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

The Rotterdam Study

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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.

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. The decline in the MMSE score was considered statistically significant if p < 0.05. Mean age at baseline was 75.9 years for those with cognitive decline and 75.0 years for those without 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.
adjusting for confounding variables. The cross-sectional

cognitive impairment and subsequent cognitive decline,

association between total homocysteine and concurrent

odds ratios and 95 percent confidence intervals for the

analytical cognitive decline sample. Differences were
tested among subjects from the random sample plus the addi-
tional sample of persons with cognitive decline. Subjects who did not participate at the follow-up examination were tested with analysis of covariance after adjustment for age. We
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ous variables and the chi-square test for categorical vari-
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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 ques-

tionnaire (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 voca-
tional training, college, or university (32)); and hyperten-
sive medication.

Given previous studies (8, 9, 11), atherosclerosis was considered as a possible intermediate of the associa-
tion 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
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product terms in the model (2, 4, 8).

The following two extreme options were examined:

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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 com-
pared. 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 concentra-
tion was 15.6 (standard error, 0.35) μmol/liter. Age-
adjusted baseline MMSE score and homocysteine concentra-
tion 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

<table>
<thead>
<tr>
<th>Participants at follow-up</th>
<th>Random sample† (n = 630)</th>
<th>Cognitive decline sample† (n = 72)</th>
<th>Nonparticipants at follow-up (n = 117)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) (mean (SD†))</td>
<td>67.7 (7.1)</td>
<td>69.9 (9.4)</td>
<td>78.1 (9.6)*</td>
</tr>
<tr>
<td>Total homocysteine concentration (µmol/liter) (mean (SE†))</td>
<td>16.8 (0.35)</td>
<td>14.7 (0.95)</td>
<td>17.2 (0.65)</td>
</tr>
<tr>
<td>MMSE score (mean (SD))</td>
<td>28.1 (0.07)</td>
<td>28.1 (0.20)</td>
<td>25.4 (0.25)</td>
</tr>
<tr>
<td>Sex (% female)</td>
<td>60</td>
<td>64</td>
<td>65</td>
</tr>
<tr>
<td>Primary education only (%)</td>
<td>35.2</td>
<td>41.8</td>
<td>42.5</td>
</tr>
<tr>
<td>Current smokers (%)</td>
<td>21.9</td>
<td>21.3</td>
<td>30.9**</td>
</tr>
<tr>
<td>Alcohol consumers (%)</td>
<td>71.6</td>
<td>56.7*</td>
<td>35.3*</td>
</tr>
<tr>
<td>Alcohol consumption (g/day) (mean (SD))§</td>
<td>14.7 (0.62)</td>
<td>14.3 (2.72)</td>
<td>19.9 (3.11)</td>
</tr>
<tr>
<td>Intima-media thickness £0.9 mm (%)¶</td>
<td>14.4</td>
<td>9.0</td>
<td>21.8</td>
</tr>
<tr>
<td>Plaques in carotid arteries (%)¶</td>
<td>48.3</td>
<td>62.1</td>
<td>46.9</td>
</tr>
<tr>
<td>Peripheral arterial disease (%)¶</td>
<td>8.5</td>
<td>12.8</td>
<td>39.0*</td>
</tr>
<tr>
<td>History of stroke (%)</td>
<td>3.5</td>
<td>9.9</td>
<td>6.0</td>
</tr>
</tbody>
</table>

* 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 impairment was stronger than the association with concurrent cognitive impairment (OR for highest vs. lowest tertile = 1.89, 95 percent CI: 0.78, 4.62) (table 2). After adjustment for age, sex, and education, this OR decreased to 1.28 (95 percent CI: 0.72, 2.12) (table 2).

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

<table>
<thead>
<tr>
<th>Total homocysteine tertiles (µmol/liter)</th>
<th>Crude OR†</th>
<th>95% CI†</th>
<th>Adjusted OR†</th>
<th>95% CI†</th>
<th>Crude OR†</th>
<th>95% CI†</th>
<th>Adjusted OR†</th>
<th>95% CI†</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;12.9</td>
<td>231</td>
<td>13.9</td>
<td>Reference</td>
<td>Reference</td>
<td>1.89</td>
<td>0.76, 4.62</td>
<td>1.63</td>
<td>0.65, 4.11</td>
</tr>
<tr>
<td>12.9-15.7</td>
<td>229</td>
<td>17.0</td>
<td>1.70</td>
<td>0.70, 4.15</td>
<td>1.70</td>
<td>0.65, 4.11</td>
<td>1.50</td>
<td>0.50, 4.38</td>
</tr>
<tr>
<td>≥15.8</td>
<td>242</td>
<td>16.1</td>
<td>1.70</td>
<td>0.70, 4.15</td>
<td>1.70</td>
<td>0.50, 4.38</td>
<td>1.60</td>
<td>0.50, 4.28</td>
</tr>
</tbody>
</table>

* 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: 0.52, 1.54) (table 3). If those who were lost to follow-up had all shown a cognitive decline, the adjusted odds ratio for the highest 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, the results would have been similar. This makes selection bias a less probable explanation for 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,

**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.

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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 x 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 −20ºC for approximately 4 years. Serum total homocysteine is stable for at least 10 years when stored at −20ºC (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 collection 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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