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High homocysteine and low B vitamins predict cognitive decline in aging men: the Veterans Affairs Normative Aging Study[1–4]

Katherine L Tucker, Ning Qiao, Tammy Scott, Irwin Rosenberg, and Avron Spiro III

ABSTRACT

Background: Elevated homocysteine concentrations may contribute to cognitive impairment. Most elevations in homocysteine result from inadequate folate, vitamin B-12, or vitamin B-6 intake. It is not clear whether the observed associations between homocysteine and cognitive measures are causal or whether they are due to homocysteine, to independent actions of the B vitamins, or to both.

Objective: We aimed to assess the individual and independent effects of baseline plasma homocysteine, folate, vitamin B-12, and vitamin B-6 and of dietary B vitamin intakes on 3-y changes in cognitive measures in 321 aging men.

Design: Participants were from the Veterans Affairs Normative Aging Study. Cognitive function was assessed with the Mini-Mental State Examination and on the basis of measures of memory, verbal fluency, and constructional praxis, which were adapted from the revised Wechsler Adult Intelligence Scale and the Consortium to Establish a Registry for Alzheimer's Disease batteries at 2 time points. At baseline, dietary intakes were assessed with a food-frequency questionnaire, and blood was drawn for the measurement of B vitamins and homocysteine.

Results: Over a mean 3-y follow-up, declines in constructional praxis, measured by spatial copying, were significantly associated with plasma homocysteine, folate, and vitamins B-6 and B-12 and with the dietary intake of each vitamin. Folate (plasma and dietary) remained independently protective against a decline in spatial copying score after adjustment for other vitamins and for plasma homocysteine. Dietary folate was also protective against a decline in verbal fluency. A high homocysteine concentration was associated with a decline in recall memory.

Conclusions: Low B vitamin and high homocysteine concentrations predict cognitive decline. Spatial copying measures appear to be most sensitive to these effects in a general population of aging men. *Am J Clin Nutr* 2005;82:627–35.

KEY WORDS Folate, vitamin B-6, vitamin B-12, homocysteine, cognitive function

INTRODUCTION

It has long been known that a deficiency of several B vitamins, including vitamin B-12, can lead to neurologic deterioration and cognitive decline. However, there is increasing evidence to suggest that even moderately low or subclinical B vitamin concentrations may be associated with cognitive impairment (1). Homocysteine, an amino acid that becomes elevated in the presence of inadequate folate, vitamin B-12, or vitamin B-6, is a risk factor for cardiovascular disease (2). Several prospective studies have shown positive risk associations with myocardial infarction and stroke (3–7), although some have reported null findings (8–10).

Several mechanisms for the effects of homocysteine on cognitive decline have been proposed (11–13). Because the effects on the vasculature that contribute to heart disease and stroke are also likely to increase the risk of vascular dementia, it has been hypothesized that inadequate B vitamin status and high homocysteine concentrations may contribute to cognitive decline through silent brain infarction (11, 14). Homocysteine may also be directly neurotoxic through overstimulation of *N*-methyl-D-aspartate receptors, which results in calcium influx and apoptosis (12, 13). However, a recent study suggests that the oxidized forms of homocysteine, homocysteinesulfinic acid, and homocysteic acid, rather than homocysteine itself, are the toxic compounds (15). In addition, low concentrations of folate or vitamin B-12 may impair methylation reactions important to the maintenance of brain tissue. Folate and vitamin B-12 are required for methionine synthesis and the subsequent formation of *S*-adenosylmethionine, a universal methyl donor important to the formation of neurotransmitters, phospholipids, and myelin (1).

[1] From the Jean Mayer US Department of Agriculture Human Nutrition Research Center on Aging at Tufts University, Boston, MA (KLT, NQ, and IR); the New England Medical Center Department of Psychiatry, Boston, MA (TS); and the Boston University School of Public Health and the Veterans Affairs Boston Healthcare System, Boston, MA (AS).

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[4] Reprints not available. Address correspondence to KL Tucker, Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University, 711 Washington Street, Boston, MA 02111. E-mail: katherine.tucker@tufts.edu.

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Recent reviews of homocysteine, B vitamins, and cognitive function or decline have reached different conclusions. In 2001, Calvaresi and Bryan (16) reviewed evidence from 8cross-sectional, 2 longitudinal, and 4 experimental studies and concluded that there was good evidence to suggest that B vitamins are related to cognitive performance and decline. More recently, Ellinson et al (17) concluded that total homocysteine is negatively associated but that folate or vitamin B-12 are inconsistently associated with cognitive scores. Other reviews (18, 19) concluded that, with the exception of clear deficiency, the evidence for a role of B vitamins in preventing cognitive decline remains unclear. Most recently, reviews of clinical trials of folic acid, vitamin B-12, or vitamin B-6 noted no conclusive effect of treatment on dementia (20–22). However, the authors identified only 4 qualifying trials for folic acid, 2 for vitamin B-12, and 2 for vitamin B-6; sample sizes were small (n = 11–139) and the durations short (1–5 mo). All of these reviews noted that more research is needed to understand these relations.

We previously reported that homocysteine is negatively and B vitamins positively associated with cross-sectional measures of cognitive function in 68 male participants aged 54–81 y in the VA Normative Aging Study (NAS) (23). In this report we examined the association between homocysteine and associated B vitamins and cognitive decline in 321 men from the NAS over a 3-y follow-up period.

SUBJECTS AND METHODS

Subjects

The NAS began in 1963 by recruiting men in the Boston area who were originally free of heart disease or other major health problems. Participating men return every 3–5 y for a health examination, at which time they complete a series of questionnaires. Since 1993, a brief cognitive examination was added to these visits. Dietary intake data have been collected since 1987, and assessments of plasma B vitamins and homocysteine were added in 1993. In this analysis, we examined the relation between baseline plasma homocysteine, folate, vitamin B-12, and vitamin B-6 and cognitive decline in 321 men who completed 2 cycles of cognitive testing ≈3 y apart. This protocol was approved by the Institutional Review Boards of both the Boston Veterans Affairs Medical Center and Tufts New England Medical Center. All participants gave written informed consent.

Cognitive measures

We selected those tests that were significantly correlated with at least one of the B vitamins—folate, vitamin B-12, or vitamin B-6—in our earlier cross-sectional assessment of 68 men. These included measures of working memory (backward digit span), recall (word list memory test), language (verbal fluency), and spatial copying (constructional praxis). We also examined changes in Mini-Mental State Examination (MMSE) scores as a global measure of cognitive function (24).

The Backward Digit Span test is from the Revised Wechsler Adult Intelligence Scale (25). Participants are read a list of digits and asked to recall these in backward sequence. The score is the longest span of digits recalled correctly in backward order, with a maximum of 8. The word list memory test is adapted from the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) battery (26). Ten words are presented on a computer screen consecutively, for 2 s each, and participants are then asked to recall these words. Three consecutive trials are administered, and the score is the sum of words remembered; the maximum score is 30. The verbal fluency test is also from the CERAD. Participants are asked to name as many animals as they can within 1 min.

In the spatial copying task, participants are asked to copy a circle, crossed rectangles, a vertical diamond, and a cube (from the CERAD battery) as well as tilted triangles, an 8-dot circle, a horizontal diamond, and a tapered box (from the Developmental Test of Visual-Motor Integration; VMI) (25, 27). The accuracy of the copied figures is scored by trained staff using criteria from the CERAD and VMI. The resulting score is the total number of figures drawn correctly; the maximum score is 9. A second score is weighted by the degree of difficulty of the figure, resulting in a maximum score of 26.

Plasma analysis

Fasting plasma samples were drawn at the VA field site and stored at −80 °C. Batches were transferred on dry ice to the Jean Mayer US Department of Agriculture Human Nutrition Research Center on Aging, where they were again stored at −80 °C and later analyzed for total homocysteine, vitamin B-12, folate, and vitamin B-6 (as pyridoxal-5'-phosphate; PLP). The time between blood draw and analysis averaged 1.7 ± 1.2 y. Total homocysteine in plasma was measured by using an adaptation of the method described by Araki and Sako (28). The CV for this assay in our laboratory is 4.0%. PLP was measured enzymatically, by using tyrosine decarboxylase, based on the principles described by Shin-Buehring et al (29). The CV for this assay in our laboratory is 5.0%. Plasma folate and vitamin B-12 concentrations were measured by radioassay with the use of a commercially available kit from Bio-Rad (Hercules, CA). The CVs for these assays in our laboratory are 4.7% for vitamin B-12 and 4.3% for folate.

Dietary intake

Dietary intake was assessed with a version of the Willett semiquantitative food- frequency questionnaire. This scannable form, which requests participants to record the number of times they consume each of 126 food items per month, week, or day was mailed to NAS participants before their examination visit and checked for completeness at the examination. Forms were processed through a nutrient database at the Channing Laboratory at Harvard University to obtain estimates of usual daily nutrient intake. Vitamin and mineral supplement use was also asked on this questionnaire and was included in the total nutrient intake estimates. Questionnaires with improbable intakes (>16.75 or <2.51 MJ) were excluded from further analysis.

Statistical analysis

Test scores (backward digit span, word list recall, verbal fluency, figure copying, and MMSE) at follow-up were regressed, on baseline total homocysteine, plasma vitamin B-12, plasma folate, and PLP by using the regression procedure in SAS (version 9.1; SAS Institute Inc, Cary, NC). Because of skewness, homocysteine and all nutrient measures were log transformed. Models were adjusted for time (mo) between cognitive measures as well as for respective baseline score, age, education level (y), body mass index (in kg/m^2), smoking (current, past, or never),

alcohol use (≤2 drinks/d, >2 drinks/d, or none), serum creatinine, systolic blood pressure, and diabetes diagnosis. Because folic acid fortification of cereal grain products was initiated in 1996, we further adjusted for the time of the second cognitive measurement relative to the start of fortification (1 October 1996) and to the completion of the phase-in period (1 August 1997) to note whether the measure was taken before implementation, during the transition, or after full implementation of folic acid fortification. All baseline dietary and plasma nutrient measures were assessed before fortification of the food supply with folic acid. In a final set of linear models, all measures of either plasma B vitamins and homocysteine or of dietary B vitamin intake were included jointly in the fully adjusted models to determine whether one or more of these contributed independently to the result.

We also regressed the follow-up cognitive scores on initial dietary intake measures for folate, vitamin B-6, and B-12 by using the same set of covariates described for the plasma analyses, except that serum creatinine was replaced with total energy intake. Dietary measures were also skewed and, therefore, were log transformed before inclusion in the regression models.

In addition to the linear analyses, we created tertile categories for each of the nutrient measures and homocysteine to examine the change in cognitive scores graphically for those with relatively low, average, and high intakes and the status of these measures. These analyses were conducted by using the general linear models procedure in SAS; each tertile variable was defined as a class variable. For those with a significant test for trend (across median values for each tertile), least-squares means were compared across tertile categories, with Tukey's adjustment for multiple comparisons. The same set of covariates described above for the linear regression models was used in these analyses with categorical change measures.

RESULTS

The mean age of the 321 men included was 67 y at baseline (**Table 1**). They were relatively highly educated and had a mean of 2 y of education after high school. Only 6% were current smokers at baseline, and the mean alcohol intake was 14 g, or ≈1 drink/d. Mean body mass index was in the overweight range (ie, 28), and 11% of the men reported having diabetes. Although most of the follow-up visits were scheduled at 3 y after baseline, the actual time to follow-up ranged from 1 to 4 y. Therefore, this variable was adjusted in the analysis. Mean plasma homocysteine and B vitamin measures were all within normal range and mean dietary intakes met National Research Council recommendations (30). However, ranges were large and included individuals with deficient plasma status and intake for each of these B vitamins.

The mean and ranges of cognitive scores indicated that the group of participants was not severely cognitively impaired (**Table 2**). The lowest MMSE was 22, a score that indicated that the participant was mildly impaired and likely to progress but was still able to complete the questionnaires (31); the mean score was 27, which indicated that the participants were considered generally well functioning. Other scores showed a wide range of responses; the average scores were comparable with the findings of other studies of generally healthy adults and were considerably greater than those that may be considered impaired (25, 32).

TABLE 1
Characteristics of participating men at baseline

	Value[1]	No. of subjects
Demographics and behavior		
Age (y)	67 ± 7 (50–85)	321
Education (y)	14 ± 3 (6–24)	319
Alcohol intake (g/d)	13.9 ± 19 (0–112)	309
Smoking status (%)		320
Current	5.6	
Former	59.7	
Never	34.7	
Health measures		
BMI (kg/m^2)	27.9 ± 4.0 (20.1–51.9)	320
Diabetes (%)	10.6	321
Systolic blood pressure (mm Hg)	138 ± 16.9 (97–208)	320
Serum creatinine (mg/dL)	1.2 ± 0.2 (0.7–1.9)	320
Follow-up period (d)	1092 ± 100 (370–1481)	321
Plasma measures		
Folate (nmol/L)	26 ± 12 (6–95)	314
Vitamin B-6 (nmol/L)	86 ± 84 (11–828)	313
Vitamin B-12 (pmol/L)	335 ± 136 (90–1069)	315
Homocysteine (nmol/L)	11 ± 5 (3.0–45)	317
Dietary intake		
Folate (μg)	440 ± 202 (80–1216)	321
Folate equivalents (mg)	497 ± 285 (80–1589)	321
Vitamin B-6 (mg)	3.98 ± 7.19 (0.5–85.5)	321
Vitamin B-12 (μg)	9.57 ± 5.73 (1.4–57.0)	321
Energy (kJ)	8.37 ± 2.37 (3.4–16.1)	320

[1] All values are $\bar{x}$ ± SD; range in parentheses.

Linear associations with longitudinal measures of cognitive function

The B vitamin and homocysteine concentrations were significantly predictive of several final cognitive scores, adjusted for baseline score and covariates, as described above (**Table 3**). Because the baseline cognitive measures were adjusted, the relations described with final measures approximate the effects on change in score over the follow-up period. Spatial copying score was significantly associated with each of the baseline plasma vitamins (positively) and with homocysteine (negatively). In addition, these scores were also significantly positively associated with baseline dietary intakes of folate, vitamin B-6, and vitamin B-12. Verbal fluency was significantly associated with dietary folate and tended toward being significantly associated with dietary intake of vitamin B-6 ($P < 0.1$).

Homocysteine was significantly negatively associated with recall memory, as assessed by word list memory score ($P < 0.05$); B vitamins were not. None of the measures examined were significantly associated with working memory, as measured by backward digit span or with the MMSE ($P > 0.1$).

Because folate, vitamin B-6, and vitamin B-12 are intrinsically related to homocysteine and are often correlated with each other, we examined their associations with cognitive outcomes when adjusted for each other for the 3 measures for which there was at least one significant association. This provides further evidence of the differential strength of association of these variables, after their common variance was accounted for. Plasma folate ($P < 0.01$) remained significantly associated with longitudinal measures of figure copying score when all 3 plasma B vitamins and homocysteine were included simultaneously in the same model,

TABLE 2
Baseline cognitive measures

	Value[1]	Cutoff indicating impairment
Spatial copying[2]		
Sum of drawings 1–9 ($n = 307$)	5.75 ± 1.82 (0–9)	—
Sum of weighted drawings 1–9 ($n = 307$)	15.3 ± 5.9 (0–26)	—
Language		
Verbal fluency, no. correct ($n = 260$)	19.0 ± 4.8 (7–37)	<13.6[3]
Memory		
Word list memory, 3 trials ($n = 257$)	19.6 ± 3.79 (9–29)	<15.1[3]
Backward digit span, longest span recalled ($n = 257$)	5.12 ± 1.33 (3–8)	<2.99[4]
Mini-Mental State Examination, total score ($n = 302$)	27.2 ± 1.8 (22–30)	<24[5]

[1] All values are $\bar{x}$ ± SD; range in parentheses.
[2] No published cutoffs that define impairment are available for this combination of figure copying scores.
[3] From reference 32.
[4] From reference 25.
[5] From reference 24.

as did dietary folate ($P < 0.05$) after the other 2 B vitamins were adjusted (**Table 4**). When dietary B vitamins were included together, none remained independently significant with longitudinal measures of verbal fluency. Homocysteine was almost significant with the word list memory task.

Categorical differences in change in cognitive function measures

For those associations that were significant in linear models, we repeated the analyses with regression of change in cognitive scores from baseline to subsequent (mean of 3 y) measure onto tertile categories of baseline plasma B vitamins and homocysteine as well as dietary intakes (**Figures 1–3**). On the basis of this grouping, only the figure copying scores remained significant. Men with plasma folate concentrations < 20 nmol/L or dietary folate intakes <339 μg/d had relatively large losses ($\bar{x}$: 0.68 and 0.55 of a point, respectively, from a baseline mean score of 5.8) in spatial copying ability, whereas those >30 nmol/L or 523 μg/d showed, on average, no apparent loss. Similarly, men with a plasma PLP concentration <46 nmol/L or a dietary intake <2.1 mg/d showed losses similar to those seen with low folate, whereas those with values greater than these cutoffs showed little

TABLE 3
Association between individual baseline plasma and dietary intake measures and 3-y cognitive measures[1]

	β[2]			
	Folate	Vitamin B-6	Vitamin B-12	Homocysteine
Constructional praxis: spatial copying, sum of drawings[3]				
Plasma ($n = 280–284$)	1.00[4]	0.38[5]	0.59[6]	−1.31[7]
Diet ($n = 287$)	0.67[5]	0.30[6]	0.37[6]	—
Language: verbal fluency, no. correct				
Plasma ($n = 239–243$)	0.76	0.56	0.06	−0.12
Diet ($n = 245$)	1.44[6]	0.81[8]	0.38	—
Working memory: backward digit span, longest span recalled				
Plasma ($n = 236–240$)	−0.28	−0.03	0.18	−0.12
Diet ($n = 242$)	0.11	0.04	0.12	—
Recall memory: word lists, total of 3 trials				
Plasma ($n = 235–239$)	0.43	0.33	−0.20	−1.43[6]
Diet ($n = 241$)	0.31	0.21	−0.01	—
Mini-Mental State Examination				
Plasma ($n = 271–275$)	0.12	0.15	−0.16	−0.49
Diet ($n = 278$)	0.08	−0.05	0.14	—

[1] Final cognitive measures regressed onto baseline diet and plasma measures (log transformed), adjusted for baseline cognitive measures, age, education, smoking, alcohol intake, BMI, diabetes (yes or no), systolic blood pressure, time of second measure relative to folic acid fortification, time interval between the 2 cognitive measures, and serum creatinine (for plasma) or total energy intake (for diet).
[2] Adjusted regression coefficient from a multiple linear regression model.
[3] Results for the weighted sums did not differ significantly from those for the unweighted sums (data not shown).
[4] $P < 0.0001$.
[5] $P < 0.01$.
[6] $P < 0.05$.
[7] $P < 0.001$.
[8] $P < 0.1$.

TABLE 4
Association between simultaneously included baseline plasma and dietary measures and 3-y cognitive measures[1]

	β[2]			
	Folate	Vitamin B-6	Vitamin B-12	Homocysteine
Constructional praxis: spatial copying, sum of drawings[3]				
Plasma	0.71[4]	0.17	0.06	−0.65
Diet	0.71[5]	−0.09	0.07	—
Language: verbal fluency, no. correct				
Plasma	0.69	0.39	−0.51	0.30
Diet	1.35	0.32	−0.38	—
Recall memory: word list, total of 3 trials				
Plasma	−0.07	0.36	−0.71	−1.34[6]
Diet	0.28	0.16	−0.22	—

[1] Final cognitive measures regressed onto baseline plasma or dietary measures (log transformed), adjusted for baseline cognitive measures, age, education, smoking, alcohol intake, BMI, diabetes (yes or no), systolic blood pressure, time of second measure relative to folic acid fortification, time interval between the 2 cognitive measures, and serum creatinine (for plasma) or total energy intake (for diet).
[2] Adjusted regression coefficient from a multiple linear regression model.
[3] Results for the weighted sums did not differ significantly from those for the unweighted sums (data not shown).
[4] $P < 0.01$.
[5] $P < 0.05$.
[6] $P < 0.1$.

if any loss in function (Figure 2). Those with a plasma PLP concentration >85 nmol/L or intakes >3.1 mg/d were significantly less likely to have a decrease in this measure ($P < 0.01$). The linear effect of homocysteine was also evident (Figure 3). Men with homocysteine concentrations >11 nmol/L were significantly more likely to lose spatial ability than were those with a homocysteine concentration <9 nmol/L, for whom scores remained similar to those at baseline. Tertile comparisons of plasma vitamin B-12 or intake of vitamin B-12 were not significantly associated with a change in figure copying score.

When examined in tertile categories, the highest intake categories for plasma and dietary folate and for PLP and dietary

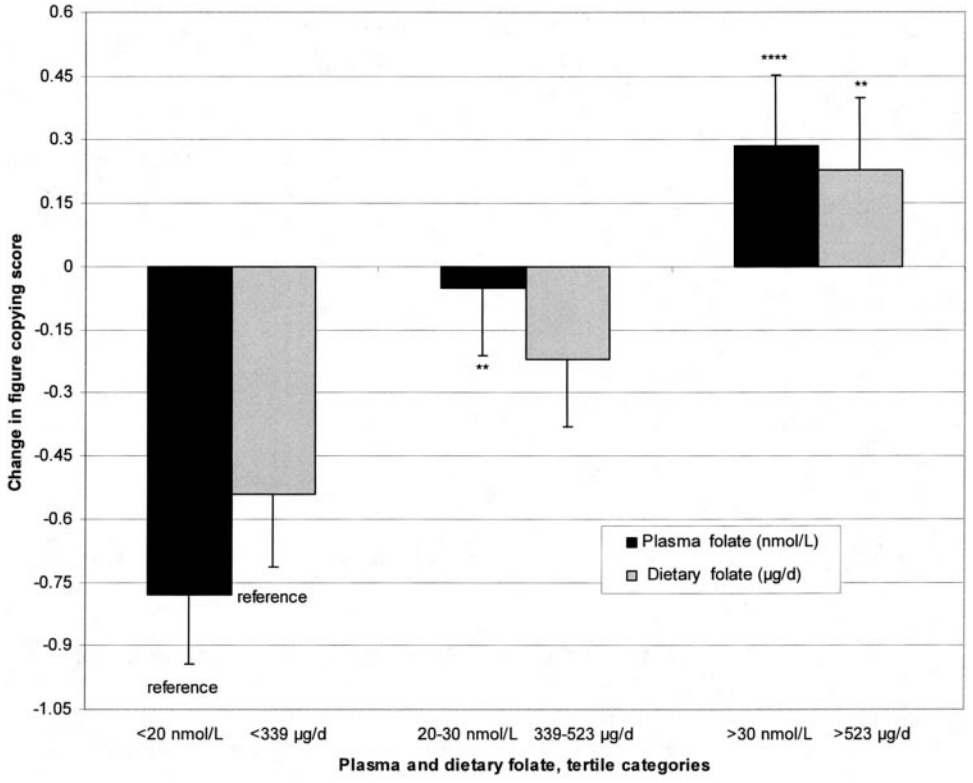

FIGURE 1. Change in figure copying score by tertile category for plasma and dietary folate. *P* for trend (on the basis of tertile medians) <0.0001 for plasma and <0.01 for diet. **, ****Significantly different from lowest tertile (*t* test comparisons of least-squares means from general linear models, with Tukey's adjustment for multiple comparisons): $^{**}P < 0.01$, $^{****}P < 0.0001$.

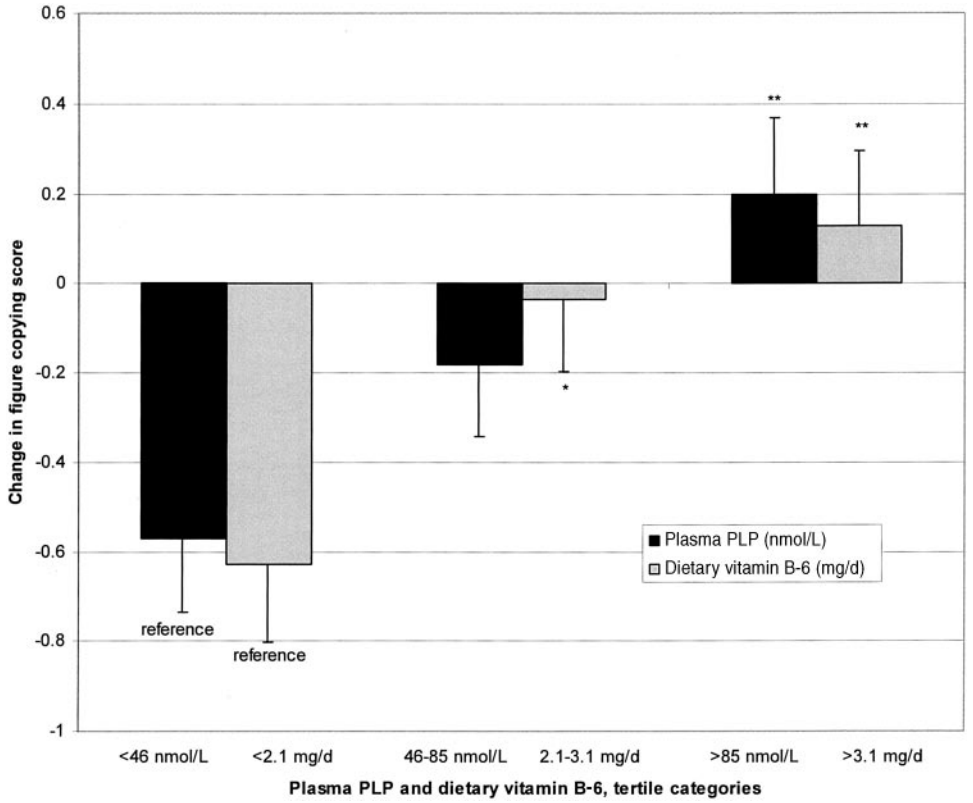

FIGURE 2. Change in figure copying score by tertile category for plasma pyridoxal-5'-phosphate (PLP) and dietary vitamin B-6. *P* for trend (on the basis of tertile medians) <0.01 for plasma and <0.01 for diet. *,**Significantly different from lowest tertile (*t* test comparisons of least-squares means from general linear models, with Tukey's adjustment for multiple comparisons): $^{*}P < 0.05$, $^{**}P < 0.01$.

vitamin B-6 each approached significance ($P < 0.1$) in relation to those with respective lowest intakes for verbal fluency with evidence of linear pattern (data not shown).

DISCUSSION

These results support the protective role of B vitamins, particularly folate and vitamin B-6, as well as the role of homocysteine, as a risk factor for cognitive decline. As in our earlier and smaller cross-sectional study (23), we found that the strongest associations were seen with measures of spatial copying. Other studies support the sensitivity of complex tasks, such as spatial copying to respond to homocysteine. McCaddon et al (33) also found that homocysteine was more strongly associated with declines in spatial copying over 5 y than with other measures of cognitive decline, and a recent study (34) found clear significant associations between homocysteine and the Stroop test, a measure of executive function and cognitive flexibility, but not with simpler measures of verbal memory or with the MMSE.

Unlike our earlier study, in which homocysteine remained independently associated with figure copying score after adjustment for B vitamins, we found stronger and independent associations with folate and these 3-y longitudinal changes in figure copying. The independent contributions of plasma folate, after adjustment for homocysteine and other B vitamins, and of dietary folate, after adjustment for dietary vitamins B-6 and B-12, suggest that this vitamin may have effects other than through elevating homocysteine. The larger sample size and longitudinal design of the present study, along with the consistency of results across plasma and dietary measures, suggest that folate itself may be the important factor in preventing decline in this complex measure of constructional praxis. In contrast, we found that homocysteine was more strongly associated with recall memory (23).

Existing studies show a mixture of associations of B vitamins and homocysteine with different measures of cognitive outcome; however, the primary mechanisms for the association are unclear. Most studies have used the MMSE as their measure of cognitive function, and most have been conducted in patient populations with dementia. Patients with Alzheimer disease have both lower B vitamin and higher total homocysteine concentrations than do nondemented patients (35–38). Furthermore, lower concentrations of B vitamins and elevated homocysteine have been related to the severity of disease (39, 40). Imaging studies of brain morphology generally support associations between hippocampal atrophy and white matter hyperintensities and high homocysteine concentrations (36, 40–42). In a group of psychiatric inpatients, we previously found that both high homocysteine and low folate concentrations were significantly associated with white matter hyperintensities but that only low folate was associated with low hippocampal and amygdal volumes (43).

Results from patient populations are highly suggestive but cannot clarify the question of whether the associations between poor B vitamin status and elevated homocysteine and cognitive impairment are a product of the disease or whether these micronutrient inadequacies are responsible for some of the cognitive impairments. Fewer longitudinal population-based studies exist, but those do generally support the hypothesis that low B vitamin

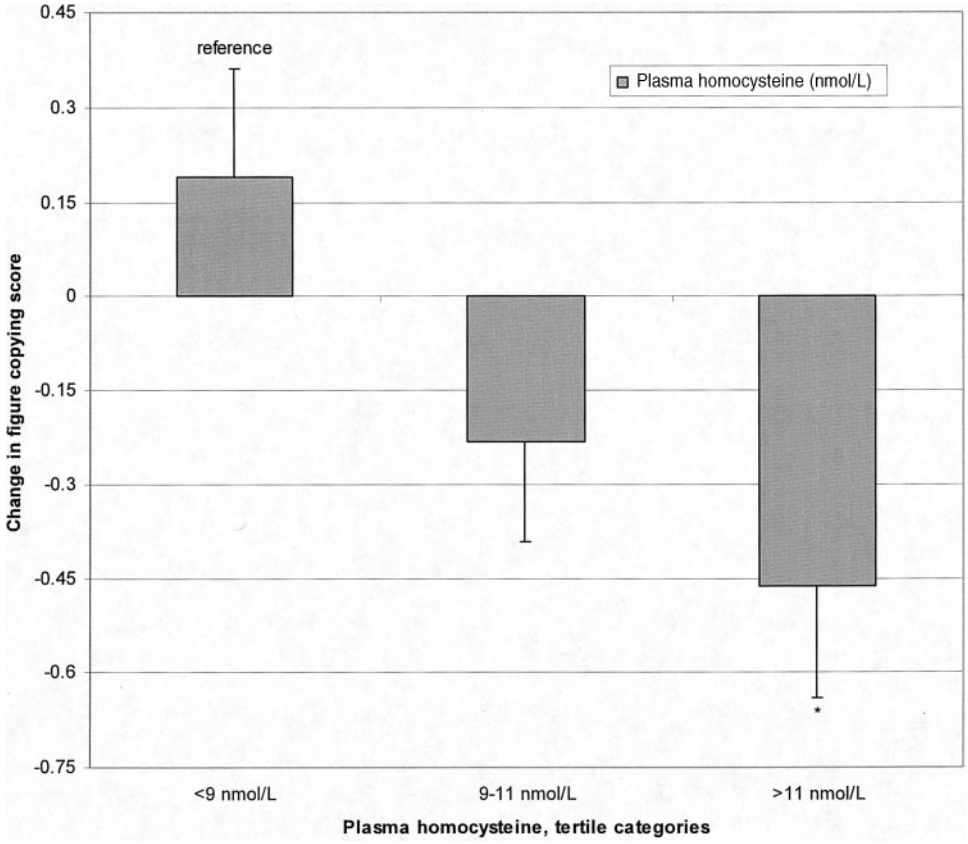

FIGURE 3. Change in figure copying score by tertile category for plasma homocysteine. *P* for trend (on the basis of tertile medians) <0.05. *Significantly different from lowest tertile, $P < 0.05$ (*t* test comparisons of least-squares means from general linear models, with Tukey's adjustment for multiple comparisons).

status and high homocysteine concentrations are causal contributors to cognitive decline and dementia; the strongest associations are generally seen for the most complex tasks (33, 34, 44, 45). The Rotterdam Study did not initially find an association between homocysteine and a decline in MMSE score over a 2.7 y follow-up (46). However, more recent analyses from the same study found that elevated homocysteine concentrations are associated with significantly poorer psychomotor speed (47). In Sweden, a 3-y follow-up study showed that subjects with low baseline folate or vitamin B-12 were twice as likely to develop Alzheimer disease (48). Results from the Framingham Heart Study showed clear associations between baseline homocysteine concentration and incidence of dementia over an 8-y follow-up period (49). In contrast, the Epidemiology of Vascular Aging Study in France found only a nonsignificant ($P = 0.09$) trend for the association between high homocysteine concentrations and the presence of white matter hyperintensities after magnetic resonance imaging 2 y later (45).

The suggested negative effects of elevated homocysteine on cognitive function may result from atherosclerosis, from vasotoxic effects, or from excitotoxic effects (36, 50–56). In addition to direct effects on the vasculature, homocysteine may be neurotoxic, by activating the *N*-methyl-D-aspartate receptor and leading to cell death (53, 57). However, a recent study, which used dissociated neurons from embryonic Wistar rats, found that oxidized forms of homocysteine, but not homocysteine itself, resulted in a rapid dose-response–related inhibition of network activity (15). A study of cultured murine cortical neurons with homocysteine noted increases in reactive oxygen species, phospho-tau immunoreactivity, and other indicators of apoptosis (58).

A second mechanism that may contribute to the observed associations is hypomethylation, which results from the lower availability of methyl donors due to B vitamin deficiency (59). Hypomethylation interferes with protein synthesis and affects neurotransmitter metabolism (60–62). Low concentrations of folate, vitamin B-6, and vitamin B12 may, therefore, lead directly to cognitive impairment through the accumulation of neuronal DNA damage (11, 63). A study of hippocampal cultures in folic acid–deficient medium noted DNA damage that potentiated amyloid β toxicity (64).

Most of the previous work in this area has assumed that elevated homocysteine is the causal factor associated with cognitive decline. Our results support the negative influence of homocysteine on several measures of cognitive decline, including spatial copying, measures of memory, and MMSE score. However, our results further suggest that low folate may have independent effects on constructional praxis beyond its effect on raising homocysteine. When adjusted for each other, low plasma folate rather than high homocysteine was independently significantly associated with declines in spatial copying ability, whereas high

homocysteine remained more predictive of declines in memory and overall MMSE score.

Despite the continuing concern that the observed associations between homocysteine and cognitive function may indicate a response to cognitive decline or dementia rather than be a cause of such, more recent longitudinal studies support the likelihood of a causal connection with either homocysteine or its associated B vitamins. Our data suggest that the effects are complex and involve multiple pathways. Although homocysteine is likely to affect vascular changes that contribute to cognitive decline, other mechanisms involving B vitamins, particularly folate, may also be contributory. The consistency of findings across plasma and dietary measures shown here also argues against the suggestion that processes involved in cognitive decline also contribute to higher homocysteine. Although one may argue that the reverse causality may be through poorer dietary intake (or less accurate reporting of intake) with cognitive decline, this is unlikely in a population with early decline, as this group of men represents.

Further studies to confirm and refine the observed associations are needed along with long-term randomized trials to demonstrate the effect of vitamin supplementation in the general population. Whether due to low vitamin availability, high homocysteine concentrations, or both, B vitamin intakes and status appear to be important in reducing cognitive decline in men. Since the baseline measures were taken for this study, the food supply has been fortified with folic acid, which has led to reductions in homocysteine concentrations in the US population. Further study is needed to determine whether this change will translate to reductions in cognitive decline. More attention to B vitamin and homocysteine status could have a major effect on the health and well being of our aging population.

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KLT designed the analysis and drafted the manuscript. NQ performed the statistical analysis. TS assisted with the interpretation of cognitive measures. IR assisted with the discussion of potential mechanisms of action. AS was responsible for the data collection, coding, and definition of variables. None of the authors had a conflict of interest associated with this manuscript, and all authors contributed to the final version.

REFERENCES

1. Selhub J, Bagley LC, Miller J, Rosenberg IH. B vitamins, homocysteine, and neurocognitive function in the elderly. Am J Clin Nutr 2000; 71(suppl):614S–20S.
2. Boushey CJ, Beresford SA, Omenn GS, Motulsky AG. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. Probable benefits of increasing folic acid intakes. JAMA 1995;274: 1049–57.
3. Perry IJ, Refsum H, Morris RW, Ebrahim SB, Ueland PM, Shaper AG. Prospective study of serum total homocysteine concentration and risk of stroke in middle-aged British men. Lancet 1995;346:1395–8.
4. Ridker PM, Manson JE, Buring JE, Shih J, Matias M, Hennekens CH. Homocysteine and risk of cardiovascular disease among postmenopausal women. JAMA 1999;281:1817–21.
5. Bostom AG, Rosenberg IH, Silbershatz H, et al. Nonfasting plasma total homocysteine levels and stroke incidence in elderly persons: the Framingham Study. Ann Intern Med 1999;131:352–5.
6. Bots ML, Launer LJ, Lindemans J, et al. Homocysteine and short-term risk of myocardial infarction and stroke in the elderly: the Rotterdam Study. Arch Intern Med 1999;159:38–44.
7. Maxwell CJ, Hogan DB, Ebly EM. Serum folate levels and subsequent adverse cerebrovascular outcomes in elderly persons. Dement Geriatr Cogn Disord 2002;13:225–34.
8. Evans RW, Shaten BJ, Hempel JD, Cutler JA, Kuller LH. Homocyst(e)ine and risk of cardiovascular disease in the Multiple Risk Factor Intervention Trial. Arterioscler Thromb Vasc Biol 1997;17:1947–53.
9. Folsom AR, Nieto FJ, McGovern PG, et al. Prospective study of coronary heart disease incidence in relation to fasting total homocysteine, related genetic polymorphisms, and B vitamins: the Atherosclerosis Risk in Communities (ARIC) study. Circulation 1998;98:204–10.
10. Fallon UB, Elwood P, Ben-Shlomo Y, Ubbink JB, Greenwood R, Smith GD. Homocysteine and ischaemic stroke in men: the Caerphilly study. J Epidemiol Community Health 2001;55:91–6.
11. Rosenberg IH, Miller JW. Nutritional factors in physical and cognitive functions of elderly people. Am J Clin Nutr 1992;55(suppl):1237S–43S.
12. McCaddon A, Kelly CL. Alzheimer's disease: a 'cobalaminergic' hypothesis. Med Hypotheses 1992;37:161–5.
13. Ho PI, Collins SC, Dhitavat S, et al. Homocysteine potentiates beta-amyloid neurotoxicity: role of oxidative stress. J Neurochem 2001;78: 249–53.
14. Matsui T, Arai H, Yuzuriha T, et al. Elevated plasma homocysteine levels and risk of silent brain infarction in elderly people. Stroke 2001; 32:1116–9.
15. Gortz P, Hoinkes A, Fleischer W, et al. Implications for hyperhomocysteinemia: not homocysteine but its oxidized forms strongly inhibit neuronal network activity. J Neurol Sci 2004;218:109–14.
16. Calvaresi E, Bryan J. B vitamins, cognition, and aging: a review. J Gerontol B Psychol Sci Soc Sci 2001;56:P327–39.
17. Ellinson M, Thomas J, Patterson A. A critical evaluation of the relationship between serum vitamin B, folate and total homocysteine with cognitive impairment in the elderly. J Hum Nutr Diet 2004;17:371–83; quiz 385–7.
18. Luchsinger JA, Mayeux R. Dietary factors and Alzheimer's disease. Lancet Neurol 2004;3:579–87.
19. Moretti R, Torre P, Antonello RM, Cattaruzza T, Cazzato G, Bava A. Vitamin B12 and folate depletion in cognition: a review. Neurol India 2004;52:310–8.
20. Malouf M, Grimley EJ, Areosa SA. Folic acid with or without vitamin B12 for cognition and dementia. Cochrane Database Syst Rev 2004;3.
21. Malouf R, Areosa SA. Vitamin B12 for cognition. Cochrane Database Syst Rev 2004;3.
22. Malouf R, Grimley Evans J. Vitamin B6 for cognition. Cochrane Database Syst Rev 2004;3.
23. Riggs KM, Spiro A III, Tucker K, Rush D. Relations of vitamin B-12, vitamin B-6, folate, and homocysteine to cognitive performance in the Normative Aging Study. Am J Clin Nutr 1996;63:306–14.
24. Folstein MF, Folstein SE. "Mini-Mental State:" a practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975;12:189–98.
25. Wechsler D. Manual for the Wechsler Adult Intelligence Scale–revised. New York, NY: Psychological Corporation, 1981.
26. Morris JC, Heyman A, Mohs RC, et al. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part I. Clinical and neuropsychological assessment of Alzheimer's disease. Neurology 1989;39: 1159–65.
27. Beery K. The Developmental Test of Visual-Motor Integration Manual, revised ed. Cleveland, OH: Modern Curriculum Press, 1989.
28. Araki A, Sako Y. Determination of free and total homocysteine in human plasma by high-performance liquid chromatography with fluorescence detection. J Chromatogr 1987;422:43–52.
29. Shin-Buehring Y, Rasshofer R, Endres W. A new enzymatic method for pyridoxal-5'-phosphate determination. J Inherit Metab Disord 1981;4: 123–4.
30. Food and Nutrition Board, Institute of Medicine. Dietary reference intakes for thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid, biotin, and choline. Washington, DC: National Academy Press, 1998.
31. Feinberg LF, Whitlatch CJ. Are persons with cognitive impairment able to state consistent choices? Gerontologist 2001;41:374–82.
32. Welsh KA, Butters N, Mohs RC, et al. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part V. A normative study of the neuropsychological battery. Neurology 1994;44:609–14.
33. McCaddon A, Hudson P, Davies G, Hughes A, Williams JH, Wilkinson C. Homocysteine and cognitive decline in healthy elderly. Dement Geriatr Cogn Disord 2001;12:309–13.
34. Garcia A, Haron Y, Pulman K, Hua L, Freedman M. Increases in homocysteine are related to worsening of stroop scores in healthy elderly

persons: a prospective follow-up study. J Gerontol A Biol Sci Med Sci 2004;59:1323–7.
35. Joosten E, Lesaffre E, Riezler R, et al. Is metabolic evidence for vitamin B-12 and folate deficiency more frequent in elderly patients with Alzheimer's disease? J Gerontol 1997;52A:M76–9.
36. Clarke R, Smith AD, Jobst KA, Refsum H, Sutton L, Ueland PM. Folate, vitamin B12, and serum total homocysteine levels in confirmed Alzheimer disease. Arch Neurol 1998;55:1449–55.
37. Gottfries CG, Lehmann W, Regland B. Early diagnosis of cognitive impairment in the elderly with the focus on Alzheimer's disease. J Neural Transm 1998;105:773–86.
38. Leblhuber F, Walli J, Widner B, Artner-Dworzak E, Fuchs D, Vrecko K. Homocysteine and B vitamins in dementia. Am J Clin Nutr 2001;73: 127–8.
39. Sommer BR, Wolkowitz OM. RBC folic acid levels and cognitive performance in elderly patients: a preliminary report. Biol Psychiatry 1988; 24:352–4.
40. Snowdon DA, Tully CL, Smith CD, Riley KP, Markesbery WR. Serum folate and the severity of atrophy of the neocortex in Alzheimer disease: findings from the Nun study. Am J Clin Nutr 2000;71:993–8.
41. Williams JH, Pereira EA, Budge MM, Bradley KM. Minimal hippocampal width relates to plasma homocysteine in community—dwelling older people. Age Aging 2002;31:440–4.
42. Hogervorst E, Ribeiro HM, Molyneux A, Budge M, Smith AD. Plasma homocysteine levels, cerebrovascular risk factors, and cerebral white matter changes (leukoaraiosis) in patients with Alzheimer disease. Arch Neurol 2002;59:787–93.
43. Scott TM, Tucker KL, Bhadelia A, et al. Homocysteine and B vitamins relate to brain volume and white-matter changes in geriatric patients with psychiatric disorders. Am J Geriatr Psychiatry 2004;12:631–8.
44. Ebly EM, Schaefer JP, Campbell NR, Hogan DB. Folate status, vascular disease and cognition in elderly Canadians. Age Ageing 1998;27:485–91.
45. Dufouil C, Alperovitch A, Ducros V, Tzourio C. Homocysteine, white matter hyperintensities, and cognition in healthy elderly people. Ann Neurol 2003;53:214–21.
46. Kalmijn S, Launer LJ, Lindemans J, Bots ML, Hofman A, Breteler MM. Total homocysteine and cognitive decline in a community-based sample of elderly subjects: the Rotterdam Study. Am J Epidemiol 1999;150: 283–9.
47. Prins ND, Den Heijer T, Hofman A, et al. Homocysteine and cognitive function in the elderly: the Rotterdam Scan Study. Neurology 2002;59: 1375–80.
48. Wang HX, Wahlin A, Basun H, Fastbom J, Winblad B, Fratiglioni L. Vitamin B(12) and folate in relation to the development of Alzheimer's disease. Neurology 2001;56:1188–94.
49. Seshadri S, Beiser A, Selhub J, et al. Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. N Engl J Med 2002;346:476–83.
50. Beal MF, Kowall NW, Swartz KJ, Ferrante RJ. Homocysteic acid lesions in rat striatum spare somatostatin-neuropeptide Y (NADPH-diaphorase) neurons. Neurosci Lett 1990;108:36–42.
51. Beal MF, Swartz KJ, Finn SF, Mazurek MF, Kowall NW. Neurochemical characterization of excitotoxin lesions in the cerebral cortex. J Neurosci 1991;11:147–58.
52. Fassbender K, Mielke O, Bertsch T, Nafe B, Froeschen S, Hennerici M. Homocysteine in cerebral macroangiography and microangiopathy. Lancet 1999;353:1586–7.
53. Lipton SA, Kim WK, Choi YB, et al. Neurotoxicity associated with dual actions of homocysteine at the *N*-methyl-D-aspartate receptor. Proc Natl Acad Sci U S A 1997;94:5923–8.
54. Yoo JH, Chung CS, Kang SS. Relation of plasma homocyst(e)ine to cerebral infarction and cerebral atherosclerosis. Stroke 1998;29:2478–83.
55. Brattstrom L, Lindgren A, Israelsson B, et al. Hyperhomocysteinaemia in stroke: prevalence, cause, and relationships to type of stroke and stroke risk factors. Eur J Clin Invest 1992;22:214–21.
56. Giles WH, Croft JB, Greenlund KJ, Ford ES, Kittner SJ. Total homocyst(e)ine concentration and the likelihood of nonfatal stroke: results from the Third National Health and Nutrition Examination Survey, 1988–1994. Stroke 1998;29:2473–7.
57. Parnetti L, Bottiglieri T, Lowenthal D. Role of homocysteine in age-related vascular and non-vascular diseases. Aging (Milano) 1997;9:241–57.
58. Ho PI, Ortiz D, Rogers E, Shea TB. Multiple aspects of homocysteine neurotoxicity: glutamate excitotoxicity, kinase hyperactivation and DNA damage. J Neurosci Res 2002;70:694–702.
59. James SJ, Melnyk S, Pogribna M, Pogribny IP, Caudill MA. Elevation in *S*-adenosylhomocysteine and DNA hypomethylation: potential epigenetic mechanism for homocysteine-related pathology. J Nutr 2002;132: 2361S–6S.
60. Bottiglieri T, Crellin RF, Reynolds EH. Folate and neuropsychiatry. In: Bailey LB, ed. Folate in health and disease. New York, NY: Marcel Dekker, 1995:435–62.
61. Alpert JE, Fava M. Nutrition and depression: the role of folate. Nutr Rev 1997;55:145–9.
62. Bailey LB, Gregory JF. Folate metabolism and requirements. J Nutr 1999;129:779–82.
63. Mischoulon D. The role of folate in major depression: mechanisms and clinical implications. Am Soc Clin Psychopharmacol Prog Notes 1996; 7:4–5.
64. Kruman II, Kumaravel TS, Lohani A, et al. Folic acid deficiency and homocysteine impair DNA repair in hippocampal neurons and sensitize them to amyloid toxicity in experimental models of Alzheimer's disease. J Neurosci 2002;22:1752–62.