Relations of vitamin B-12, vitamin B-6, folate, and homocysteine to cognitive performance in the Normative Aging Study

Karen M Riggs, Avron Spiro III, Katherine Tucker, and David Rush

ABSTRACT We investigated the relations between plasma concentrations of homocysteine and vitamins B-12 and B-6 and folate, and scores from a battery of cognitive tests for 70 male subjects, aged 54–81 y, in the Normative Aging Study. Lower concentrations of vitamin B-12 (P = 0.04) and folate (P = 0.003) and higher concentrations of homocysteine (P = 0.0009) were associated with poorer spatial copying skills. Plasma homocysteine was a stronger predictor of spatial copying performance than either vitamin B-12 or folate. The association of homocysteine with spatial copying performance was not explained by clinical diagnoses of vascular disease. Higher concentrations of vitamin B-6 were related to better performance on two measures of memory (P = 0.03 and P = 0.05). The results suggest that vitamins (and homocysteine) may have differential effects on cognitive abilities. Individual vitamins and homocysteine should be explored further as determinants of patterns of cognitive impairment.

KEY WORDS Vitamin B-12, vitamin B-6, folate, homocysteine, age, psychological testing, cognition

INTRODUCTION

Clinical deficiencies of B vitamins have been implicated in brain-related disorders, including reversible dementia (vitamin B-12 and possibly folate), depression (folate), and electrophysiological dysfunction, including convulsions (vitamin B-6) (1–3). In healthy older adults serum concentrations of B vitamins usually considered to be in the normal range were associated with poorer scores on tests of delayed recall, abstract reasoning, and selective attention (4, 5).

Plasma homocysteine is a marker for individual or composite status of vitamin B-12, folate, and vitamin B-6 (6). High plasma concentrations of homocysteine can be largely attributed to inadequate plasma concentrations of B vitamins (7), and it has been suggested that measurement of homocysteine is necessary to adequately identify persons with low concentrations of vitamin B-12 and folate who are actually deficient (8). Research using measurements of serum concentrations of homocysteine suggests that deficiencies of vitamin B-12, folate, and vitamin B-6 increase with age and are common in older adults (9, 10). Hyperhomocysteinemia may also occur because of impaired homocysteine metabolism as a result of genetic factors (11) or from reduced clearance because of impaired renal function (12). High plasma concentrations of homocysteine are associated with increased risk of cardiovascular, cerebrovascular, and peripheral vascular diseases (13).

Decrements in psychomotor speed and on tests measuring fluid and visual abilities were associated with vascular disease in several studies (14–18). Such cognitive dysfunction could result either from infarcts associated with clinically evident cerebrovascular disease or possibly from less easily measured gradual cell attrition due to inadequate perfusion (19). Therefore, high plasma homocysteine concentrations may be implicated in cognitive impairment because of the association between homocysteine and vascular pathology, or alternatively, as a marker of B vitamin insufficiency.

This study focused on relations of plasma vitamin B and homocysteine concentrations to cognitive performance in participants of the Boston Veterans Affairs Normative Aging Study (NAS). Plasma concentrations of homocysteine, vitamin B-12, vitamin B-6, and folate were assessed in the first 70 men who completed a 1-h cognitive battery of tests instituted during 1993 to determine relations of associated B vitamins and homocysteine with performance on tests measuring abilities in spatial copying, memory, language, perceptual speed, and spatial reasoning. For those tests for which an association with plasma homocysteine was found, we assessed the following hypotheses: 1) that the effect of homocysteine is a reflection of vascular disease status and 2) that it is predicated on B vitamin concentrations.

SUBJECTS AND METHODS

Subjects

Seventy NAS participants, aged 54–81 y (̅ x ± SD: 66.1 ± 7.0 y), participated in this study. Education ranged from 9 to

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complete the full battery of tests, which are being administered to consenting NAS subjects as part of an ongoing study of 20 y (13.9 ± 2.4 y). These subjects were the first 70 to be notified by the study protocol and were the first 70 to agree on by both committees.

Measures

Blood was collected from fasting participants. Plasma obtained by low-speed, refrigerated centrifugation was stored at −70 °C. Total homocysteine was determined by the method of Araki and Sako (20), vitamin B-12 and folate by using a radioassay kit from Ciba-Corning (Magic; Medfield, MA), and vitamin B-6 (pyridoxal phosphate) by the procedure of Bühmann Laboratories (Allschwill, Switzerland) (21).

Because participants in the larger study will be followed over time, the battery of cognitive tests includes tests appropriate for an aging sample, including tests specifically chosen to assess cognitive status and changes in adults with various pathologic conditions such as Alzheimer disease [Consortium to Establish a Registry for Alzheimer’s Disease (CERAD)] (22). Some tests are also included from the Neurobehavioral Evaluation System (NES2; 23), a computerized battery originally developed to evaluate cognitive effects of occupational exposure to toxins.

Language tests

Verbal tests [Wechsler Adult Intelligence Scale-Revised (WAIS-R; 24)]. Subjects identify 15 line-drawn objects by name (maximum score 15).

Vocabulary [Wechsler Adult Intelligence Scale-Revised (WAIS-R; 24)]. Subjects define words of increasing difficulty, which are scored according to quality of definition (maximum score 70).

Tests of perceptual speed and attention

Pattern Comparison (NES2). Subjects choose the odd pattern from three similar patterns displayed on a computer monitor. The scores are the number of correct responses (maximum 25) and the mean response latency for correct decisions.

Continuous Performance Test (NES2). Subjects press a button when they see a large letter “S”, but no other letter, on a computer monitor. The score is the mean response latency for items in the best two of six trials (10 target items in each trial). Best trials are defined as trials on which no or minimal errors are made and for which the mean response latencies are the fastest (25).

Memory tests

Word List Memory Test (adapted from CERAD). The score used for this test is the sum of words (maximum score 30) recalled over three consecutive trials, in which 10 words are presented (at a rate of one word every 2 s) on the computer screen. An associated test is the Word List Delayed Recall Test; the score is the number of words (maximum score 10) recalled after an intervening (spatial copying) task from the 10-word list previously presented in three serial trials. An alternative method of scoring delayed recall relies on measurement of Word List Savings (26): the score is the proportion of words (maximum 100%) recalled on the Delayed Recall Test compared with the third trial of the Word List Memory Test.

Backward Digit Span (WAIS-R). The scores are longest span of digits (maximum score 8) and total number of items (maximum score 12) repeated correctly in a backward sequence.

Activity Memory (adapted from 27). In this test of incidental memory we test the ability to remember information that was not explicitly presented as part of a memory task. Subjects number the first eight tests according to the order in which they completed them. The score is the proportion of consecutively paired items recalled correctly according to order, for which points (maximum 9) are given for correct sequencing of each set of two tests and correct order of the first and eighth tests.

Pattern Memory (NES2). One pattern is presented on the computer screen, which is followed after a brief interval by three similar patterns, from which the original pattern must be identified. The scores are the number of correct responses (maximum 25) and mean response latency for correct decisions.

Spatial copying test—Constructional Praxis (CERAD)

Subjects copy a circle, crossed rectangles, a vertical diamond, and a cube. These figures are augmented by the tilted triangles, eight-dot circle, horizontal diamond, and tapered box from the Developmental Test of Visual-Motor Integration (VMI) (28) and the overlapping pentagons from the Mini-Mental State Exam (29). The four CERAD items are scored with CERAD criteria (maximum score 11); all items are scored additionally by VMI scoring criteria or criteria derived from comparable VMI figures, with the score calculated as the total number of figures drawn correctly (maximum score 9).

Spatial reasoning test—Paper Folding

Subjects decide on 12 trials whether, when unfolded, a computer-displayed square piece of paper with one or two successive folds and a hole punched through the folded paper resembles the pattern of holes portrayed in another unfolded paper (30). The scores are number of correct responses (maximum 12) and mean response latencies for processing steps and decisions.

Statistical analyses

The distance-weighted least-squares method (31) was used to visually inspect the shape of possible associations between the variables of interest; plots were generated by using SYGRAPH (32). Because associations could be adequately described by a linear model, correlations were calculated between age, education, plasma B vitamins, plasma homocysteine, and cognitive test scores. Regression analyses were used to further examine significant associations (P < 0.05) or those marginally significant (P < 0.10) in these initial analyses.

Age, years of education, and diagnosis of disease from the previous exam that might affect cognitive performance, either directly or because of factors related to the disease or treatment of the disease, were also considered as possible covariates in the regression analyses. These diseases included cancer (33), diabetes (34), and hypertension (35, 36). In those cases in which age, education, and/or disease were significantly related...
to performance on a particular test, they were entered as covariates. Disease status was unknown for five persons. When adjustments were made for disease variables, these subjects were not included in the analyses.

Because multiple cognitive tests were administered, a Bonferroni adjustment to the $P$ value (0.05/11) was calculated. The resulting $P$ value of 0.004 was used as a conservative test of the significance of results of the regression analyses. Because this work was primarily exploratory in nature, all results of regression analyses that were significant or marginally significant at a more liberal $P$ value of 0.05 are also reported. The SAS computer software statistics program (SAS Institute, Cary, NC) was used for all statistical analyses except for the plots generated by the distance-weighted least-squares method.

**RESULTS**

Most participants completed all cognitive tests. One subject did not complete the computerized tests because of a computer problem, and five other subjects did not complete the full battery either because of conflicting time commitments or, in one case, a complaint of tiredness. Normal or near-normal distributions of test scores measuring accuracy were found on all tests except the CERAD short version of the Boston Naming Test, on which participants generally performed at a high level. For subsequent analyses, log-transformed vitamin B-12, vitamin B-6, folate, and homocysteine values and also log-transformed scores for cognitive response times, which were positively skewed, were used to reduce the effect of outliers on statistical analyses.

No significant relations were found between plasma concentrations of the B vitamins or homocysteine and age or years of education of the subjects. Concentrations of plasma homocysteine were negatively associated with blood concentrations of vitamin B-12 ($r = -0.36, P < 0.01$) and folate ($r = -0.58, P < 0.001$). However, homocysteine and vitamin B-6 concentrations were not associated, as might be expected, given that fasting plasma homocysteine concentrations generally do not reflect vitamin B-6 status, unlike homocysteine measured postprandially or after a large oral dose of L-methionine (37). Plasma folate and vitamin B-12 concentrations were positively related ($r = 0.42, P < 0.001$). The correlation between folate and vitamin B-6 were nearly significant ($r = 0.21, P = 0.08$).

In Table 1 the means and ranges of plasma B vitamin and homocysteine concentrations are given.

In Table 2 are unadjusted correlations among age, education, B vitamins, homocysteine, and cognitive test scores. In the case of response times, for which data were log-transformed for statistical analyses, means are reported after conversion back to the original units.

After completing the regression analyses described in the Methods section and after appropriate covariates were adjusted for, significant or near-significant relations between B vitamins and/or homocysteine and test scores were found only for the CERAD Constructional Praxis and VMI-augmented spatial copying tests and for the Activity Memory and Backward Digit Span memory tests. These results are described below. No significant relations were found between plasma B vitamins or homocysteine and any of the other tests, including those measuring perceptual speed, spatial reasoning, and language abilities.

**Spatial copying**

None of the possible covariates were related to scores on either of two versions of the spatial copying task (CERAD Constructional Praxis and the augmented set of CERAD and VMI figures scored by VMI criteria). A significant main effect for homocysteine concentration ($P < 0.004$ in both cases) was found for spatial copying scores on both of these versions. In Figure 1 the Constructional Praxis scores of the subjects according to their plasma homocysteine concentrations, indicated by quartile, are shown. For comparison purposes, the dotted lines indicate the mean scores on this test of the CERAD subjects described by Morris et al (22). The 18 NAS subjects in the highest (fourth) quartile of homocysteine concentrations ($\geq 12.6 \mu $mol/L), on average, scored similarly to subjects with clinically significant deficits, that is, to subjects judged to have mild Alzheimer disease. Scores for subjects in the fourth quartile ranged from 6 to 11, in comparison with scores of subjects with mild Alzheimer disease, which ranged from 1 to 11 (22).

The mean proportion of criteria scored correct for each of the four Constructional Praxis figures by subject quartiles of homocysteine are shown in Figure 2. These results demonstrate that subjects with the highest plasma homocysteine concentration were able to fulfill the fewest criteria for at least the most difficult figure, the cube.

Performance on the extended set of CERAD and VMI figures, for which at least two figures can be assessed for each of three graded difficulty levels, is shown in Figure 3. The pattern here is more marked, corroborating the hypothesis suggested by Figure 2 that spatial copying errors occurred in response to complexity. Subjects with high plasma homocysteine concentrations had difficulty with the more complex figures but performed like subjects with lower homocysteine concentrations.

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**TABLE 1**

<table>
<thead>
<tr>
<th>B vitamin and homocysteine concentrations in study subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Observed</strong></td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>Vitamin B-12 (pmol/L)</td>
</tr>
<tr>
<td>Vitamin B-6 (nmol/L)$^4$</td>
</tr>
<tr>
<td>Folate (nmol/L)</td>
</tr>
<tr>
<td>Homocysteine (μmol/L)</td>
</tr>
</tbody>
</table>

$^1$ For comparison purposes, ranges of values for B vitamins and homocysteine were taken from Human Nutrition Research Center Metabolic Research Unit laboratory values falling between the 5th and 95th percentiles.

$^2$ ± SD; range in parentheses; the number of subjects whose values were lower (38–40), or in the case of homocysteine (7), higher, than suggested normal values in brackets.

$^3$ As pyridoxal phosphate.

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TABLE 2
Correlations between age, education, plasma B vitamin and homocysteine concentrations, and test scores

<table>
<thead>
<tr>
<th>Test and mean score</th>
<th>Age</th>
<th>Education</th>
<th>Vitamin B-12</th>
<th>Folate</th>
<th>Vitamin B-6</th>
<th>Homocysteine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Language</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Verbal fluency</td>
<td>-0.22^1</td>
<td>0.19</td>
<td>-0.08</td>
<td>0.08</td>
<td>0.23^1</td>
<td>0.07</td>
</tr>
<tr>
<td>(n = 69)</td>
<td>(19.6 ± 4.6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boston Naming Test</td>
<td>-0.20^1</td>
<td>0.16</td>
<td>0.15</td>
<td>0.14</td>
<td>0.07</td>
<td>-0.08</td>
</tr>
<tr>
<td>(n = 69)</td>
<td>(14.6 ± 0.92)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vocabulary</td>
<td>-0.12</td>
<td>0.52^2</td>
<td>0.14</td>
<td>0.17</td>
<td>0.05</td>
<td>-0.09</td>
</tr>
<tr>
<td>(n = 69)</td>
<td>(49.7 ± 10.8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perceptual speed and attention</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Pattern Comparison Test (n = 69)</td>
<td>-0.28^3</td>
<td>0.32^2</td>
<td>0.05</td>
<td>0.10</td>
<td>-0.09</td>
<td>-0.01</td>
</tr>
<tr>
<td>Total correct (24.0 ± 1.4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean response time, correct trials (5.2 s)</td>
<td>0.18</td>
<td>-0.15</td>
<td>-0.10</td>
<td>-0.002</td>
<td>0.19</td>
<td>-0.10</td>
</tr>
<tr>
<td>Continuous Performance Test (n = 68)</td>
<td>0.20</td>
<td>-0.10</td>
<td>0.14</td>
<td>0.01</td>
<td>0.09</td>
<td>-0.02</td>
</tr>
<tr>
<td>Mean response time, best of two trials (330.3 ms)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Memory</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Word List Memory Task (n = 68)</td>
<td>-0.33^2</td>
<td>0.36^2</td>
<td>-0.22^1</td>
<td>-0.18</td>
<td>-0.02</td>
<td>0.14</td>
</tr>
<tr>
<td>(n = 68)</td>
<td>(19.2 ± 4.0)</td>
<td>(5.3 ± 1.2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Word List Delayed Recall (n = 68)</td>
<td>-0.27^3</td>
<td>0.41^2</td>
<td>-0.21^1</td>
<td>-0.21^1</td>
<td>-0.08</td>
<td>0.09</td>
</tr>
<tr>
<td>(n = 68)</td>
<td>(6.3 ± 2.0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Word List Savings (n = 68)</td>
<td>-0.12</td>
<td>0.34^2</td>
<td>-0.12</td>
<td>-0.12</td>
<td>-0.03</td>
<td>-0.02</td>
</tr>
<tr>
<td>(n = 68)</td>
<td>(80 ± 17.7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Backward Digit Span</td>
<td>-0.18</td>
<td>0.13</td>
<td>0.13</td>
<td>0.02</td>
<td>0.26^4</td>
<td>-0.005</td>
</tr>
<tr>
<td>Total (n = 68)</td>
<td>-0.26^4</td>
<td>0.17</td>
<td>0.15</td>
<td>0.04</td>
<td>0.27^3</td>
<td>0.0005</td>
</tr>
<tr>
<td>(n = 68)</td>
<td>(5.4 ± 2.0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Activity Memory (n = 65)</td>
<td>-0.21^1</td>
<td>0.35^2</td>
<td>-0.03</td>
<td>0.12</td>
<td>0.23^1</td>
<td>-0.14</td>
</tr>
<tr>
<td>(n = 65)</td>
<td>(0.56 ± 0.22)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pattern Memory (n = 69)</td>
<td>-0.37^2</td>
<td>0.25^3</td>
<td>0.06</td>
<td>0.04</td>
<td>0.05</td>
<td>-0.15</td>
</tr>
<tr>
<td>Total correct (19.8 ± 2.4)</td>
<td>0.25^4</td>
<td>-0.06</td>
<td>0.06</td>
<td>0.05</td>
<td>0.08</td>
<td>-0.06</td>
</tr>
</tbody>
</table>

Spatial copying

CERAD Constructional Praxis (n = 68) | 0.14 | 0.19 | 0.25^4 | 0.35^3 | -0.14 | -0.39^2 |
| (9.0 ± 1.5) | | | | | | |
| DT-VM augmented version (n = 68) | 0.08 | 0.15 | 0.27^3 | 0.21^4 | -0.01 | -0.34^2 |
| (14.6 ± 5.5) | | | | | | |

Spatial reasoning

Paper Folding Test (n = 69) | -0.31^1 | 0.27^3 | 0.14 | 0.14 | 0.03 | -0.02 |
| Total correct (8.6 ± 1.9) | 0.40^2 | -0.19 | -0.02 | 0.05 | 0.07 | 0.02 |
| Decision time (3.4 s) | | | | | | |

^1 P < 0.10.
^2 P < 0.01.
^3 P < 0.05.

on less complex designs. Therefore, it appears that the decrement associated with a high plasma homocysteine concentration is not due to such factors as poor motivation or a speed-accuracy tradeoff, but to difficulty in organizing the parts in more complex figures.

The pattern of relation of the plasma B vitamin concentrations to spatial copying performance was less straightforward. A significant main effect of plasma folate concentration (P < 0.01) was found for scores on the CERAD test, but the main effect was not significant (P = 0.08) for scores on the augmented set of figures. Lower plasma folate concentrations were related to lower scores on the CERAD test, but not on the augmented set of figures. In separate analyses, significant main effects of vitamin B-12 concentration were found both for the CERAD test (P < 0.05) and for the augmented set of figures (P < 0.05). According to an analysis of accuracy by figure difficulty (as in Figure 3 above), it appears that the lowest plasma concentrations of vitamin B-12 and folate were associated with the poorest performance on the most difficult figures and that, as in the case of homocysteine concentration, performance on easy and moderately difficult figures remained fairly consistent for subjects across plasma concentrations of the vitamins. Therefore, increasing the number of subjects for future analyses may result in significant results for both vitamins on both versions of the test.

In attempting to answer the question of whether plasma homocysteine is acting primarily as an index of B vitamin insufficiency or as a pathogenic factor in vascular disease, first, we statistically controlled for the influence of B vitamins to measure the consequent degree of attenuation of the homocysteine-related variance for the CERAD spatial copying test. The folate and vitamin B-12 effects, as shown in Table 3, were no
longer significant when they were entered into the regression equation with homocysteine, and homocysteine concentration makes a unique contribution to performance on the CERAD test separate from possible effects of folate and vitamin B-12. Second, we compared prevalence of vascular disease by quartiles of homocysteine concentration to assess whether subjects with high plasma homocysteine concentrations were more likely to have vascular disease than those with lower concentrations. [Vascular disease diagnoses were coded by using the International Classification of Diseases (ICDA8) (41).] Only 1 of the 70 subjects received a diagnosis of cerebrovascular disease, 22 a diagnosis of hypertension, and 8 a diagnosis of ischemic heart disease. According to this analysis, subjects from different quartiles of homocysteine concentration did not differ according to diagnoses of cerebrovascular disease \[\chi^2(3) = 2.93, P = 0.40\]; hypertension \[\chi^2(3) = 1.52, P = 0.67\]; or ischemic heart disease \[\chi^2(3) = 1.01, P = 0.79\].

As well as being an effective marker of composite B vitamin concentrations, specifically vitamin B-12 and folate, homocysteine may be associated with cognitive performance in its own right. Although homocysteine does not appear to be acting as a predictor of clinically diagnosed vascular disease in this sample, it is possible that the homocysteine concentration is associated with subclinical vascular or other changes that may affect cognitive function.

**Memory**

**Backward Digit Span Test**

Of possible covariates, only age (\(r = -0.26, P < 0.05\)) was related significantly to Backward Digit Span total score (the total number of items answered correctly), but not to backward digit span (the longest span correctly recalled). A main effect of vitamin B-6 concentration was significant (\(P < 0.05\)) for both backward digit span and Backward Digit Span total scores, the latter after adjustment for age. As shown in Figure 4, subjects with the highest plasma concentration of vitamin B-6 had the best performance on both of these measures. These subjects both remembered the longest spans and were most consistent in their ability to recall spans correctly in a backward order. The interaction between age and plasma vitamin B-6 concentration for this test was not significant.

**Activity Memory Test**

Age (\(r = -0.32, P < 0.01\)), education (\(r = 0.27, P < 0.05\)), cancer (\(r = -0.34, P < 0.01\)), and diabetes (\(r = -0.23, P < 0.10\)) were related to the proportion of items remembered.

**TABLE 3**

<table>
<thead>
<tr>
<th>Equation number and variable</th>
<th>(R^2)</th>
<th>Increment (R^2)</th>
<th>(F)</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, Vitamin B-12</td>
<td>0.06</td>
<td>2.08</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>2, Folate</td>
<td>0.12</td>
<td>3.00</td>
<td>0.003</td>
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</tr>
<tr>
<td>3, Homocysteine</td>
<td>0.15</td>
<td>-3.47</td>
<td>0.0009</td>
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<tr>
<td>4, Vitamin B-12</td>
<td>0.06</td>
<td>1.07</td>
<td>0.28</td>
<td></td>
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<tr>
<td>+ Homocysteine</td>
<td>0.17</td>
<td>-2.90</td>
<td>0.005</td>
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<tr>
<td>5, Folate</td>
<td>0.12</td>
<td>1.31</td>
<td>0.19</td>
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<tr>
<td>+ Homocysteine</td>
<td>0.18</td>
<td>-2.10</td>
<td>0.03</td>
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</tr>
<tr>
<td>6, Vitamin B-12</td>
<td>0.06</td>
<td>1.09</td>
<td>0.27</td>
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<td>+ Folate</td>
<td>0.14</td>
<td>2.37</td>
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<td>6, Vitamin B-12</td>
<td>0.06</td>
<td>0.79</td>
<td>0.43</td>
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<tr>
<td>+ Folate</td>
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<td>1.08</td>
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<tr>
<td>+ Homocysteine</td>
<td>0.18</td>
<td>-1.93</td>
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</tr>
</tbody>
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Increment \(R^2\) indicates the increment in \(R^2\) associated with adding the variable to the regression equation; the \(F\) value evaluates the statistical significance of \(R^2\) for the first variable entered or the increment in \(R^2\) associated with the addition of the second or third variable. CERAD, Consortium to Establish a Registry of Alzheimer’s Disease.
correctly in sequence. Shown in Figure 5 is the mean proportion recalled correctly according to subject quartiles of plasma vitamin B-6 concentration, with the proportion adjusted by years of education, cancer, and diabetes. A main effect of plasma vitamin B-6 concentration was marginally significant (P = 0.05), as was the interaction between vitamin B-6 concentration and age (P = 0.06). The relatively younger subjects, but not the older subjects, appeared to perform on this test according to vitamin B-6 concentration. The interactions between covariates other than age and vitamin B-6 concentration for this test were not significant.

**Word List Memory Test**

Of possible covariates, age (r = -0.33, P < 0.01), education (r = 0.36, P < 0.01), cancer (r = -0.36, P < 0.01), and diabetes (r = -0.31, P < 0.05) were related to the total number of items remembered across all three learning trials. After adjustment for these covariates, the marginal relation between plasma vitamin B-12 concentration and performance on this test was attenuated further (P = 0.13). The surprising trend for higher plasma concentrations of vitamin B-12 to be related to decreased recall on this test appears to be an artifact resulting from subjects with certain diseases such as cancer and diabetes having higher vitamin B-12 concentrations, most likely caused by those with illness using vitamin supplements.

**DISCUSSION**

Plasma vitamin B-12 and folate concentrations appear to be related to cognitive performance in a different manner than is vitamin B-6 concentration. High fasting concentrations of homocysteine and low concentrations of vitamin B-12 and folate were associated with deficits in spatial copying. These associations were observed despite the fact that few subjects had what are currently considered low concentrations of vitamins B-12 (<200 ng/L) (38) and folate (<3 μg/L) (40). Higher concentrations of vitamin B-6 were associated with better performance on two tests of memory.

Plasma homocysteine concentration was more strongly related to spatial copying performance than was serum B vitamin concentration and it remained a significant predictor even with the use of a more conservative test of statistical significance (Bonferroni adjustment resulting in a P value of 0.004). High plasma homocysteine concentration was associated with scores that fell within the clinically significant range on the CERAD Constructions Praxis test. When compared with CERAD patients and control subjects, the NAS subjects with the highest homocysteine concentrations performed on average like patients with mild Alzheimer disease. They also exhibited difficulty in copying the most complex spatial figures. Few subjects in the highest quartile of homocysteine concentration completed the cube (22%) and tapered box (17%) correctly according to VMI criteria. In comparison, these figures are mastered by 50% of schoolchildren by the age of 13 y (28). On the other hand, subjects with the lowest concentration of homocysteine performed most like CERAD control subjects, ostensibly demonstrating the best maintenance of spatial copying skills. (Although there was no association between age and spatial copying performance in this sample, we hypothesize that poor performance is indicative of organic deficit that might be age-related within individuals. Another alternative, that correct copying of the most difficult figures was dependent on prior learning and that subjects in the highest homocysteine concentration quartile had not learned the proper methods, is unlikely because of the lack of association between education and performance on this task.)

The explanation for many subjects' poor performance on this particular test is unclear. Similar associations between plasma homocysteine concentration and performance on other tests of spatial skills, Pattern Memory and Paper Folding, were not found. Each test requires somewhat different skills and is particularly susceptible to different kinds of errors, which may explain the lack of further associations. Certainly, the correlations between scores on the two versions of the spatial copying test and accuracy scores for the Pattern Memory and Paper Folding tests were generally low, 0.0 and 0.17 for the Paper Folding Test and 0.16 and 0.31 for the Pattern Memory Test (for the Constructions Praxis and extended set of figures), respectively.

Furthermore, the subjects' performance on other tests from the CERAD battery was generally in the average to above-average range, as judged by comparison of mean scores for NAS subjects with mean scores of CERAD elderly control subjects. In comparison, in this study, subjects' mean score on the Constructions Praxis test fell at the 13th percentile of mean scores of CERAD control subjects (26). Although the subjects in these studies may not be perfectly comparable, this discrepancy suggests that difficulties with spatial copying are a relatively isolated problem in the NAS subjects.

Plasma vitamin B-6 concentration, on the other hand, was associated with performance on two tests of memory. The
highest quartile (87.6 ± 48.1 nmol/L, range: 51.2–215.8 nmol/L) of vitamin B-6 concentration was related to the best performance on the Backward Digit Span Test, and performance improved on the Activity Memory Test with increased plasma vitamin B-6 concentration. Although normative data are not available for the Activity Memory Test, scores on the Backward Digit Span Test for subjects with lower plasma concentrations of vitamin B-6 are similar to scores of healthy older adults and are not indicative of a deficit (42).

The Backward Digit Span Test assesses working memory, requiring simultaneous storage and processing of new information. The Activity Memory Test measures incidental memory, for which a good score depends both on whether information has been incidentally encoded and on the subject’s motivation and ability to access this information. Both tests require complex, sequential processing of information, in comparison with the visually based Pattern Memory Test, on which a more global processing strategy may be associated with better performance. Also, these tests do not allow for learning, as does the Word List Memory Test, in which words in a list are presented repeatedly in three trials.

Nearly one-half the subjects in this study had low plasma vitamin B-6 (pyridoxal phosphate) concentrations according to the suggested value of Leklem (< 30 μmol/L) (39). (There is no commonly accepted normal value for vitamin B-6 concentration.) Although there were a few persons with very high concentrations of vitamin B-6 consistent with the use of nutritional supplements, most persons in the highest quartile had concentrations consistent with a normal, healthy diet (43). Although the exact nature of the relation between plasma vitamin B-6 and homocysteine is unclear because fasting homocysteine was measured (37), it appears that vitamin B-6 itself (and not homocysteine nor any vascular-related deficit possibly associated with homocysteine) is more likely as a locus of an effect on memory performance.

All of the B vitamins measured in this study are important to brain function. Vitamin B-12 and folate are required as coenzymes in the synthesis of serotonin and catecholamine neurotransmitters and also of S-adenosylmethionine, which has antidepressant properties (44-46). Vitamin B-12 deficiency may also result in demyelination (3). Vitamin B-6 is a cofactor in the production of neurotransmitters including dopamine, noradrenaline, serotonin, γ-amino butyric acid (GABA), and taurine, and in rats, deficiencies have been associated with defects in myelin in the central nervous system (47).

Homocysteine appears to cause endothelial cell damage, thus promoting atherosclerosis (48). Plasma homocysteine concentrations have also been associated with degree of carotid stenosis in older adults (49) and with severity of white matter hyperintensities on magnetic resonance imaging of the brain (50). Changes in the brain related to B vitamin and homocysteine concentrations thus might occur at metabolic and/or structural levels, with either focal or diffuse effects on brain function possibly depending on the specific pattern and persistence of concentrations of B vitamins and homocysteine. Certainly, elevated homocysteine concentrations may result in damage that is focal in nature, because of heightened risk of cerebrovascular disease. Alternatively, it has been suggested that a more direct effect of homocysteine could occur through the action of excitatory amino acid derivatives, including homocysteic acid (51). Administration of increasing dosages of homocysteic acid to rats has been associated with selective vulnerability of cortical neurons to cell death and concomitant changes in concentrations of neurotransmitters (52). However, despite the plausibility of B vitamins and homocysteine affecting cognitive performance, the particular mechanisms by which the relations with specific cognitive abilities found in this study might have occurred are as yet unclear.

The associations that we report are generally consistent, even if occasionally specifically inconsistent with those reported in previous studies. They are generally consistent in that low concentrations of B vitamins have been associated with poorer performance on some cognitive tasks. However, they are inconsistent in that some associations between specific vitamins and cognitive abilities were not replicated in this study. For example, in a previous study, low vitamin B-12 concentrations (the lowest 5% compared with the upper 95%) were associated with poorer memory performance in normal older adults (5).

The lack of similar associations in the present study could be attributed to one or more of the following reasons. First, vitamin B-12 may have acted as a surrogate for some associated vitamin, health, or other factor in previous studies or in the current study. For instance, it seems likely that the surprising negative, although marginal association between vitamin B-12 concentration and performance on the Word List Memory Test is attributable to vitamin supplementation among those under medical care for such diseases as cancer and diabetes. These diseases, which were more frequent among those with high concentrations of vitamin B-12, might directly or indirectly affect memory performance. Second, larger numbers of subjects with low concentrations of vitamins may be needed to find some associations. Even among persons with concentrations of vitamin B-12 or folate low enough to be defined as deficiencies, approximately one-third may demonstrate few or no clinically discernible signs of cognitive deficits (53), which suggests the existence of individual differences in the association between B vitamin concentrations and cognitive function.

Third, more sensitive tests of some cognitive abilities may be required to differentiate subjects by nutritional status. The importance of a match between subjects’ competence level and the difficulty of cognitive tests has been noted by some researchers (35, 54). Although the tests used in this study appear in general to discriminate adequately between subjects, further research is necessary to determine their effectiveness in discerning nutritional differences, particularly when such nutritional deficits are uncommon.

Finally, the cognitive differences associated with age in this study are not the result of differences in B vitamin or homocysteine concentrations. It will be important, however, to follow these and other subjects over time to track true, within-individual cognitive changes with age because it is possible that there are age effects that are not being measured crosssectionally and that low plasma concentrations of B vitamins and a high concentration of homocysteine may account for some age effects when plasma vitamin concentrations and cognitive changes are studied longitudinally. In support of the possibility of age effects that could not be measured in the present study, there were no age effects for some tests on which such effects might be expected. For instance, there was no relation between age and response latencies on the Pattern Comparison and the Continuous Performance tests nor between age and spatial copying skills. The lack of these effects may result either from the
limited range of ages of subjects, which is smaller than in many studies for which age effects for these or similar measures have been reported, or from the nature of the NAS sample. Participants were originally chosen because of their good health, which means that older subjects had health status similar to younger subjects when they entered the study. The nonrandom nature of this selection procedure means that any cohort effects as a function of health possibly would favor older subjects, compared with other more representative samples. Thus, it is important to test change longitudinally within individuals.

In conclusion, we expect that results from this study and from future analyses of data from the NAS will help to elucidate the relations between B vitamins, homocysteine, and cognitive functioning, and the concentrations of vitamins and homocysteine at which risk of cognitive deficit and/or the benefit of improved performance may occur. These results were obtained for men only, and exploration of possible sex differences will be necessary. Other future directions for this research include use of larger samples of subjects to test further the associations that were suggested by this and by previous research, and longitudinal study of changes in performance of subjects, to see if and how nutrition affects the presence and rate of changes in cognitive abilities.

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