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Total Homocysteine and Cognitive Decline in a Community-based Sample of Elderly Subjects

The Rotterdam Study

Sandra Kalmijn,[1-3] Lenore J. Launer,[1,2] Jan Lindemans,[4] Michiel L. Bots,[5] Albert Hofman,[1] and Monique M. B. Breteler[1]

Homocysteine has been associated with an increased risk of cardiovascular disease. Cardiovascular diseases have been related to cognitive decline. The authors investigated the association of homocysteine with concurrent cognitive impairment and subsequent cognitive decline in a random sample of 702 community-dwelling respondents aged 55 years or over to the prospective Rotterdam Study in 1990–1994. Multiple logistic regression was used to calculate odds ratios and 95 percent confidence intervals for the association between total homocysteine levels and cognitive impairment (Mini-Mental State Examination (MMSE) score <26) and cognitive decline (drop in MMSE score of >1 point/year). Mean duration of follow-up was 2.7 years. After adjustment for age, sex, and education, there was no relation between total homocysteine and cognitive impairment (highest vs. lowest tertile: odds ratio (OR) = 1.30, 95% confidence interval (CI): 0.50, 3.38) or cognitive decline (middle vs. lowest tertile: OR = 1.14, 95% CI: 0.67, 1.93; highest vs. lowest tertile: OR = 0.91, 95% CI: 0.52, 1.58). Subjects who were lost to follow-up due to death or nonresponse had slightly higher age-adjusted homocysteine levels and lower MMSE scores at baseline. Sensitivity analyses showed that selective loss to follow-up was not a likely explanation for the absence of an association in the participants. Although a relation between homocysteine and reduced cognitive function is biologically plausible, this study suggests no such association in a community-based sample of the elderly. *Am J Epidemiol* 1999;150:283–9.

atherosclerosis; cognition; dementia; homocysteine; mortality; prospective studies

The prevalence and incidence of dementia increases dramatically with age. Cognitive decline is one of the major symptoms of dementia. As the proportion of older people in our society increases, we can expect a rise in the number of people with decreased cognitive function. Therefore, it is important to search for modifiable risk factors. The amino acid homocysteine may be such a risk factor (1). Several cross-sectional and longitudinal studies have shown that homocysteine is associated with an increased risk of stroke (2–5), coronary heart disease (2, 6, 7), and carotid artery atherosclerosis (2, 8, 9). Both cardiovascular disease and carotid atherosclerosis have been related to cognitive impairment and dementia (10–12). Additionally, homocysteine could be associated with cognitive function through other mechanisms, such as thrombosis (13) or a direct neurotoxic effect (14). Furthermore, elevated total homocysteine levels may be regarded as an indicator of vitamin B12 and folate deficiency, which have been related to cognitive impairment and dementia in a number of studies (15–17). Homocysteine is a potentially modifiable risk factor, as several intervention studies have shown a decrease in homocysteine concentration after supplementation with folate (18).

Previously, a small number of studies found an association between homocysteine and cognitive function (19–22). However, these studies were all cross-sectional, most were not population based (19–21), and results were based on small numbers (19, 20). We investigated the association between nonfasting total homocysteine levels and cognitive function in a Dutch study that was prospective, community based, and rel-

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Abbreviations: CI, confidence interval; MMSE, Mini-Mental State Examination; OR, odds ratio; SD, standard deviation.

[1] Department of Epidemiology and Biostatistics, Erasmus University Medical School, Rotterdam, the Netherlands.
[2] Department of Chronic Diseases and Environmental Epidemiology, National Institute of Public Health and the Environment, Bilthoven, the Netherlands.
[3] Netherlands Institute for Health Sciences, Rotterdam, the Netherlands.
[4] Department of Clinical Chemistry, University Hospital Rotterdam, Rotterdam, the Netherlands.
[5] Julius Center for Patient Oriented Research, Utrecht University, Utrecht, the Netherlands.
Reprint requests to Dr. Sandra Kalmijn, Department of Epidemiology and Biostatistics, Erasmus University Rotterdam, P. O. Box 1738, 3000 DR Rotterdam, the Netherlands.

atively large. In addition, several confounding and mediating factors were taken into account.

MATERIALS AND METHODS

Study population

Data are from the Rotterdam Study, which is a single-center, prospective, population-based study designed to investigate determinants of selected chronic diseases and disabilities in older persons (23). The design of the study was approved by the Medical Ethics Committee of Erasmus University, Rotterdam, the Netherlands, and written informed consent was obtained from all participants. All residents of a suburb of Rotterdam who were aged 55 years or more were invited to participate. The baseline examination (between 1990 and 1993) included 7,983 subjects (a response rate of 78 percent). During a home visit, trained interviewers administered a questionnaire covering, among other areas, sociodemographic background, medical history, and medication use. This was followed by two visits to the research center, where subjects underwent clinical examinations, including neuropsychologic testing. Subjects living in homes for the elderly were visited at their residence. The follow-up examination took place at the research center in 1993 and 1994. Of the 7,215 subjects who were still alive, 6,315 (88 percent) participated in the follow-up examination.

We randomly selected 630 subjects from all those who were given a Mini-Mental State Examination (MMSE) at baseline and at follow-up (n = 5,535) for determination of serum total homocysteine. The sample did not differ in age, sex, or education from the population from which they were selected. To investigate the association between homocysteine and different outcomes, we performed a number of nested case-control studies. For this study, we selected a random sample of cases, defined as subjects with a decline in the MMSE score of more than 1 point/year (n = 72). This resulted in a total sample size of 702 subjects, including 110 subjects with cognitive decline.

Previously, it was shown with these data that the cross-sectional association between high homocysteine levels and cardiovascular disease was confined to subjects under age 75 years (2). The lack of an association in participants aged 75 years or more may have been the result of selection; those susceptible to the adverse effects of elevated homocysteine levels may have died before age 75, leaving only the elderly, who, in our study, were less susceptible to elevated homocysteine levels. To assess whether our results on cognitive function could be affected by selective mortality and nonresponse, we randomly selected blood samples from subjects with a baseline MMSE score who had died during follow-up (n = 56) or who for other reasons did not participate in the follow-up examination (n = 61). The sampling probabilities of the three samples, i.e., the random sample, the additional sample of subjects with cognitive decline, and the sample of nonparticipants, were similar.

Homocysteine determination

Nonfasting serum samples were obtained at baseline. They were put on ice immediately and were processed within 60 minutes, which has been shown to be sufficient to prevent increases in total homocysteine concentration due to ex vivo generation (24). Serum was kept frozen at –20°C until determination of total homocysteine in 1995 and 1996. Average storage duration was 4.1 (standard deviation (SD), 0.5) years for subjects without cognitive decline and 4.3 (SD, 0.8) years for those with cognitive decline (p = 0.04). Total homocysteine was determined at the clinical chemistry laboratory of the University Hospital Rotterdam, the Netherlands, as a fluorescence derivative, using high-pressure liquid chromatography according to Araki and Sako (25), as modified by Ubbink et al. (26). A number of quality control samples were incorporated into runs. The estimation of the total homocysteine concentration of these samples had to be within two standard deviations of the level of the control serum. The within-run coefficient of variation ranged from 2.3 to 4.0 percent, and the day-to-day coefficient of variation ranged from 3.2 to 4.0 percent for elevated and normal total homocysteine concentrations, respectively.

MMSE

Global cognitive function was tested with the Dutch language version of the 30-point MMSE administered during the visit to the research center both at baseline and at follow-up (27). The test was administered by specially trained research assistants. The MMSE includes questions on orientation to time and place, registration, attention and calculation, recall, language, and visual construction. This screening test was originally created for a clinical setting (27) and is used extensively in epidemiologic studies (28). If fewer than four individual items (out of 20) were not answered by the subject, these were rated as errors (29). If a subject did not answer four or more individual items, the total MMSE score was considered missing. A score of less than 26 points on the MMSE at baseline indicated cognitive impairment (30). Cognitive decline was defined as a drop in the MMSE score from baseline to follow-up of more than 1

point/year (approximately >1 SD). Mean follow-up duration between the first and second MMSEs was 2.7 (SD, 0.5) years.

Other measurements

The following variables were considered as possible confounders: age (continuous); sex; cigarette smoking (current, former, never); alcohol consumption (none, <1 drink (13.2 g), 1–2 drinks, and ≥3 drinks/day), assessed with a semiquantitative food frequency questionnaire (31); baseline MMSE score (continuous) in the analyses on cognitive decline; level of education, grouped into four levels (completed primary education, lower vocational or general education, intermediate vocational or general education, and higher vocational training, college, or university (32)); and hypertension, which was defined in accordance with the World Health Organization (33) as a systolic blood pressure of 160 mmHg or more, a diastolic blood pressure of 95 mmHg or more, or the use of antihypertensive medication.

Given previous studies (8, 9, 11), atherosclerosis was considered as a possible intermediate of the association between total homocysteine and cognitive decline. Three indicators of atherosclerosis measured at baseline were used: thickening of the carotid artery intima-media wall (≥0.9 mm) (34), presence of plaques in the carotid arteries (35), and presence of peripheral arterial disease (ankle-brachial index <0.90) (34). We also examined whether stroke mediated the association between total homocysteine and cognitive decline. A history of stroke was considered present if a self-reported event was confirmed by a detailed history, neuroimaging, or discharge reports collected from the general practitioner or neurologist (36).

Statistical analysis

Differences in baseline characteristics between the random sample, the additional sample of persons with cognitive decline, and the sample of those who did not participate in the follow-up examination were tested with analysis of covariance after adjustment for age. We compared baseline characteristics according to tertiles of homocysteine level and presence of cognitive decline among subjects from the random sample plus the additional cognitive decline sample. Differences were tested with the nonparametric Kruskall-Wallis test for continuous variables and the chi-square test for categorical variables. We used multiple logistic regression to estimate odds ratios and 95 percent confidence intervals for the association between total homocysteine and concurrent cognitive impairment and subsequent cognitive decline, adjusting for confounding variables. The cross-sectional analyses on cognitive impairment were performed on the random sample ($n = 630$). For the longitudinal analyses on cognitive decline, the random sample of those with cognitive decline was added to the random sample ($n = 702$). Homocysteine levels were categorized into tertiles, with the lowest tertile as the reference category. Multiple linear regression analyses with yearly cognitive decline as a continuous outcome were also performed.

We investigated whether atherosclerosis or stroke was an intermediate in the association between total homocysteine and cognitive decline by including these variables separately as covariates in the model. The analysis on atherosclerosis included 515 subjects because of missing data on the atherosclerosis indicators. We tested for interaction between total homocysteine and sex, age, or hypertension by putting their product terms in the model (2, 4, 8).

To investigate selective loss to follow-up, we performed a sensitivity analysis in which we included the sample of those who were lost to follow-up ($n = 56$ who had died and 61 nonparticipants) in our analysis. The following two extreme options were examined: first, we assumed that those lost to follow-up would all have shown cognitive decline, and second, we assumed that none of them would have shown cognitive decline. All tests were two-sided, and a p value of less than 0.05 was considered to be statistically significant. Data analyses were performed using BMDP statistical software (BMDP Statistical Software, Inc., Los Angeles, California).

RESULTS

In table 1, the random sample, the additional sample of subjects with cognitive decline, and the sample of persons who did not participate at follow-up are compared. At baseline, the mean age of the participants from the random sample was 67.7 (SD, 7.1) years. Sixty percent of the sample was female. The mean age-adjusted baseline MMSE score was 28.1 (SD, 0.07), and the mean age-adjusted total homocysteine concentration was 15.6 (standard error, 0.35) μmol/liter. Age-adjusted baseline MMSE score and homocysteine concentration were not different between the cognitive decline sample and the random sample. Those with cognitive decline consumed alcohol less often than did subjects from the random sample. Subjects who did not participate at the follow-up examination were older and had a lower age-adjusted MMSE score than did those from the random sample. They also had a higher age-adjusted total homocysteine concentration, although this was not significant. Furthermore, they smoked more often, consumed alcohol less often, and had a higher prevalence of peripheral arterial disease than did persons from the random sample.

TABLE 1. Age-adjusted baseline characteristics among a random sample, a sample of those with cognitive decline, and a sample of nonparticipants, the Rotterdam Study, 1990–1993

	Participants at follow-up		Nonparticipants at follow-up (n = 117)
	Random sample† (n = 630)	Cognitive decline sample† (n = 72)	
Age (years) (mean (SD‡))	67.7 (7.1)	69.9 (9.4)	78.1 (9.6)*
Total homocysteine concentration (μmol/liter) (mean (SE‡))	15.6 (0.35)	14.7 (0.95)	17.2 (0.85)
MMSE score (mean (SD))	28.1 (0.07)	28.1 (0.20)	25.4 (0.25)
Sex (% female)	60	64	65
Primary education only (%)	35.2	41.8	42.5
Current smokers (%)	21.9	21.3	30.9**
Alcohol consumers (%)	71.8	56.7**	35.3*
Alcohol consumption (g/day) (mean (SD))§	14.7 (0.82)	14.3 (2.72)	19.9 (3.11)
Intima-media thickness ≥0.9 mm (%)¶	14.4	9.0	21.8
Plaques in carotid arteries (%)¶	48.3	62.1	46.9
Peripheral arterial disease (%)¶	8.5	12.8	39.0*
History of stroke (%)	3.5	9.9	6.0

* $p < 0.001$, for the difference with the random sample.
** $p < 0.05$.
† The random sample consisted of persons who performed a Mini-Mental State Examination (MMSE) at baseline and at follow-up and included 38 subjects with cognitive decline (drop of greater than 1 point/year in the MMSE). The cognitive decline sample consisted of a random sample of all subjects who showed cognitive decline.
‡ SD, standard deviation; SE, standard error.
§ Among consumers of alcohol.
¶ Based on 547 subjects because of missing values.

Comparison of baseline characteristics according to homocysteine tertiles showed that the average age increased from 65.0 (SD, 6.3) years in the lowest homocysteine tertile to 70.3 (SD, 6.9) years in the highest homocysteine tertile ($p < 0.001$). The percentage of women decreased from 68.8 to 51.6 percent with rising total homocysteine levels ($p < 0.001$). Thickening of the carotid artery intima-media wall and a history of stroke were more frequent in subjects with higher homocysteine levels. The yearly drop in the MMSE score was 0.23 (SD, 0.69) among participants in the lowest homocysteine tertile and 0.30 (SD, 0.87) among those in the highest tertile ($p = 0.32$). Subjects with a drop in the MMSE score of more than 1 point/year (n = 110, 15.7 percent) were older (70.1 years; SD, 8.9) than were those without such a drop (67.5 years; SD, 7.0; $p = 0.01$). Participants with cognitive decline more often had a primary education as the highest attained level than did those without cognitive decline (45.5 vs. 33.3 percent, $p = 0.03$). In addition, they had lower alcohol consumption, and peripheral arterial disease and a history of stroke were more frequent (results not shown).

There was a nonsignificant positive crude association between homocysteine and concurrent cognitive impairment (OR for highest vs. lowest tertile = 1.70, 95 percent CI: 0.70, 4.15) (table 2). After adjustment for age, sex, and education, this OR decreased to 1.30 (95 percent CI: 0.50, 3.38). The crude association between high total homocysteine levels and cognitive

TABLE 2. Odds ratios and 95% confidence intervals for cognitive impairment (cross-sectional) and cognitive decline (longitudinal) according to tertiles of total homocysteine level, the Rotterdam Study, 1993

Total homocysteine tertiles (μmol/liter)	No.	% with cognitive decline	Risk of cognitive impairment* (n = 36/630)				Risk of cognitive decline† (n = 110/702)			
			Crude OR‡	95% CI‡	Adjusted OR§	95% CI	Crude OR	95% CI	Adjusted OR§	95% CI
<12.9	231	13.9	Reference		Reference		Reference		Reference	
12.9–15.7	229	17.0	1.89	0.78, 4.62	1.63	0.65, 4.11	1.28	0.77, 2.12	1.14	0.67, 1.93
≥15.8	242	16.1	1.70	0.70, 4.15	1.30	0.50, 3.38	1.19	0.72, 1.98	0.91	0.52, 1.58

* Defined as a Mini-Mental State Examination (MMSE) score of <26 at baseline.
† Defined as a drop in the MMSE score of >1 point/year.
‡ OR, odds ratio; CI, confidence interval.
§ Adjusted for age, sex, education, and, in the analyses on cognitive decline, baseline MMSE score.

decline also was not significant. After adjustment for age, sex, education, and baseline MMSE score and compared with the lowest tertile, the OR was 1.14 (95 percent CI: 0.67, 1.93) for the middle tertile and 0.91 (95 percent CI: 0.52, 1.58) for the highest tertile. Additional adjustment for alcohol consumption, smoking, or hypertension did not alter these estimates. The results were the same without adjustment for baseline MMSE score. There was no interaction between age and homocysteine (p = 0.41), sex and homocysteine (p = 0.56), or hypertension and homocysteine (p = 0.90). When the indicators of atherosclerosis were included in the model, the odds ratio for cognitive decline did not change at all (n = 515; OR = 1.4, 95 percent CI: 0.7, 2.8). The odds ratio was also not altered after adjustment for a history of stroke. Entering homocysteine in the model as a continuous variable yielded an odds ratio per unit increase in the homocysteine level of 1.00 (95 percent CI: 0.97, 1.04) for cognitive impairment and of 0.99 (95 percent CI: 0.95, 1.03) for cognitive decline. Multiple linear regression analyses with yearly cognitive decline as a continuous outcome (negative values indicate a drop in the MMSE score) did not show a significant association either (middle vs. lowest tertile: beta = –0.04, 95 percent CI: –0.19, 0.11; highest vs. lowest tertile: beta = 0.02, 95 percent CI: –0.14, 0.17).

Sensitivity analyses

If we put subjects who did not participate at the follow-up examination (n = 117) in the group with no cognitive decline, the adjusted odds ratio for the highest compared with the lowest tertile was 0.89 (95 percent CI: 0.52, 1.54) (table 3). If those who were lost to follow-up had all shown a cognitive decline of more than 1 point/year, the crude OR would be 2.12 (95 percent CI: 1.43, 3.14). However, after adjustment for age and other major confounders, this OR decreased to 1.01 (95 percent CI: 0.65, 1.59).

DISCUSSION

High homocysteine levels have been associated with an increased risk of stroke and other cardiovascular events (37), which, in turn, have been related to decreased cognitive function and dementia (10–12). Therefore, we hypothesized that a high homocysteine level was associated with cognitive function. However, in this prospective, community-based study of elderly subjects, there was no significant association of elevated total homocysteine levels with concurrent cognitive impairment or subsequent cognitive decline.

This study has a number of strong points. We took a random sample of subjects who participated at baseline and at follow-up in a population-based study. Furthermore, in the longitudinal analyses, total homocysteine levels, which were measured at baseline, were less likely to be influenced by the outcome, cognitive decline. Finally, we were able to investigate whether selective loss to follow-up explained our findings. If subjects who were lost to follow-up had all shown cognitive decline, the results would have been similar. This makes selection bias a less probable explanation for our findings.

Several methodological considerations arise when we try to explain our negative findings. We used only one global measure of cognitive function, the MMSE. However, the MMSE is a valid and reliable test (38) and has been used extensively in epidemiologic studies (28). The mainly cortical functions that it measures are important to daily functioning and are severely affected in dementia. The MMSE was not developed to estimate change in cognitive function, however. One study examined the reliability of change in the MMSE among patients with dementia. It found that for a time interval between the MMSEs of 1 year or more, the reliability was around 0.74, which is reasonable (39). To our knowledge, no such studies have been performed in a general population. We defined cognitive decline as a drop in the MMSE of more than 1 point/year, which is,

TABLE 3. Sensitivity analyses—adjusted odds ratios for cognitive decline according to tertiles of total homocysteine level, the Rotterdam Study, 1993–1994

Total homocysteine tertiles (μmol/liter)	*Subjects lost to follow-up* placed in group with*							
	No cognitive decline				Cognitive decline†			
	No.	% with cognitive decline	Adjusted OR‡,§	95% CI‡	No.	% with cognitive decline	Adjusted OR§	95% CI
<12.9	248	12.9	Reference		248	19.8	Reference	
12.9–15.8	262	14.9	1.14	0.68, 1.93	262	27.5	1.05	0.67, 1.65
>15.8	309	12.6	0.89	0.52, 1.54	309	34.3	1.01	0.65, 1.59

* Due to death or nonresponse (n = 117).
† Defined as a drop in the Mini-Mental State Examination (MMSE) score of >1 point/year.
‡ OR, odds ratio; CI, confidence interval.
§ Adjusted for age, sex, education, and baseline MMSE score.

on average, more than 2.7 points for the total follow-up period. This change would be significant at the 5 percent level (one-tailed) for an individual, based on the standard error of measurement (1.96 × standard error of the mean = 2.6) (40). In addition, with our definition of cognitive decline, we were able to find an association with known risk factors, such as age, education, and stroke. Still, random misclassification may have diluted our results for cognitive decline.

Furthermore, the results for cognitive decline may have been influenced by regression to the mean and a ceiling effect of the MMSE. If subjects with high homocysteine levels had low baseline MMSE scores, then regression to the mean of these subjects would have attenuated an association between high homocysteine levels and cognitive decline. Especially when subjects with high homocysteine levels scored high on the MMSE, a ceiling effect of the MMSE would have attenuated an association between high homocysteine and cognitive decline. However, there was no association between homocysteine level and baseline MMSE score.

Total homocysteine was measured in nonfasting serum that had been stored at –20°C for approximately 4 years. Serum total homocysteine is stable for at least 10 years when stored at –20°C (41). Still, differences in food intake before blood was obtained and reduction of total homocysteine levels over time stored can lead to misclassification (24, 41). If subjects with cognitive impairment or decline had eaten less before blood obtainment than did those without decline, the homocysteine levels of the former group may have been lower, resulting in a smaller difference in homocysteine level between those with and those without reduced cognitive function (41). The duration of storage was, on average, 0.2 years longer in subjects with cognitive decline compared with those without. Although the same technique and protocol were used for the estimation of total homocysteine during the entire study period and the day-to-day variation of the estimation was small, the slightly longer duration of storage might have led to somewhat lower total homocysteine levels in subjects with cognitive decline. This would make it more difficult to find a relation if one existed.

Homocysteine levels were relatively high in this population (37). The explanation for this may be that participants were older than those from most studies. In addition, homocysteine was measured in nonfasting serum. Finally, the use of vitamin supplementation and food fortification is less frequent in the Netherlands than in the United States, perhaps leading to a higher frequency of (subclinical) vitamin B12 and folate deficiencies and, thus, of hyperhomocysteinemia. Another study among elderly in the Netherlands showed similar high homocysteine levels (42). Although the variation in the homocysteine level was not smaller or larger than in other studies, the upward shift of the homocysteine distribution may not leave enough people with low homocysteine levels, making it difficult to find an association.

Finally, the follow-up time of this study was 2.7 years, which might have been too short to detect an effect of homocysteine on cognitive decline. We hypothesized that homocysteine would be related to cognitive decline through its effect on cerebral blood vessels. This is a chronic process, with cognitive decline as a later consequence than cerebral atherosclerosis.

Few studies have investigated the association between homocysteine and cognitive function. A study among 70 male participants from the Normative Aging Study has investigated the cross-sectional association between homocysteine and performance on 18 different cognitive tests (19). They found that homocysteine was strongly related to spatial coping performance after adjustment for major confounders and multiple testing, but not to any of the other cognitive domains. A study among 27 depressed elderly inpatients showed a correlation between homocysteine levels and poor cognition, but only in those without cardiovascular disease (20). Another study compared 510 psychogeriatric patients, including 295 patients with dementia, with 163 controls, who were slightly but not significantly younger (21). Both demented and nondemented psychogeriatric patients had higher homocysteine levels than did the controls. These latter two studies did not adjust for age or other possible confounders, however. Finally, a case-control study among individuals aged 85 years or more observed an association between a high homocysteine concentration and a low MMSE score (22). All of these studies were cross-sectional, making it difficult to ascertain the direction of the association. In addition, three of four were not population based (19–21), thereby reducing the generalizability of the results.

In summary, although an association between homocysteine and cognitive function is biologically plausible, total homocysteine did not appear to be a risk factor for cognitive impairment and decline in this general population of elderly. Several methodological explanations for the absence of an association were offered, but the possibility that homocysteine is truly not related to cognitive function can not be discarded.

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