Homocysteine and cognitive function in healthy elderly community dwellers in Italy[1–3]

Giovanni Ravaglia, Paola Forti, Fabiola Maioli, Antonio Muscari, Loredana Sacchetti, Giorgia Arnone, Valeria Nativio, Teresa Talerico, and Erminia Mariani

ABSTRACT

Background: Elevated plasma total homocysteine (tHcy) concentrations are common in the elderly and have been suggested to be a risk factor for dementia.

Objective: In an elderly population, we examined the relation between plasma tHcy and scores on the Mini-Mental State Examination (MMSE), a commonly used screening measure of cognitive impairment in general practice.

Design: Fasting plasma tHcy concentrations were measured in 650 healthy, cognitively normal Italian community dwellers aged ≥ 65 y ($\bar{x} \pm$ SD: 72.8 ± 6.0 y). Socioeconomic status; serum folate, vitamin B-12, and creatinine; other potential dietary and lifestyle determinants of tHcy; and conventional vascular disease risk factors were also assessed.

Results: Subjects with MMSE scores of 26–28 had higher plasma tHcy concentrations (12.7 μmol/L; range: 12.2–13.2 μmol/L) than did those with scores > 28 (11.9 μmol/L; 11.4–12.3 μmol/L; $P < 0.01$). Subjects with scores of 24–25 had higher plasma tHcy concentrations (14.5 μmol/L; 13.5–15.6 μmol/L) than did subjects with scores of 26–28 ($P < 0.01$) or > 28 ($P < 0.001$). The risk of hyperhomocysteinemia (plasma tHcy > 15 μmol/L) was higher in subjects with scores of 24–25 (odds ratio: 3.81; 95% CI: 1.9, 7.5) or 26–28 (odds ratio: 1.96; 95% CI: 1.3, 3.0) than in those with scores > 28. The results did not change after adjustment for conventional vascular risk factors and for age, medical, dietary, and lifestyle determinants of plasma tHcy.

Conclusion: Elevated plasma tHcy has an independent, graded association with concurrent cognitive impairment as measured with the MMSE in healthy elderly community dwellers. *Am J Clin Nutr* 2003;77:668–73.

INTRODUCTION

As the proportion of elderly people in our society increases, we can expect an increase in the number of people with cognitive impairment (1). Therefore, it is important to identify modifiable risk factors for age-related cognitive decline.

The sulfur amino acid homocysteine is a unique candidate for this role because of its association with cerebrovascular disease (2) and its direct neurotoxicity (3). Moreover, an elevated concentration of plasma total homocysteine (tHcy) is an indicator of inadequate folate and vitamin B-12 status (4). Insufficient methylation of homocysteine to methionine because of a deficit in these vitamins leads to an insufficient supply of methyl groups, which are necessary for the synthesis of myelin, neurotransmitters, and membrane phospholipids (3, 5).

Elevated tHcy concentrations have been found in both vascular and Alzheimer dementia (6, 7) as well as in other psychogeriatric conditions (8). In the nondemented elderly population, hyperhomocysteinemia has been reported to be associated with poor performance on neuropsychological tests measuring specific cognitive abilities (9–13).

The Mini-Mental State Examination (MMSE) is the most widely used general measure of cognitive function (14). Many studies consistently reported an association between plasma tHcy concentrations and MMSE scores in demented elderly patients (15–17) but not in centenarians (18). On the contrary, results from analogous studies in healthy elderly populations are conflicting (11–13, 19, 20). The differences may have resulted from the small numbers of participants in these studies (11, 13, 19), from methodologic issues (20), and from the different possible confounders taken into account (12).

Indeed, B vitamin status, along with age and reduced renal function, are responsible for most cases of mild hyperhomocysteinemia in older people (4, 21), but several other dietary, lifestyle, genetic, and clinical factors have also been suggested to affect plasma tHcy concentrations (22–25).

In the current study, we used baseline data from a community-based and relatively large Italian study of brain aging (26) to estimate the relations between plasma tHcy concentrations in healthy elderly community dwellers and their performance (ie, scores) on the MMSE. Several potential confounders of tHcy concentrations were also analyzed.

SUBJECTS AND METHODS

Subjects

Data are from the Conselice Study, which is a single-center population-based study designed to investigate determinants of cognitive performance in older persons. Details on study design and data collection have been published elsewhere (26). The study was approved by the Institutional Review Board of the Department of Internal Medicine, Cardioangiology, and Hepatology, University of Bologna, and written informed consent was obtained from all participants.

Briefly, between May 1999 and May 2000, 1016 (75%) of the 1353 persons aged ≥65 y residing in the Italian municipality of Conselice, province of Ravenna, Emilia Romagna region, underwent the following procedures: *1*) a standardized personal interview covering, among other issues, medical history, medication use, and sociodemographic, lifestyle, and dietary information; *2*) an extensive medical and neurologic examination; and *3*) venous blood drawing and urine sample collection for routine biochemical blood and urine analyses.

The Italian version of the 30-point MMSE (27) was also administered to all participants by the same specially trained lay assistant. Because of the poor educational background of our study population (<20% of the participants had >5 y of formal education), we chose the standard cutoff score of <24 to identify subjects with possible cognitive impairment; scores of 24–30 indicated nondementia (27, 28).

Of all the participants in the Conselice Study who scored ≥24 on the MMSE (*n* = 857), 157 were excluded because of a medical condition or because they were taking drugs known to affect plasma tHcy concentrations: history of acute myocardial infarction, stroke or transient ischemic attacks, current liver disease, reduced renal excretion (serum creatinine > 133 μmol/L), cancer, and treatment with vitamins, theophylline, psychotropic drugs, cytotoxic drugs, or estrogen replacements. Another 50 subjects were excluded because they had conditions indicating cognitive impairment: epilepsy, major psychiatric illnesses, sensory-motor impairments affecting neuropsychological testing, a score of ≤6 (which signifies cognitive impairment) on the clock drawing test (CDT) according to the Wolf-Klein method (29), and dependency in one or more of the tasks included in the Instrumental Activities of Daily Living scale (30). This left us with 650 community dwellers (295 men and 355 women) aged 65–91 y ($\bar{x}$ ± SD: 72.8 ± 6.0 y). The study sample did not differ significantly by age, sex, or education level from the population from which they were selected.

Sociodemographic, lifestyle, and clinical variables

The standardized interview provided information about age, sex, years of formal education (≤3 y or >3 y, because at the time that the subjects went to school, the first educational degree was achieved after 3 y of schooling), income, smoking habit (never smokers, exsmokers, or current smokers), physical activity (sedentary or active lifestyle, on the basis of whether moderate physical activity was performed for ≥4 h/wk), coffee consumption (classified according to the number of cups consumed per day), wine and liquor consumption (classified according to the number of drinks consumed per day), meat consumption (classified according to the number of servings consumed per week), body mass index (BMI; calculated as weight in kilograms divided by the square of the height in meters), hypertension (defined as a systolic blood pressure of ≥140 mm Hg, a diastolic blood pressure of ≥90 mm Hg, or the use of antihypertensive medication), diabetes mellitus, and cardiovascular diseases other than myocardial infarction (angina, peripheral vascular disease, congestive heart failure, or atrial fibrillation). A diagnosis of diabetes mellitus or cardiovascular disease was based on medical histories provided by the patients and confirmed by their general practitioners. Whenever available, previous medical records were also reviewed.

Laboratory measurements

Venous blood samples were taken between 0730 and 0900 after the subjects had fasted overnight. Blood samples for plasma tHcy measurements were collected in tubes containing EDTA and placed in a refrigerator (−4 °C) within 15–30 min of collection. Plasma was separated within 1–3 h, and the samples were stored at −70 °C for ≤12 mo until the tHcy analysis was performed. Plasma tHcy concentrations were measured with the fully automatized IMx assay (Abbott Laboratories, Abbott Park, IL). Intra- and interassay CVs were 2.1% and 3.2%, respectively.

Blood samples for serum folate and vitamin B-12 measurements were collected in empty tubes and sent to the biochemical laboratory for immediate immunoelectrochemiluminescence analysis (Elecsys Folate Immunoassay and Elecsys B12 Immunoassay for Elecsys 2010 System; Roche Diagnostics Italia SpA Monza, Milano, Italy). Lower reference values at our laboratory are 5.7 nmol/L for serum folate and 148 pmol/L for vitamin B-12 concentrations.

Serum total cholesterol was measured in fresh blood by enzymatic assay (Roche Diagnostics, Monza, Italy) with a Hitachi 917 System autoanalyzer (Boehringer Mannheim, Mannheim, Germany). Serum creatinine was measured with the use of the Jaffé method, adapted for autoanalyzers.

Statistical methods

The results for the continuous variables are presented as means ± SDs (continuous) and the results for the categorical variables are presented as numbers and percentages, except for plasma tHcy, serum folate, and serum vitamin B-12. Because of the highly skewed distribution of these 3 variables, logarithmic transformations were applied and the values were reported as geometric means and 95% CIs. Given previous reports (4), low serum vitamin B-12 concentrations may be less important than are low serum folate concentrations as a cause of hyperhomocysteinemia. To account for this association, without the use of complex mathematical models, we used in all statistical analyses a combined serum B vitamin index of 4 categories based on the median value of each serum vitamin (serum folate: 12 nmol/L; serum vitamin B-12: 245 pmol/L): category 1 (subjects with both serum folate and vitamin B-12 concentrations above the median of the respective distribution; B vitamin replete), category 2 (subjects with only serum vitamin B-12 concentrations below the median), category 3 (subjects with only serum folate concentrations below the median), and category 4 (subjects with both serum folate and vitamin B-12 below the median).

Hyperhomocysteinemia was defined as a plasma tHcy concentration > 15 μmol/L, corresponding to the 95th percentile for homocysteine among the individuals of category 1 of the serum B vitamin index.

The subjects were categorized into 3 groups according to their MMSE score (24–25, 26–28, and >28), and differences among the 3 MMSE groups were evaluated by using analysis of variance

TABLE 1
Characteristics of the study participants by Mini-Mental State Examination (MMSE) score[1]

	MMSE score			
Variable	24–25 (n = 46)	26–28 (n = 259)	>28 (n = 345)	P[2]
Men (%)	37 [17]	49 [127]	44 [151]	0.215
Age (y)[3]	78.6 ± 7.0[a]	73.1 ± 6.1[b]	71.8 ± 5.2[c]	<0.001
Education ≤ 3 y (%)	48 [22]	37 [95]	21 [71]	<0.001
Income < 6200 Euro/y (%)[4]	28 [13]	31 [80]	18 [62]	0.001
Plasma tHcy (μmol/L)[5]	14.5 (13.5, 15.6)[a]	12.7 (12.2, 13.2)[b]	11.9 (11.4, 12.3)[c]	<0.001
Hyperhomocysteinemia (%)	37 [17]	23 [60]	13 [46]	<0.001
Serum creatinine (μmol/L)[3]	90.1 ± 16.4[a]	85.6 ± 15.9[a,b]	83.6 ± 15.3[b]	0.018
Serum folate (nmol/L)[5]	11.4 (10.0, 13.0)	11.3 (10.7, 11.9)	11.6 (11.1, 12.2)	0.638
Serum vitamin B-12 (pmol/L)[5]	233 (206, 264)	240 (228, 253)	237 (227, 248)	0.879
Serum B vitamin index (%)[6]				0.805
Category 1, highest	28 [13]	26 [66]	29 [99]	
Category 2	22 [10]	23 [60]	20 [68]	
Category 3	28 [13]	23 [60]	23 [80]	
Category 4, lowest	22 [10]	28 [73]	28 [98]	
Smoking habit (%)				0.568
Never smoker	63 [29]	64 [166]	57 [198]	
Exsmoker	28 [13]	27 [70]	32 [110]	
Current smoker	9 [4]	9 [23]	11 [37]	
Physical activity (%)	50 [23]	66 [172]	68 [233]	0.060
Coffee consumption (%)[7]				0.581
0 cups/d	37 [17]	26 [68]	13 [98]	
1–2 cups/d	54 [25]	59 [153]	59 [202]	
>2 cups/d	9 [4]	15 [38]	28 [45]	
Wine and liquor consumption				0.351
0 drinks/d	39 [18]	42 [109]	46 [159]	
1–2 drinks/d	29 [13]	35 [92]	34 [117]	
≥2 drinks/d	33 [15]	22 [58]	46 [69]	
Meat < 3 servings/wk	30 [14]	29 [75]	27 [95]	0.879
BMI (kg/m²)	28.2 ± 5.6	28.6 ± 4.0	28.9 ± 4.2	0.400
Cholesterol (mmol/L)	6.1 ± 1.2	6.3 ± 1.1	6.3 ± 1.0	0.342
Hypertension (%)	50.5 [49]	56.2 [117]	57.4 [198]	0.482
Cardiovascular disease (%)	13 [6]	19 [50]	14 [49]	0.202
Diabetes mellitus (%)	11 [5]	6 [15]	7 [24]	0.441

[1] n in brackets. tHcy, total homocysteine. Means in a row with different superscript letters are significantly different, $P < 0.05$ (Tukey's t test for all pairwise multiple comparisons).

[2] Comparison between groups were performed by ANOVA or chi-square test as appropriate.

[3] $\bar{x}$ ± SD.

[4] Equivalent to ≈US $5800/y.

[5] Geometric $\bar{x}$; 95% CI in parentheses.

[6] Category 1: serum folate > 12 nmol/L and serum vitamin B-12 > 245 pmol/L; category 2, serum vitamin B-12 ≤ 245 pmol/L; category 3, serum folate ≤ 12 nmol/L; category 4, serum folate ≤ 12 nmol/L and serum vitamin B-12 ≤ 245 pmol/L.

[7] 1 cup = 0.24 L.

(Tukey's test for all pairwise multiple comparisons) or chi-square tests as appropriate.

In agreement with previous reports (4, 21–24), preliminary analyses showed that, in this population, the following variables had a mutually independent association with elevated plasma tHcy concentrations: age, education level ≤3 y, income ≤6200 Euro/y (equivalent to ≈US$5800/y), serum creatinine, serum B vitamin index, active lifestyle, and coffee and meat consumption. Education level and income are also well-known potential confounders of MMSE score (14). All of these variables were entered as covariates in the general linear model that was used to estimate adjusted geometric means of tHcy concentrations by MMSE score group.

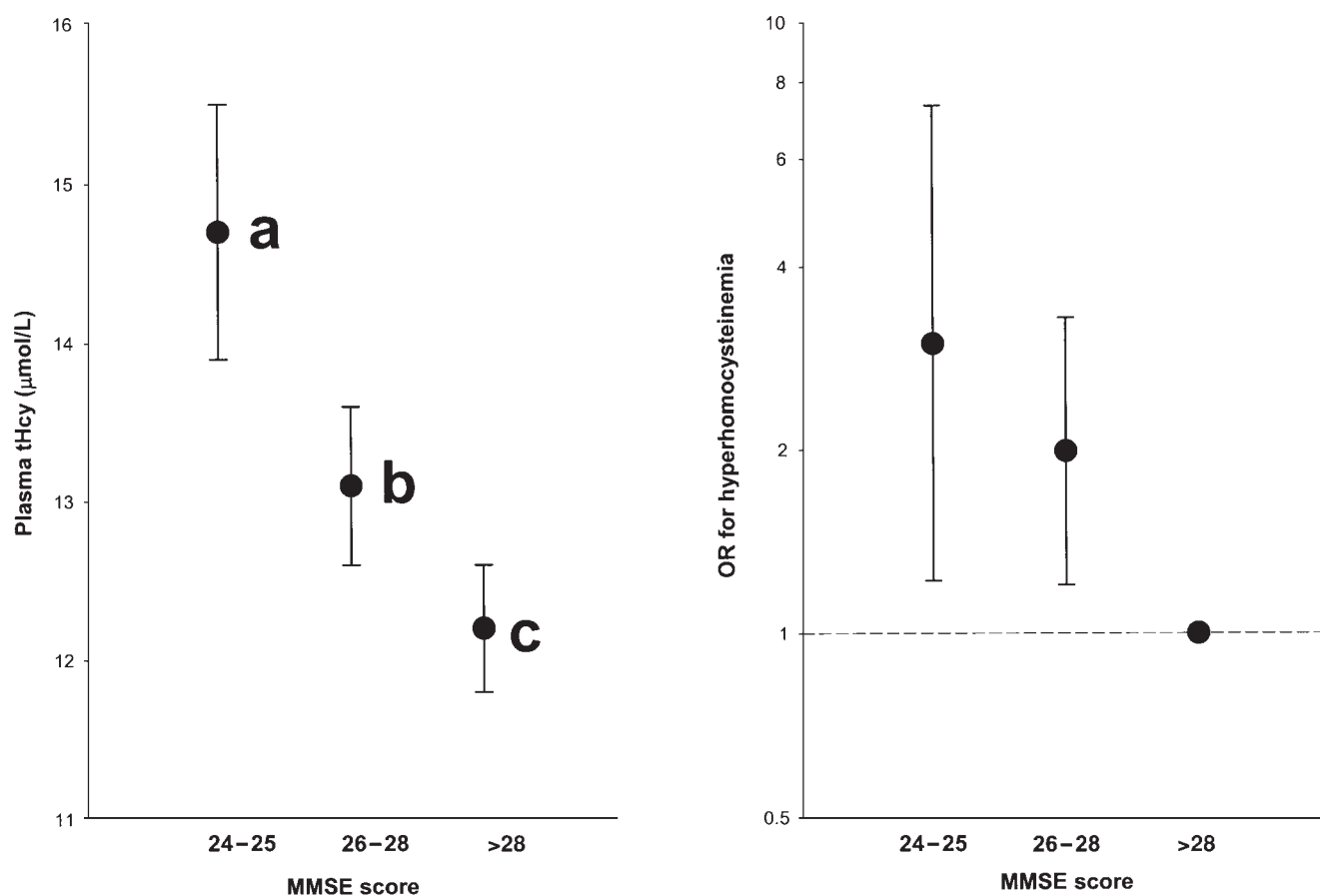Crude and multivariable-adjusted odds ratios (ORs) for hyperhomocysteinemia by MMSE score group were also determined by logistic regression, with subjects with an MMSE score > 28 as the reference category. The multivariable-adjusted logistic model included the same covariates as the general linear model.

Information about consumption of tea, milk, eggs, vegetables, and fruit was also available for all participants, but additional adjustment of the multivariate model for these variables had a minimal influence on the results; therefore, they were not included in this report.

The statistical analyses were performed with SYSTAT10 (SPSS Inc, Chicago). All tests were two-tailed, and a P value < 0.050 was considered significant.

RESULTS

The characteristics of the study population by MMSE score group are shown in **Table 1**. MMSE scores of the population

FIGURE 1. Multivariable-adjusted plasma total homocysteine (tHcy) concentrations and multivariable-adjusted odds ratios (OR) for hyperhomocysteinemia by Mini-Mental State Examination (MMSE) score: 24–25 (n = 46), 26–28 (n = 259), and > 28 (n = 345). Means with different letters are significantly different, $P < 0.05$ (Tukey's test for all pairwise multiple comparisons).

ranged from 24 to 30 (median: 29), and, as expected, subjects with lower MMSE scores were older, were less educated, and had a lower socioeconomic status than did subjects with higher MMSE scores. Plasma tHcy concentrations ranged from 5.5 to 47 μmol/L, with an overall geometric mean of 12.3 μmol/L. The overall prevalence of hyperhomocysteinemia was 18.9%. Because MMSE score groups and plasma tHcy concentrations did not differ significantly by sex, the data for men and women were pooled.

Mean plasma tHcy concentrations were significantly higher in subjects with an MMSE score of 26–28 than in those with a score > 28 and were significantly higher in subjects with an MMSE score of 24–25 than in those with a score of ≥ 26. The crude risk of hyperhomocysteinemia was significantly higher in subjects with a score of 24–25 (OR: 3.81; 95% CI: 1.9, 7.5) or 26–28 (OR: 1.96; 95% CI: 1.3, 3.0) than in the reference group (MMSE score > 28). None of the other study variables differed significantly among MMSE score groups, except for serum creatinine concentrations, which were significantly higher in subjects with an MMSE score of 24–25 than in those with a score > 28.

As shown in **Figure 1**, after adjustment for all of the study covariates, all differences in plasma tHcy concentrations among the MMSE score groups remained significant (analysis

of variance: F = 8.517, $P < 0.001$), as did the inverse association between the risk of hyperhomocysteinemia and MMSE score. Additional adjustment for sex, BMI, hypertension, cardiovascular disease, or diabetes mellitus did not significantly change these estimates. No significant association with MMSE score was found for vitamin B-12 or folate serum concentration.

DISCUSSION

In this relatively large sample of elderly Italian community dwellers with no clinical evidence of cognitive impairment, we found an inverse association between mild hyperhomocysteinemia and MMSE score. The association was independent of age, socioeconomic status, renal function, vitamin B status, other lifestyle determinants of plasma tHcy, and conventional risk factors for cardiovascular disease.

The results of this study disagree with the findings in a nondemented elderly population by Budge et al (13), who showed an association between tHcy concentrations and lower scores on the cognitive subscale of the Cambridge Dementia Inventory but not with MMSE score. The current results also disagree with our previous negative findings in centenarians with different degrees of cognitive impairment (18) and in a subsample of cognitively

healthy participants in the Conselice Study (19). It is possible, however, that the sample size in both of our prior studies and in the study by Budge et al (13) was not large enough to detect any difference that may have been present. Moreover, as far as centenarians are concerned, they have many peculiar biological and sociocultural characteristics; therefore, caution is needed when comparing this population with younger elderly people.

In a prospective analysis of elderly participants in the Rotterdam Study, Kalmijn et al (20) found no significant association between elevated tHcy concentrations and cognitive decline, as measured by a change of ≥1 point/y in the MMSE score. In their study, however, plasma tHcy was assayed in nonfasting samples. On the contrary, McCaddon et al (11) reported that fasting tHcy concentrations predicted 5-y follow-up changes in the MMSE scores of healthy elderly persons independently of several tHcy determinants. Their study, however, included only 32 subjects.

None of these studies reported significant associations between plasma tHcy and baseline MMSE score. It must be noted, however, that Kalmijn et al (20) chose an MMSE score cutoff of 26 for participant selection, whereas in McCaddon et al's study (11), initial MMSEs score ranged from 29 to 30. With so narrow an interval, even if subjects with mild hyperhomocysteinemia had low baseline MMSE scores, the ceiling effect of the test may have attenuated the association.

Finally, in a recent study (12) of 2 cohorts of elderly community dwellers (one aged 63 y and the other aged 78 y), plasma tHcy concentrations were reported in association with both scores on a test of specific cognitive domains and on the MMSE, but only among subjects from the older cohort. In the current study, however, age and childhood intelligence quotient were the only possible confounders taken into account along with vitamin B status.

The MMSE, by itself, cannot be used as a diagnostic tool to identify dementia, but it is widely used in both clinical and epidemiologic settings for detecting cognitive impairment, and studies in different populations showed a sensitivity of ≈87% for the traditional cutoff of 23–24 (21). A meta-analysis of studies in community dwellers, however, indicated that MMSE scores ≥26 significantly decrease the likelihood of dementia in the elderly, whereas scores <26 are less useful in determining the probability of disease (31).

In the current study, according to current guidelines for detection of cognitive impairment in general practice (32), a normal result on the CDT, the ability to perform independently in everyday activities, and an MMSE score of ≥24 were required for all participants. The CDT focuses on visuospatial and constructional abilities and, combined with the MMSE score, is an extremely efficient screening test for mild-to-moderate dementia (33). Taking into account the ability to independently perform the activities listed in the Instrumental Activities of Daily Living scale additionally improves the predictive value of the MMSE score, especially among poorly educated people (34), because functional impairment in social and occupational activities is among the current criteria for dementia (35).

At the mildest and generally unrecognized stage of cognitive impairment, however, symptoms are subtle and may develop insidiously over years (36). Individuals in this preclinical stage often still perform within normal limits on cognitive tests but have lower scores on neuropsychological tests than do their dementia-free counterparts (37).

Taken as a whole, these data suggest that some of our study participants had clinically unrecognized cases of early or very mild

cognitive impairment, especially those with MMSE scores in the lower range. Might then mild hyperhomocysteinemia be an early marker of cognitive impairment?

There are several mechanisms through which homocysteine might affect cognition independently of vitamin B status. Hyperhomocysteinemia has been recognized as a risk factor for cerebral micro- and macroangiopathy through pathologic changes in arterial walls and blood coagulation systems (2). Moreover, homocysteine might cause direct neuronal damage through activation of *N*-methyl-D-aspartate receptors (3) or apoptosis triggered by DNA damage (38).

Vitamin B-6 (39), riboflavin (9), and the homozygous state of a common mutation (677C T) in the methylenetetrahydrofolate reductase (EC 1.7.995) gene (40) may also influence homocysteine metabolism; however, none of these factors were studied in the current study. However, fasting plasma tHcy concentrations generally do not reflect vitamin B-6 status (39), whereas the effect of the methylenetetrahydrofolate reductase 677C T polymorphism and riboflavin status are essentially confined to homozygous individuals with folic acid depletion (10, 41).

Finally, it must be noted that, in disagreement with previous studies (6, 12), no independent relation was found between MMSE score and serum vitamin B-12 or folate concentration in the current study. This could have been due to the exclusion of patients with overt dementia (6) and to differences in the array of confounders taken into account (12).

In conclusion, we found an inverse association between mild hyperhomocysteinemia and MMSE score in healthy elderly community dwellers. The results of this cross-sectional study require confirmation in a longitudinal study but support the hypothesis that homocysteine is a marker of cognitive impairment in the elderly. ■

REFERENCES

1. Ritchie K, Kildea D. Is senile dementia "age-related" or "ageing-related"? Evidence from meta-analysis of dementia prevalence in the oldest-old. Lancet 1995;346:931–4.
2. Eikelboom JW, Hankey GJ, Anand SS, L0fthouse E, Staples N, Baker RI. Association between high homocyst(e)ine and ischemic stroke due to large- and small-artery disease but not other etiologic subtypes of ischemic stroke. Stroke 2000;31:1069–75.
3. Parnetti L, Bottiglieri T, Lowenthal D. Role of homocysteine in age-related vascular and non-vascular diseases. Aging Clin Exp Res 1997;9: 241–57.
4. Selhub J, Jacques PF, Wilson PWF, Rush D, Rosenberg IH. Vitamin

status and intake as primary determinants of homocysteinemia in an elderly population. JAMA 1993;270:2693–8.

5. Nourhashemi F, Gillette-Guyonnet S, Andrieu S, et al. Alzheimer disease: protective factors. Am J Clin Nutr 2000;71(suppl):643S–9S.

6. Clarke R, Smith DA, Jobst KA, Refsum H, Sutton L, Ueland PM. Folate, vitamin B12, and serum total homocysteine in confirmed Alzheimer disease. Arch Neurol 1998;55:1449–55.

7. 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.

8. Nilsson K, Gustafson L, Hultberg B. Plasma homocysteine is a sensitive marker for tissue deficiency of both cobalamines and folates in a psychogeriatric population. Dement Geriatr Cogn Disord 1999;10: 476–82.

9. Morris MS, Jacques PF, Rosenberg IH, Selhub J. Hyerhomocysteinemia associated with poor recall in the third National Health and Nutrition Examination Survey. Am J Clin Nutr 2001;73:927–33.

10. Riggs KM, Spiro A, Tucker K, Rush D. Relations of vitamin B12, vitamin B6, folate, and homocysteine to cognitive performance in the Normative Aging Study. Am J Clin Nutr 1996;63:306–14.

11. McCaddon A, Huddon P, Davies G, Hughes A, Williams JHH, Wilkinson C. Homocysteine and cognitive decline in healthy elderly people. Dement Geriatr Cogn Disord 2001;12:309–13.

12. Duthie SJ, Whalley LJ, Collins AR, Leaper S, Berger K, Deary IJ. Homocysteine, B vitamin status, and cognitive function in the elderly. Am J Clin Nutr 2002;75:908–13.

13. Budge M, Johnston C, Hogervorst E, et al. Plasma total homocysteine and cognitive performance in a volunteer elderly population. Ann N Y Acad Sci 2000;903:407–10.

14. Tombaugh TN, McIntyre NJ. The Mini-Mental State Examination: a comprehensive review. J Am Geriatr Soc 1992;40:922–35.

15. 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: 127–34.

16. Bottiglieri T, Parnetti L, Arning E, et al. Plasma total homocysteine levels and the C677 mutation in the methylenetetrahydrofolate reductase (MTHFR) gene: a study in an Italian population with dementia Mech Ageing Dev 2001;122:2013–23.

17. Lehmann M, Gottfries CG, Regland B. Identification of cognitive impairment in the elderly: homocysteine is an early marker. Dement Geriatr Cogn Disord 1999;10:12–20.

18. Ravaglia G, Forti P, Maioli F, et al. Elevated plasma homocysteine levels in centenarians are not associated with cognitive impairment. Mech Ageing Dev 2000;121:251–61.

19. Ravaglia G, Forti P, Maioli F, et al. Blood homocysteine and vitamin B levels are not associated with cognitive skills in healthy normally ageing subjects. J Nutr Health Aging 2000;4:218–21.

20. Kalmijn S, Launer L, Lindemans J, Bots M, Hofman A, Breteler MMB. Total homocysteine and cognitive decline in a community-based sample of elderly subjects. Am J Epidemiol 1999;150:283–9.

21. Herrmann W, Quast S, Ullrich M, Schultze H, Bodis M, Geisel J. Hyperhomocysteinemia in high-aged subjects: relation of B-vitamins, folic acid, renal function and the methylenetetrahydrofolate reductase mutation. Atherosclerosis 1999;144:91–100.

22. Nygard O, Refsum H, Ueland PM, Vollset SE. Major lifestyle determinants of plasma total homocysteine distribution: the Hordaland Homocysteine Study. Am J Clin Nutr 1998;67:263–70.

23. Appel LJ, Miller ER III, Jee SH, et al. Effect of dietary patterns on serum homocysteine: results of a randomized, controlled feeding study. Circulation 2000;102:852–7.

24. Jacques PF, Bostom AG, Wilson PWF, Rich S, Rosenberg IH, Selhub J. Determinants of plasma total homocysteine concentration in the Framingham Offspring cohort. Am J Clin Nutr 2001;73: 613–21.

25. Ueland PM, Hustad S, Schneede J, Refsum H, Vollset SE. Biological and clinical implications of the MTHFR C677T polymorphism. Trends Pharmacol Sci 2001;22:195–201.

26. Ravaglia G, Forti P, Maioli F, et al. Conselice Study: a population based study of brain aging in a municipality of the Emilia Romagna Region: Conselice, A.U.S.L. Ravenna. Design and methods. Arch Gerontol Geriatr 2001;33(suppl):325–31.

27. Valente C, Maione P, Lippi A, et al. Validation of the Mini Mental State Examination (MMSE) as a screening instrument for dementia in an Italian Population. G Gerontol 1992;40:161–5.

28. Scurti R, Pennese F, Palombo V, Cesauri O, Puddu GM, Abate G. Validation of the Mini Mental State in a geriatric Italian population. G Gerontol 1993;41:407–11.

29. Wolf-Klein GP, Silverstone FA, Levy AP, Brod MS, Breuer J. Screening for Alzheimer's disease by clock drawing. J Am Geriatr Soc 1989; 37:730–4.

30. Lawton MP, Brody EM. Assessment of older people: self-maintaining and instrumental activities of daily living. Gerontologist 1969;9: 179–85.

31. Siu AL. Screening for dementia and investigating its causes. Ann Intern Med 1991;115:122–32.

32. Eccles M, Clarke J, Livingstone M, Freemantle N, Mason J. North of England evidence based guidelines development project: guideline for the primary care management of dementia. BMJ 1998; 317:802–8.

33. Brodaty H. The clock drawing test for dementia of the Alzheimer's type: a comparison of three scoring methods in a memory disorders clinic. Int J Geriatr Psychiatry 1997;12: 619–27.

34. Barberger-Gateau P, Commenges D, Gagnon M, Letenneur L, Sauvel C, Dartigues JF. Instrumental activities of daily living as a screening tool for cognitive impairment and dementia in elderly community dwellers. J Am Geriatr Soc 1992;40:1129–34.

35. American Psychiatric Association. Diagnostic and statistical manual of mental disorders. 4th ed. Washington, DC: APA Press, 1994.

36. Villareal DT, Morris JC. The diagnosis of Alzheimer's disease. Alzheimer Dis Rev 1998;3:142–52.

37. Slowinski M, Lipton RB, Buschke H, Stewart W. The effects of preclinical dementia on estimates of normal cognitive functioning in aging. J Gerontol 1996;51B:P217–25.

38. 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.

39. Ubbink JB, van der Merwe A, Delport R, Allen RH, Stabler SP, Riezier R. The effect of a subnormal vitamin B-6 status on homocysteine metabolism. J Clin Invest 1996;98:177–84.

40. Deloughery TG, Evans A, Sadeghi A, et al. Common mutation in methylenetetrahydrofolate reductase. Correlation with homocysteine metabolism and late-onset vascular disease. Circulation 1996;94: 3074–8.

41. Jacques PK, Kalmbach R, Bagley PJ, et al. The relation between riboflavin and plasma total homocysteine in the Framingham Offspring cohort is influenced by folate status and the C677 transition in the methylenetetrahydrofolate reductase gene J Nutr 2002; 132:283–8.