 to −0.14 SD.

Vitamins

The linear regression coefficients in Table 4 express the associations between natural log folate (nl-folate) concentrations and cognitive performance. For the Basic model, nl-folate was positively and significantly related to the Global, Working Memory, and Scanning-Tracking composites and Similarities. With adjustment for the Basic+CVD-RF+CVD covariate set, nl-folate was significantly and positively related only to the Global and Scanning-Tracking composite scores.

Linear regression coefficients in Table 5 express the associations between natural log B_6 (nl-B_6) and cognitive performance. Natural log B_6 was positively related to every cognitive outcome variable for the Basic covariate model. It was also positively and significantly related to every cognitive domain for the Basic+CVD-RF+CVD model with one exception, i.e., the Verbal Memory composite ($p > .05$).

Natural log vitamin B_{12} was not significantly associated with the Global Composite score, and was related only to Similarities in both the Basic model ($\beta = 0.2175$, $p < .001$), and the Basic+CVD-RF+CVD model ($\beta = 0.1920$, $p < .01$).

TABLE 4. Regression Coefficients (β) and Standard Errors (SEβ) Showing the Association Between Natural Log Serum Folate Concentrations and Test Scores in SD Units (*z* Scores)

Cognitive Test	Model	(*N* = 812)	
		β	SEβ
Global Composite	Basic	0.2236**	0.0815
	Basic+CVD-RF+CVD	0.1763*	0.0815
Visual-Spatial Organization Composite	Basic	0.1256	0.0872
	Basic+CVD-RF+CVD	0.0926	0.0870
Verbal Memory Composite	Basic	0.1450	0.0976
	Basic+CVD-RF+CVD	0.1343	0.0996
Working Memory Composite	Basic	0.2240*	0.1000
	Basic+CVD-RF+CVD	0.1751	0.1025
Scanning Tracking Composite	Basic	0.2151**	0.0814
	Basic+CVD-RF+CVD	0.1733*	0.0824
Similarities (Abstract Reasoning)	Basic	0.2382*	0.0956
	Basic+CVD-RF+CVD	0.1818***	0.0968

* $p < .05$; ** $p < .01$, ^ $p < .07$.

TABLE 5. Regression Coefficients (β) and Standard Errors (SEβ) Showing the Association Between Natural Log Plasma Vitamin B_6 (PLP) Concentrations and Test Scores in SD Units (*z* scores)

Cognitive Test	Model	(*N* = 812)	
		β	SEβ
Global Composite	Basic	0.1667***	0.0339
	Basic+CVD-RF+CVD	0.1361***	0.0346
Visual-Spatial Organization Composite	Basic	0.1455***	0.0364
	Basic+CVD-RF+CVD	0.1200**	0.0370
Verbal Memory Composite	Basic	0.0927*	0.0408
	Basic+CVD-RF+CVD	0.0743	0.0423
Working Memory Composite	Basic	0.1382***	0.0418
	Basic+CVD-RF+CVD	0.1134**	0.0437
Scanning Tracking Composite	Basic	0.1235***	0.0341
	Basic+CVD-RF+CVD	0.1010**	0.0351
Similarities (Abstract Reasoning)	Basic	0.1837***	0.0398
	Basic+CVD-RF+CVD	0.1474***	0.0411

* $p < .05$; ** $p < .01$; *** $p < .001$.

None of our participants had serum concentrations of folate suggestive of deficiency. Relatively few persons exhibited blood concentrations of vitamin B_6 (13.8%) and vitamin B_{12} (3.7%) suggestive of deficiency (see Table 1 for definitions). Consequently, to illustrate the practical importance of the associations of folate and B_6 with cognition, we compared persons with values of folate and B_6 concentrations above the lowest quintile (folate $\geq$12 ng/ml; and B_6 $\geq$35 nmol/l) with persons for whom folate and B_6 concentrations were in the lowest quintile. Folate and B_6 blood concentrations above the lowest quintile were positively associated with cognitive performance. Increments in Global composite scores for persons above the lowest quintile in B_6 were 0.31 SD ($\beta = 0.3072$, $p < .001$) for the Basic model and 0.24 SD ($\beta = 0.2391$, $p < .001$) for the Basic+CVD-RF+CVD model. Increments in Global composite scores for persons above the lowest quintile in folate were 0.16 SD ($\beta = 0.1619$, $p < .05$) with adjustment for the Basic model and 0.12 SD ($\beta = 0.1236$, $p < .08$) for the Basic+CVD-RF+CVD covariate model.

Additional Analyses

Three additional sets of separate analyses were performed. First, where vitamin deficiency was observed (B_6 and B_{12}) we replaced the B_6 and B_{12} concentration values with vitamin deficiency (yes/no) variables. Relations between tHcy and cognition remained significant and were stronger. Second, women ($n = 85$) treated with hormone replacement therapy, a correlate of tHcy, were excluded from the sample. The pattern of significant results was the same. Third, a class variable (yes/no) for vitamin supplementation was added to the Basic+CVD-RF+CVD model. We considered vitamin supplementation as a healthy lifestyle variable, possibly affecting the relations seen between nl-folate, nl-B_6 and cognition. Essentially, results for the vitamins were the same when vitamin supplementation was added to the covariate models.

DISCUSSION

Plasma tHcy

We found inverse associations between tHcy and cognitive performance for the Global composite score and four out of five cognitive domains examined. While attenuated modestly in magnitude, these associations remained statistically significant (p values $<.05$) despite adjustment for the vitamin cofactors. The modest reduction in associations between tHcy and cognitive performance with control for the vitamins may be related to the fact that the prevalence of vitamin deficiency was relatively low in our study. Nevertheless, our study and previous studies (9–11) indicate that associations between tHcy and cognitive performance are not necessarily attributable to vitamin status or deficiency.

While relations between tHcy and cognitive performance were attenuated by adjustment for the Basic+CVD-RF+CVD covariate model, they remained statistically significant. This finding is consistent with a recent study using a large population-based sample, i.e., the Framingham Offspring Study, where inverse relations between tHcy and multiple cognitive test scores were maintained when adjusted for a composite index of risk for future stroke and additional CVD risk factors (9).

It appears, then, that tHcy is not simply a proxy for CVD or CVD-RF in the absence of clinical manifestation of CVD. Other mechanisms explaining the association between tHcy and cognition must be considered. Although untested in the present study, neurotoxicity leading to cell death (14,15) and excitotoxicity (15,16) leading to degradation in neurotransmission (16,19) are possible mechanisms. These adverse events appear to operate at very high levels of tHcy, and thus it can be argued that they cannot account for linear relations between tHcy and cognitive performance over less extreme tHcy concentrations (23).

It is also possible that subclinical vascular disease serves as one of a number of mechanisms linking tHcy and cognitive performance. Plasma tHcy may influence cognitive performance by way of silent infarcts, white matter lesions, and brain atrophy (45–47).

Similar results were found in the Framingham Offspring tHcy study (9) and our study. However, our study differed in several important respects. We employed a more comprehensive test battery and thus were able to associate tHcy with very specific domains of cognitive ability. Our sample included minority subjects but excluded individuals on kidney dialysis treatment. We included C-reactive protein and depressed mood in the CVD-RF covariate set.

In the Framingham Offspring Study, tHcy was assessed before folic acid fortification of grain products in the United States and cognitive performance was measured after fortification (9). In the MacArthur study (23), tHcy and cognitive performance were measured before folic acid fortification. In our study, tHcy and cognitive performance were assessed after fortification. Clearly, the shift of the distribution of tHcy values to lower levels after fortification (28) does not negate relations between tHcy and cognitive performance.

Unlike the Framingham tHcy study (9), we found significant relations between tHcy and cognitive performance for persons less than 60 years of age. We have no explanation for this finding, given the various differences in design features summarized above. It seems possible, though speculative, that our more comprehensive battery, including composites of test scores, resulted in more sensitivity to tHcy-associated cognitive deficits in the younger adults.

The Vitamins

Our hypothesis that the vitamins would be positively related to multiple cognitive domains when adjusted for CVD risk factors and CVD was confirmed only for vitamin B_6. This is important because only a few studies have shown positive relations between vitamin B_6 and cognition (6,23,26). Consistent with this finding, there is evidence that B_6 supplementation may improve cognitive performance in elderly men (48).

Vitamin B_{12} was related only to the Similarities test (abstract reasoning), and folate was related only to the Scanning and Tracking composite (including measures of executive performance). The fact that vitamin B_6 was related to multiple

cognitive domains and the other vitamins were related to a single cognitive domain may relate to the fact that the prevalence of vitamin deficiency in our study was modestly higher for vitamin B_6 than for the other vitamins.

It has been hypothesized that folate and vitamins B_6 and B_{12} are related to cognitive performance because tHcy metabolism requires these vitamins (22). Consequently, disruption of methylation pathways may lead to cognitive impairment via the accumulation of S-adenosylhomocysteine, a strong inhibitor of the majority of methyl tranferase enzymes (23).

In the MacArthur study (23), when sex and age were adjusted, lower levels of folate and B_6 were associated with an increasing risk of cognitive decline, whereas B_{12} was not. Folate alone was related to cognitive performance after adjustment for various covariate models including some CVD variables. In our study, folate exhibited a positive linear association with the Global and Scanning/Tracking composite scores despite adjustment for the CVD covariate set. Clearly, however, B_6 emerged as the more consistent predictor of cognitive performance across cognitive domains. Regardless of the covariance model employed, B_6 was significantly associated with every cognitive domain but Verbal Memory. Whether these relationships are causal and whether B_6 supplementation can improve cognitive performance can only be revealed by longitudinal studies and the results of ongoing clinical trials.

Limitations of our study include: 1) the cross-sectional design; 2) the high average education level of our participants; 3) absence of measures of subclinical vascular disease; and 4) our CVD categories were based on self-report, a medical diagnostic interview, treatment history, and record confirmation. Consequently, presence of CVD may have been underestimated.

Strengths of the study include: 1) the availability of objective clinical data on multiple CVD risk factors; 2) the measurement of folate, the PLP form of vitamin B_6, and vitamin B_{12}, as well as tHcy; and 3) an extensive battery of tests permitting a factor analytic approach to the identification of cognitive domains.

Conclusions and Epidemiological Implications

Our findings indicate that tHcy is inversely associated with multiple domains of cognitive functioning and that these associations are not due simply to vitamin deficit, vitamin status, or the presence of manifest CVD risk factors or history of CVD events. Vitamin B_6 was positively related to the same cognitive domains as tHcy when adjusted for the CVD and risk factor covariates and vitamin supplementation. The magnitude of the effect of tHcy on cognitive performance does not rise to the level of serious clinical deficit. However, deficits of this magnitude are of considerable importance in large populations. In subsets of individuals, deficits of this magnitude herald more catastrophic deficits later in life (49). More studies are needed in order understand the mechanisms linking tHcy to cognitive performance. Only ongoing and future clinical trials can resolve whether these associations are causal and whether targeted folate and vitamin B_6 supplementation protects against deficits in cognitive performance.

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