Hyperhomocysteinemia associated with poor recall in the third National Health and Nutrition Examination Survey[1–4]

Martha Savaria Morris, Paul F Jacques, Irwin H Rosenberg, and Jacob Selhub

ABSTRACT

Background: High circulating total homocysteine (tHcy) concentrations are associated with stroke, which is a major cause of cognitive dysfunction. Blood homocysteine concentrations are inversely correlated with performance on some cognitive-function tests and a relation was recently shown between hyperhomocysteinemia and Alzheimer disease.

Objective: The objective was to evaluate the relation between serum tHcy concentrations and performance on short delayed-recall tests of elderly men and women participating in the third National Health and Nutrition Examination Survey, phase 2 (1991–1994).

Design: Subjects were aged ≥60 y. Subjects reported no previous stroke, completed ≥8 y of education, and took a test of delayed recall of story ideas ($n = 1200$) or words ($n = 1270$).

Results: After adjustment for sex, age, race-ethnicity, income, years of education, and serum creatinine concentration, subjects in the upper half of the folate distribution recalled, on average, >4 of 6 story ideas; subjects with lower folate status recalled significantly fewer ideas ($P < 0.001$). Of the subjects with low folate status, story recall was significantly poorer in those with serum tHcy concentrations above the 80th percentile of the distribution (13.7 μmol/L) than in those with lower tHcy concentrations ($P < 0.03$). The odds ratio relating hyperhomocysteinemia to recall of ≥1 of 3 previously learned words was 0.3 (95% CI: 0.2, 0.7) after adjustment for the 5 demographic factors alone and was 0.4 (0.2, 0.9) after further adjustment for serum folate concentration.

Conclusion: Hyperhomocysteinemia is related to poor recall and this association was partially independent of folate status. *Am J Clin Nutr* 2001;73:927–33.

KEY WORDS Cognitive function, cognitive decline, dementia, homocysteine, elderly, aging, third National Health and Nutrition Examination Survey, NHANES III

INTRODUCTION

Complaints of memory loss are common in the elderly and it is widely believed that some degree of memory loss is a normal and unpreventable aspect of aging (1). The most severe memory deficits occur with dementia after multiple strokes or in association with Alzheimer disease (2). Whether the mechanisms involved in memory loss that occurs in Alzheimer disease, vascular dementia, and cognitive impairment without dementia are the same or different is a matter of much debate (2). A connection between homocysteine and vascular dementia is expected given the amino acid's reputation as an independent risk factor for stroke (3, 4), but the relation between hyperhomocysteinemia and cognitive impairment need not always be mediated by stroke or even macrovascular disease. For example, circulating homocysteine concentrations reflect B-vitamin status, and B-vitamin deficiency might lead to hypomethylation of chemicals that are crucial to brain function (5). Alternatively, homocysteine or its conversion products might be neurotoxic (6).

A high circulating homocysteine concentration has been linked to poor cognitive function in both the demented and nondemented elderly (7–10) and was found to be related to pathologically confirmed Alzheimer disease, even among subjects who did not have concomitant histologic evidence of macroscopic cerebrovascular disease (6). Microvascular disease remains a possible explanation, however.

We sought to expand the body of literature on homocysteine and cognitive function in the elderly by considering a possible relation between serum total homocysteine (tHcy) concentrations and performance on 2 delayed-recall tests administered to subjects aged ≥60 y who participated in phase 2 (1991–1994) of the third National Health and Nutrition Examination Survey (NHANES III).

SUBJECTS AND METHODS

Subjects

Subjects were men and women aged ≥60 y who participated in phase 2 of NHANES III (1991–1994). The survey was conducted by the National Center for Health Statistics (NCHS),

[1] From the Jean Mayer US Department of Agriculture Human Nutrition Research Center on Aging at Tufts University, Boston.

[2] The contents of this publication do not necessarily reflect the views or policies of the US Department of Agriculture, nor does mention of trade names, commercial products, or organizations imply endorsement by the US government.

[3] Supported in part by the US Department of Agriculture, Agricultural Research Service (contract no. 58-1950-9-001), and NIH/NHLBI grant no. R01 HL52630.

[4] Address reprint requests to M Savaria Morris, Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University, 711 Washington Street, 9th Floor, Boston, MA 02111. E-mail: morris@hnrc.tufts.edu.

Received September 6, 2000.
Accepted for publication October 2, 2000.

Centers for Disease Control and Prevention (CDC), to obtain nationally representative data on the health and nutritional status of the civilian, noninstitutionalized US population through interviews and direct physical examinations (11). Toward this goal, some population subgroups, including young children, older persons, blacks, and Mexican Americans, were oversampled. All respondents gave their informed consent, and the NHANES III protocol was reviewed and approved by the NCHS NHANES Institutional Review Board.

Blood collection

tHcy concentrations were measured in surplus sera from phase 2 participants aged ≥12 y. Of 13 635 interviewed subjects, 10 280 agreed to be physically examined. Blood was drawn across a range of fasting states and processed according to a standard protocol (11). Whole blood was collected in serum separator tubes and held at room temperature for 30–60 min before centrifugation at 115 × *g* for 15 min at room temperature; no anticoagulant was used. Sera were separated, frozen at −20°C, and transferred on dry ice to the CDC central laboratory for priority analyses.

Laboratory determinations

Red blood cell (RBC) folate concentrations and serum concentrations of various blood constituents were measured according to standardized protocols (11) and were used in the data analyses as described below.

Surplus sera were stored at −70°C for ≤3 y and underwent 1–4 freeze-thaw cycles before tHcy analysis. Long-frozen plasma samples were shown to be acceptable for characterizing plasma tHcy concentrations at the time of phlebotomy (12). After approval by the New England Medical Center Human Investigations Review Committee and the Surplus Sera Bank Steering Committee, tHcy concentrations were measured at the US Department of Agriculture Human Nutrition Research Center on Aging by the HPLC method of Araki and Sako (13). tHcy measurements were obtained from 8585 (64%) physically examined phase 2 participants aged ≥12 y. tHcy values were missing for some participants because of a lack of either blood samples or surplus sera.

"Apple, table, penny" test

During in-home interviews, subjects aged ≥60 y were asked to commit to memory the words *apple*, *table*, and *penny*. This particular test is part of the Mini Mental State Examination (MMSE) (14)—a brief test battery used widely in psychogeriatric assessments. In the test, the interviewer repeats the 3 words up to 3 times until they have been learned. The interviewer tells the subjects that they will be asked to recall the 3 words in a few minutes and continues with a few additional questions. After the brief delay, the interviewer asks the subjects to recall the 3 words. The subjects' score on the test is a number from 0 to 3, corresponding to the number of the 3 items successfully recalled after the delay.

Paragraph delayed-recall test

In the course of physical examinations conducted in mobile examination centers, NHANES III participants aged ≥60 y were administered a paragraph delayed-recall test. The test was administered by trained lay interviewers according to a standard protocol (11). All subjects administered the test were told that a short story would be read to them, subsequent to which they would be asked to repeat the story back to the interviewer. The story was as follows: "Three children were alone at home and the house caught on fire. A brave fireman managed to climb in a back window and carry them to safety. Aside from minor cuts and bruises, all were well."

After the subjects' attempts to repeat the details of the story back to the interviewer, the interviewer continued with a few additional questions unrelated to the story or to cognitive function. Finally, the subjects were asked to repeat the story a second time. The test was scored according to the number of 6 main story ideas recalled by the subjects after the brief delay: *1*) 3 children, *2*) house on fire, *3*) fireman climbed in, *4*) children rescued, *5*) minor injuries, *6*) everyone well. The score for the test ranged from 0 (for none of the 6 ideas recalled) to 6 (for all 6 ideas recalled). This test is characteristic of a type of test commonly used to evaluate short-term verbal memory (15). There are many different forms of the test—some featuring prompting for details by the interviewer and some delaying or interfering for ≤1 h before the subjects are asked to recall the story a second time. Often, the paragraphs are longer than the one used in NHANES III, and several paragraphs are frequently used in the test. Paragraph delayed-recall tests may be particularly effective at distinguishing between subjects who will and will not progress to a diagnosis of Alzheimer disease (16).

Statistical methods

There were 3128 phase 2 participants aged ≥60 y. tHcy concentrations were available for 2243 participants; word delayed-recall test scores were available for 2153 of the participants and paragraph delayed-recall test scores were available for 2059 of the participants. To focus on age-related memory impairment or age-related cognitive decline as opposed to infarct-associated dementia or a life-long learning disability, we included in our data analyses only subjects who had completed ≥8 y of education, reported no previous stroke, and learned the words *apple*, *table*, and *penny* in one try. To control for language barriers as an alternative explanation for poor performance, we excluded subjects who had asked for the tests to be administered in a language different from that reportedly usually spoken at home. Altogether, ≈41% of the original 2202 subjects with known homocysteine values and recall-test scores were excluded for these reasons, resulting in final sample sizes of 1270 for word recall and 1200 for story recall.

To account for the unequal probability of subject selection and nonresponse, we performed all statistical analyses with SUDAAN statistical software and sample-weighted analytic procedures (17). Data files were created and manipulated with SAS (18). P values <0.05 were considered statistically significant.

A perfect score on the word delayed-recall test was the most common result and was achieved by >75% of the subjects. A perfect score on the paragraph delayed-recall test was achieved by only 17.4% of subjects; however, low scores were rare. Because of the different distributions for the 2 scores, different statistical methods were used to evaluate relations with serum tHcy concentrations. Specifically, the paragraph delayed-recall score was used as a continuous outcome variable in multiple linear regression analyses, whereas passing (ie, recalling ≥1 word; n = 1227) or failing (ie, recalling nothing; n = 43) the word delayed-recall test was used as the outcome in multiple logistic regression modeling.

In all analyses that related recall-test performance to serum tHcy concentration, we controlled for sex, age (continuous), years of education (continuous), income (<$20 000/y compared with ≥$20 000/y), and race-ethnicity (non-Hispanic white, non-Hispanic black, Mexican American, or other). Additional terms included in the multivariate models were those that proved to be confounders. Potential confounders were identified from among subject characteristics related at the $P \leq 0.2$ level to either recall-test performance or serum tHcy concentration after adjustment for the 5 demographic factors. We defined a potential confounder as a term whose addition to the multivariate model that included terms for the 5 demographic factors changed the coefficient relating serum tHcy concentration to recall-test performance by at least one-third of its SE. We defined a confounder as a term whose removal from a model containing all potential confounders had an equivalent effect. Potential confounders were identified from the following possibilities: heavy alcohol use (ever compared with never drank ≥5 drinks/d), high blood pressure (systolic pressure >160 mm Hg or diastolic pressure >90 mm Hg), blood pressure medication use, blood lead concentration (≤0.169 μmol/L compared with >0.169 μmol/L), pack-years of cigarette smoking, RBC folate concentration, and serum concentrations of folate, selenium, total cholesterol, triacylglycerol, creatinine, iron, total calcium, β-carotene, and vitamins A, B-12, C, and E. Log-transformed values were entered into models for tissue constituents whose distributions were highly skewed (eg, RBC folate and serum concentrations of folate, triacylglycerol, creatinine, β-carotene, and vitamins E and A).

Before the multivariate modeling, the population was described according to 24 characteristics. Depending on the scale of the characteristic, least-squares means (SUDAAN PROC REGRESS) or proportions (SUDAAN PROC CROSSTAB) were generated for each possible value of the paragraph delayed-recall score and for passing and failing the word delayed-recall test.

Using the final multivariate models, we graphically depicted the dose-response relations between serum tHcy concentration and performance on each test. For this purpose, we divided the tHcy distribution into fifths. For story recall, we used the linear regression program to generate the least-squares mean recall score and 95% CI associated with each one-fifth of the tHcy distribution. For word recall, we used the logistic regression program to generate the odds ratio and 95% CI for passing the test associated with each one-fifth of the distribution above the lowest compared with the lowest.

Some subjects who failed the word delayed-recall test achieved above-average scores on the paragraph delayed-recall test; however, 84% of the subjects who failed the word delayed-recall test recalled ≤3 story ideas. We arbitrarily defined poor recall as a score of ≤3 on the paragraph delayed-recall test and used a multiple logistic regression model controlled for all the demographic factors to evaluate the contribution of a serum tHcy concentration above the 80th percentile of the distribution (ie, 13.7 μmol/L) to the recall deficit associated with being aged >70 y, the median age of the study group.

RESULTS

The following descriptive data were obtained by using sample weights: the mean (±SEM) age of the participants was 70.1 ± 0.5 y and the mean number of years of education of these participants was 12.3 ± 0.2 y. Forty-two percent of the participants were men, 89.2% were non-Hispanic white, 53.7% had smoked previously, 11.8% had been heavy alcohol users at one time, 36.0% had taken vitamins or minerals within 24 h of the interview, 12.8% had high blood pressure, and 38.1% had a household income <$20 000/y. The mean number of ideas recalled from the story was 4.3 ± 0.1, the mean serum tHcy concentration was 10.4 ± 0.1 μmol/L, the mean RBC folate concentration was 507.8 ± 20.3 nmol/L, and the mean serum folate concentration was 17.8 ± 0.5 nmol/L.

Only one subject who was eligible for inclusion in the data analyses did not take the word delayed-recall test. Those phase 2 NHANES III participants who were eligible for inclusion in the data analyses but who did not take the paragraph delayed-recall test (n = 75) were older and somewhat less well educated than were those who did take the test. They were also more likely to have a low household income and were less likely to have high blood pressure. Furthermore, none of the subjects reported ever having been heavy alcohol users. There were no significant differences, however, in mean serum tHcy and mean RBC folate concentrations between the 2 groups.

As shown in **Tables 1** and **2**, few subject characteristics were significantly related to performance on either test of delayed recall after adjustment for the demographic factors. For both tests, these characteristics included serum concentrations of tHcy, folate, and calcium. Additionally, the RBC folate concentration was significantly related to story recall, and serum concentrations of triacylglycerol and creatinine were significantly related to word recall. Most of the serum constituents and exposures were significantly related to serum tHcy concentration. The only exceptions were vitamin A, selenium, total cholesterol, triacylglycerol, alcohol use, and high blood pressure. Nevertheless, the only characteristic that met our criteria for confounding relative to both tests was serum folate concentration. Serum creatinine concentration proved to be a confounder of the relation between serum tHcy concentration and story recall only.

The serum folate concentration interacted significantly with the serum tHcy concentration in relation to story recall but not to word recall. Regardless of their serum tHcy concentrations, subjects in the upper half of the serum folate distribution recalled, on average, >4 of the 6 main ideas of the story (**Figure 1**). Subjects in the lower half of the serum folate distribution, on the other hand, recalled significantly fewer story ideas ($P < 0.001$), and, in this subgroup only, having a serum tHcy concentration >13.7 μmol/L was associated with recall of significantly fewer ($P < 0.03$) story ideas than was having a lower serum tHcy concentration.

The odds ratio relating passing the word-recall test to a serum tHcy concentration >13.7 μmol/L was 0.3 (95% CI: 0.2, 0.7) after adjustment for the 5 demographic factors alone and was 0.4 (95% CI: 0.2, 0.9) with further adjustment for serum folate concentration. Only those subjects with tHcy concentrations above the fourth quintile of the distribution were less likely to have passed the word-recall test than were subjects with tHcy concentrations below the first quintile (**Figure 2**).

About 23% (n = 365) of survey participants aged ≥60 y who met eligibility criteria recalled ≤3 story ideas. The odds ratio relating such poor recall to age >70 y after adjustment for race-ethnicity, income, and years of education was 2.6 (95% CI: 1.9, 3.5). The addition of a term for a serum tHcy concentration >13.7 μmol/L compared with a lower value to this model resulted in an odds ratio for hyperhomocysteinemia of 1.8 (95% CI: 1.2, 2.8) and an odds ratio for higher age of 2.4 (95% CI: 1.8, 3.3). Clearly, hyperhomocysteinemia accounted for little of the recall deficit associated with age.

TABLE 1
Characteristics of the elderly men and women who took a paragraph delayed-recall test during phase 2 (1991–1994) of the third National Health and Nutrition Examination Survey to determine the number of 6 main story ideas recalled[1]

Characteristic	Story ideas recalled 0 (n = 37)	1 (n = 27)	2 (n = 82)	3 (n = 200)	4 (n = 333)	5 (n = 306)	6 (n = 158)
Serum total homocysteine (μmol/L)[2–4]	12.9 (11.3–14.8)	11.4 (9.3–13.8)	11.7 (10.6–12.9)	10.9 (10.1–11.8)	10.4 (9.8–11.0)	10.0 (9.4–10.6)	9.8 (9.0–10.6)
Age at examination (y)[3–6]	79.2 (76.8–81.6)	73.8 (70.1–77.4)	72.8 (69.2–76.3)	72.0 (70.1–73.9)	69.9 (68.1–71.0)	68.9 (67.6–70.2)	67.4 (66.5–68.2)
Percentage male (%)[4]	50.7	42.8	38.3	37.4	38.2	43.3	47.3
Percentage NHW (%)[3–6]	89.3	87.9	81.1	83.8	90.7	91.4	92.0
Household income < $20 000/y (%)[3–5]	68.2	54.6	65.3	50.1	38.9	31	24.2
Education (y)[3–6]	12.5 (11.4–13.5)	11.1 (9.6–12.7)	11.1 (10.5–11.7)	11.8 (11.3–12.3)	12.1 (11.8–12.4)	12.8 (12.3–13.2)	12.9 (12.3–13.5)
≥5 Alcoholic drinks/d (%)[5]	0.68	19.4	12.2	12.2	12.5	10.3	16.5
Cigarette smoking (pack-years)[5,6]	10.6 (5.0–16.3)	7.2 (4.0–10.3)	15.5 (8.8–22.3)	18.9 (13.5–24.3)	19.7 (16.4–22.9)	19.0 (16.1–21.8)	20.7 (15.6–25.7)
High blood pressure (%)[5]	25	20.7	18.5	18.5	14.5	9.5	6.6
Blood pressure medication use (%)[5]	20.7	37.7	39.9	41.8	42.9	34.3	36.3
Lead > 0.169 μmol/L (%)[5,6]	61.7	61.9	38.9	42.1	46.6	41.0	52.3
RBC folate (nmol/L)[2,3,6]	464 (389–554)	399 (328–486)	433 (377–496)	545 (484–613)	488 (451–528)	534 (494–577)	545 (494–601)
Serum folate (nmol/L)[2–4,6]	12.3 (10.4–14.7)	13.5 (10.6–17.0)	16.8 (14.1–20.0)	18.0 (15.4–21.0)	17.6 (15.7–19.8)	18.9 (17.5–20.4)	18.4 (16.6–20.2)
Serum total calcium (mmol/L)[3,5]	2.25 (2.21–2.29)	2.29 (2.25–2.33)	2.30 (2.28–2.32)	2.30 (2.28–2.32)	2.30 (2.28–2.32)	2.29 (2.27–2.31)	2.30 (2.29–2.33)
Serum selenium (nmol/L)[5]	1.58 (1.50–1.66)	1.63 (1.53–1.73)	1.63 (1.55–1.71)	1.65 (1.59–1.71)	1.63 (1.57–1.69)	1.63 (1.59–1.67)	1.64 (1.60–1.68)
Serum vitamin E:total cholesterol[2,6]	5.2	4.8	5.5	5.3	5.2	5.7	5.5
Serum β-carotene:total cholesterol[2,6]	0.07	0.06	0.07	0.07	0.07	0.07	0.07
Serum vitamin A:total cholesterol[2]	0.39	0.36	0.37	0.37	0.36	0.38	0.38
Serum iron (μmol/L)[5,6]	13.5 (10.5–16.4)	16.2 (12.9–19.5)	15.1 (13.4–16.7)	15.0 (14.0–16.0)	15.3 (14.8–15.7)	15.4 (14.7–16.1)	16.2 (15.3–17.0)
Serum vitamin B-12 (pmol/L)[2,6]	260 (218–310)	276 (190–400)	302 (263–346)	327 (302–354)	302 (285–320)	305 (287–323)	314 (296–333)
Serum vitamin C (mmol/L)[5,6]	43.8 (35.8–51.7)	50.6 (40.8–60.4)	49.5 (43.2–55.9)	50.1 (43.6–56.6)	52.3 (47.3–57.8)	54.4 (49.4–59.3)	51.8 (47.4–56.2)
Serum creatinine (μmol/L)[2,4,6]	110 (102–119)	96 (84–111)	100 (195–106)	102 (99–107)	98 (97–100)	96 (95–98)	104 (98–110)
Serum triacylglycerol (mmol/L)[2]	1.6 (1.3–1.9)	1.3 (1.0–1.6)	1.5 (1.4–1.7)	1.7 (1.6–1.8)	1.5 (1.4–1.6)	1.7 (1.6–1.9)	1.6 (1.4–1.7)
Serum total cholesterol (mmol/L)[5]	5.6 (5.4–5.9)	5.4 (4.9–5.9)	5.5 (5.3–5.8)	6.1 (5.9–6.2)	5.6 (5.5–5.7)	5.8 (5.6–6.0)	5.7 (5.5–5.8)

[1] 95% CI in parentheses. Only subjects with complete information on all 24 characteristics were included. NHW, non-Hispanic white.
[2] Geometric $\bar{x}$.
[3] Significant relation with recall score after age, sex, race-ethnicity, years of education, and income were controlled for, $P < 0.05$.
[4] Final model term.
[5] $\bar{x}$.
[6] Significant relation with serum total homocysteine after age, sex, race-ethnicity, years of education, and income were controlled for, $P < 0.05$.

DISCUSSION

In a multiethnic sample of noninstitutionalized US residents aged ≥60 y, we found an inverse association between hyperhomocysteinemia and performance on a word delayed-recall test for subjects who had completed ≥8 y of education and had never had a clinically evident stroke. Subjects with serum folate concentrations above the median of the distribution generally recalled most of the main ideas of a short story. However, lower folate status, particularly lower folate status with hyperhomocysteinemia, was associated with poorer recall.

A high homocysteine concentration has long been associated with stroke (3, 4), and it was recently linked to the risk of Alzheimer disease (6). The common finding of a high homocysteine concentration in the 2 diseases has been suggested by some to indicate an etiologic link between these 2 major causes of age-related memory impairment (2, 4). With respect to stroke, the cause of the associated cognitive impairment could be stroke rather than the hyperhomocysteinemia that precedes it. We excluded subjects who reported having had a stroke so that any association we found between hyperhomocysteinemia and poor recall could not be explained by brain damage resulting from one or more clinically confirmed brain infarcts. The possibility remains, however, that the hyperhomocysteinemic subjects were the ones with the more advanced cerebral atherosclerosis and

TABLE 2
Characteristics of 1145 men and women who took a word delayed-recall test during phase 2 (1991–1994) of the third National Health and Nutrition Examination Survey, according to number of 3 words recalled[1]

Characteristic	Words recalled	
	0 (n = 37)	>0 (n = 1108)
Serum total homocysteine (μmol/L)[2–4]	12.7 (11.3, 14.3)	10.4 (10.2, 10.6)
Age at examination (y)[3–6]	75.8 (73.0, 78.7)	69.8 (68.9, 70.7)
Male (%)[4]	49.9	41.7
NHW race or ethnicity (%)[3–5]	76.3	89.9
Household income <$20000/y (%)[4]	70.0	36.7
Education (y)[4–6]	12.2 (11.0, 13.5)	12.3 (12.0, 12.7)
≥5 Alcoholic drinks/d (%)	11.0	12.3
Cigarette smoking (pack-years)[5,6]	16.5 (4.2, 28.8)	19.0 (17.1, 20.9)
High blood pressure (%)	29.7	12.6
Blood pressure medication use (%)	28.4	38.3
Lead >0.169 μmol/L (%)[5]	52.7	45.8
RBC folate (nmol/L)[2,5]	455 (397, 522)	513 (474, 555)
Serum folate (nmol/L)[2–5]	14.2 (11.2, 17.9)	17.8 (16.8, 18.9)
Serum total calcium (mmol/L)[3]	2.26 (2.24, 2.28)	2.30 (2.28, 2.32)
Serum selenium (nmol/L)	1.57 (1.51, 1.63)	1.64 (1.60, 1.68)
Serum vitamin E:total cholesterol[2,5]	5.8	5.8
Serum β-carotene:total cholesterol[2,5]	0.11	0.08
Serum vitamin A:total cholesterol[2]	0.43	0.39
Serum iron (μmol/L)[5,6]	13.2 (10.4, 16.0)	15.5 (15.0, 15.9)
Serum vitamin B-12 (pmol/L)[2,5]	290 (248, 339)	308 (302, 314)
Serum vitamin C (mmol/L)[5]	41.8 (32.5, 51.2)	52.4 (49.5, 55.2)
Serum creatinine (μmol/L)[2–5]	111 (103, 120)	99 (98, 101)
Serum triacylglycerol (mmol/L)[2,3]	1.3 (1.2, 1.5)	1.6 (1.6, 1.7)
Serum total cholesterol (mmol/L)[6]	5.4 (4.8, 5.9)	5.7 (5.6, 5.8)

[1] 95% CI in parentheses. Only subjects with complete information on all 24 characteristics were included. NHW, non-Hispanic white.
[2] Geometric $\bar{x}$.
[3] Significant relation with passing the recall test after age, sex, race-ethnicity, years of education, and income were controlled for, $P < 0.05$.
[4] Final model term.
[5] Significant relation with serum total homocysteine concentration after age, sex, race-ethnicity, years of education, and income were controlled for, $P < 0.05$.
[6] $\bar{x}$.

that their memory impairment resulted from reduced blood flow to the brain caused by microvascular or large vessel disease.

A causal relation between low folate status and poor recall is reasonable in light of folate's involvement in the synthesis of *S*-adenosylmethionine, a cofactor in the methylation reactions of neurotransmitter metabolism (5, 19). Furthermore, folate status was consistently linked to cognitive function in previous studies (20–22). Given the cross-sectional design of NHANES III, the relation we found between paragraph recall and folate status could also be explained by a detrimental effect of memory loss on diet. However, we found associations between hyperhomocysteinemia and poorer recall that were independent of folate status—results that might reflect a direct effect of homocysteine or arteriosclerosis or the inadequacy of the serum folate measurements as indicators of folate status. A direct role for homocysteine or one of its excitotoxic derivatives on brain function was postulated previously (6).

This is not the first study to report on associations between nutritional status and scores on the short-term memory tests administered in NHANES III. In an investigation of antioxidants and memory, Perkins et al (23), who did not consider homocysteine, found no consistent association between serum folate concentration and recall-test results. Differences between Perkins et al's study design and ours included our additional exclusions for low educational status and previous stroke, different definitions of poor memory, and different samples, ie, we used data only from phase 2 (the phase in which homocysteine was measured). Further analyses (data not shown) showed that our methods applied to phase 1 data would not have shown an association between folate status and recall. This discrepancy may be due to the different methods by which serum folate concentrations were determined in the 2 different phases. (Although corrections were applied to the phase 1 values, the correction may not have been adequate.)

Paragraph and word delayed-recall tests are reputed to measure short-term verbal memory (15) but other cognitive functions are certainly involved. For recall to occur, information must have been encoded, which requires hearing, attention, motivation, language skills, and learning. We tried to eliminate language problems as a potential cause of poor performance by excluding persons who asked for the tests (which were available in both English and Spanish) in a language they did not speak regularly at home. Despite these efforts, subjects of racial or ethnic categories other than non-Hispanic white scored significantly lower on the tests than did non-Hispanic white subjects, even with years of education, income, RBC folate, and homocysteine controlled for. Neither the strength of relations between homocysteine and test scores nor the proportion of subjects with poor memories varied across racial or ethnic categories. Nevertheless, it is worth noting that the tests may not have been completely appropriate for survey participants who were not non-Hispanic white.

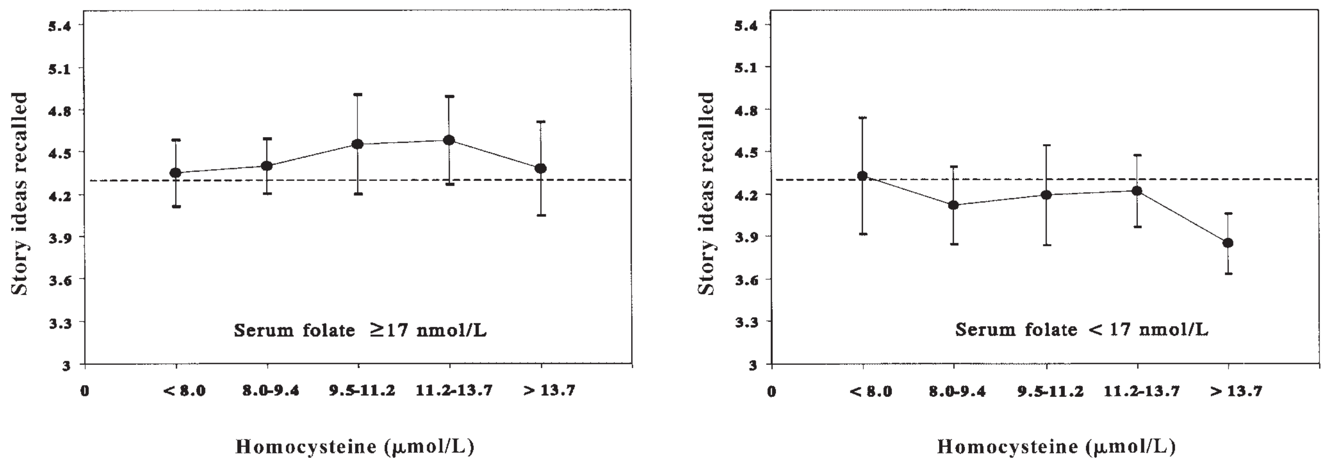

FIGURE 1. Dose-response relation between serum total homocysteine concentration and paragraph delayed-recall test score for subjects with serum folate concentrations ≥17 nmol/L (*n* = 595) and subjects with serum folate concentrations <17 nmol/L (*n* = 605). Points represent the least-squares mean of the number of story ideas recalled per fifth of the homocysteine distribution, generated by multiple linear regression analysis after adjustment for sex, age, race-ethnicity, years of education, income, and serum concentrations of folate and creatinine. Error bars represent 95% CIs. The dotted lines represent the mean number of ideas recalled for both groups combined.

Two previous studies considered a possible relation between homocysteine concentration and cognitive function. The Rotterdam Study (24), a community-based prospective study, found no association between baseline serum tHcy concentrations and performance on the MMSE at baseline or a decline in the score of at least one point per year from baseline to the end of follow-up. The "apple, table, penny" test is included in that battery, but a paragraph delayed-recall test is not. Furthermore, the MMSE evaluates cognitive functions other than memory, and homocysteine may be more strongly associated with poor memory than are other types of cognitive dysfunction. The authors of the Rotterdam Study suggest that they may not have found a link between hyperhomocysteinemia and poor cognitive function because of the dearth of lower serum tHcy concentrations among the elderly in the Netherlands.

The Normative Aging Study (8) found an association between homocysteine and results of spatial copying tests, but

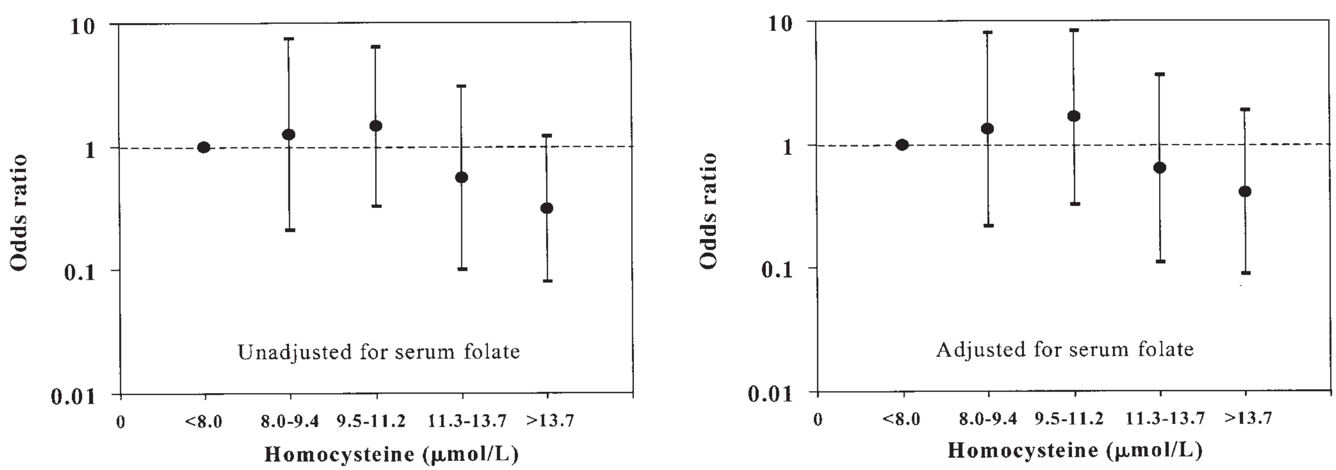

FIGURE 2. Odds ratios for passing versus failing a word delayed-recall test with increasing serum total homocysteine concentration, unadjusted and adjusted for serum folate concentration, generated by multiple logistic regression analysis after adjustment for sex, age, race-ethnicity, and income. The referent category comprises subjects with serum total homocysteine concentrations below the 20th percentile of the distribution; other categories were created by using cutoff points at the 40th, 60th, and 80th percentiles. Error bars represent 95% CIs. *n* = 1270.

not with results of 18 other tests of cognitive function, including tests of memory. In that study, vitamin B-6, which is inversely related to homocysteine concentrations but was not measured in NHANES III, was positively associated with performance on 2 tests of memory.

Because the causes of memory impairment occurring in the elderly are largely unknown, it is reasonable to ask whether age-related memory impairment is mainly due to the associated increase in homocysteine concentrations. Our results suggest that this is not the case and that aging has a large effect on recall that is independent of homocysteine. Nevertheless, hyperhomocysteinemic subjects had poorer recall for their age than did non-hyperhomocysteinemic subjects.

In conclusion, our findings indicate that hyperhomocysteinemia among the elderly is associated with poor recall. It will be interesting to see whether folate fortification, through its anticipated effect on homocysteine concentrations (25), will result in a lower prevalence of poor memory among the elderly in the United States.

REFERENCES

1. Graham JE, Rockwood K, Beattie BL, et al. Prevalence and severity of cognitive impairment with and without dementia in an elderly population. Lancet 1997;349:1793–6.
2. Miller JW. Homocysteine and Alzheimer's disease. Nutr Rev 1999;57:126–9.
3. Boushey CJ, Beresford SAA, Omenn GS, et al. 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.
4. Refsum H, Ueland PM, Nygård O, et al. Homocysteine and cardiovascular disease. Annu Rev Med 1998;49:31–62.
5. Rosenberg IH, Miller JW. Nutritional factors in physical and cognitive functions of elderly people. Am J Clin Nutr 1992;55(suppl):1237S–43S.
6. Clarke R, Smith AD, Jobst KA, Refusum H, Sutton L, Ueland PM. Vitamin B_{12}, and serum total homocysteine levels in confirmed Alzheimer disease. Arch Neurol 1998;55:1449–55.
7. Diaz-Arrastia R. Hyperhomocysteinemia: a new risk factor for Alzheimer disease? Arch Neurol 1998;55:1407–8.
8. Riggs KM, Spiro A, 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.
9. Bell I, Edman JS, Selhub J, et al. Plasma homocysteine in vascular disease and in nonvascular dementia of depressed elderly people. Acta Psychiatr Scand 1992;86:386–90.
10. McCaddon A, Davies G, Hudson P, Tandy S, Cattell H. Total serum homocysteine in senile dementia of the Alzheimer's type. Int J Geriatr Psychiatry 1998;13:235–9.
11. US Department of Health and Human Services. Third National Health and Nutrition Examination Survey (NHANES III, 1988–94) reference manuals and reports (CD-ROM). Hyattsville, MD: National Center for Health Statistics, 1996.
12. Israelsson B, Brattstrom L, Refsum H. Homocysteine in frozen plasma samples. A short cut to establish hyperhomocysteinaemia as a risk factor for arteriosclerosis? Scand J Clin Lab Invest 1993;53:465–9.
13. Araki A, Sako Y. Determination of free and total homocysteine in human plasma by high performance liquid chromatography with fluorescence detection. J Chromatogr 1987;42:43–52.
14. Folstein MF, Folstein SE, McHugh PR. "Mini-Mental State": a practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975;12:189–98.
15. Lezak MD. Neuropsychological assessment. 3rd ed. New York: Oxford University Press, 1995.
16. Kluger A, Ferris SH, Golomb J, Mittleman MS, Reisberg B. Neuropsychological prediction of decline to dementia in nondemented elderly. J Geriatr Psychiatry Neurol 1999;12:168–79.
17. Shah BV, Barnwell BG, Bieler GS. SUDAAN user's manual, release 7.5. Research Triangle Park, NC: Research Triangle Institute, 1996.
18. The SAS system for Windows, version 6.12. Cary, NC: SAS Institute Inc, 1996.
19. Bottiglieri T, Hyland K, Reynolds EH. The clinical potential of ademetionine (*S*-adenosylmethionine) in neurological disorders. Drugs 1994;48:137–52.
20. Lindeman RD, Romero LJ, Koehler KM, et al. Serum vitamin B12, C and folate concentrations in the New Mexico elder health survey: correlations with cognitive and affective functions. Am Coll Nutr 2000;19:68–76.
21. Hassing L, Wahlin A, Winblad B, Backman L. Further evidence on the effects of vitamin B12 and folate levels on episodic memory functioning: a population-based study of healthy very old adults. Biol Psychiatry 1999;45:1472–80.
22. Ebly EM, Schaefer JP, Campbell NR, Hogan DB. Folate status, vascular disease and cognition in elderly Canadians. Age Ageing 1998;27:485–91.
23. Perkins AJ, Hendrie HC, Callahan CM, et al. Association of antioxidants with memory in a multiethnic elderly sample using the Third National Health and Nutrition Examination Survey. Am J Epidemiol 1999;150:37–44.
24. 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.
25. Jacques PF, Selhub J, Bostom AG, Wilson PW, Rosenberg IH. The effect of folic acid fortification on plasma folate and total homocysteine concentrations. N Engl J Med 1999;340:1449–54.