Association of Plasma Total Homocysteine Levels With Subclinical Brain Injury

Cerebral Volumes, White Matter Hyperintensity, and Silent Brain Infarcts at Volumetric Magnetic Resonance Imaging in the Framingham Offspring Study

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Background: Elevated plasma total homocysteine (tHcy) levels have been associated with increased risk of dementia and stroke, but it is uncertain whether the mediating mechanisms are predominantly cellular, vascular, or both.

Objective: To evaluate the relationship between tHcy levels and findings at brain magnetic resonance imaging (MRI) in a community-based sample.

Design: Our sample comprised 1965 participants in the Framingham Offspring Study (1050 women; mean [SD] age, 62 [9] years) who were free of clinical stroke, dementia, or other neurologic disease affecting brain MRI and for whom at least 1 measurement of plasma tHcy level (1991-2001) and a brain MRI (1999-2002) were available. We used multivariate regression analysis to relate initial (1991-1995) and concurrent (1998-2001) plasma tHcy levels to total cerebral brain volume and lobar volumes. Initial tHcy levels were associated with a higher prevalence of silent brain infarct (relative risk, 1.5; 95% confidence interval, 1.1-2.1; P = .02) and concurrent tHcy levels, with smaller frontal (−0.14%, P = .001) and temporal lobar (−0.10%, P = .04) volumes. Prevalence of extensive white matter hyperintensity did not differ according to initial or concurrent plasma tHcy levels (relative risk, both 1.0; 95% confidence interval, 0.7-1.4 and 0.8-1.4, respectively).

Conclusions: Higher plasma tHcy levels are associated with smaller brain volume and the presence of silent brain infarcts at MRI, even in healthy, middle-aged adults. Thus, both cellular and vascular mechanisms may underlie the association of plasma tHcy level with brain aging, as reflected by the effects on both subclinical and overt disease.

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ELEVATED PLASMA TOTAL HOMOCYSTEINE (tHcy) levels have been associated with an increased risk of clinical stroke, dementia, and Alzheimer disease. The mechanisms underlying the association with clinical dementia are uncertain and may involve both vascular and neuronal pathways. Magnetic resonance imaging (MRI) of the brain provides subclinical markers that may reflect vascular or nonvascular brain injury. Thus, the presence or absence of silent brain infarct (SBI) and extensive white matter hyperintensity (WMH) are considered indicators of subclinical macrovascular and microvascular injury, respectively, whereas total cerebral brain volume (TCBV), hippocampal volume, and lobar volumes are accepted as measures of neuronal loss and other gen-

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of middle-aged adults (younger than those described in previous studies) who did not have stroke or dementia.

**METHODS**

**STUDY PARTICIPANTS**

The Framingham Offspring Study cohort comprises 5124 participants who were enrolled in 1971 and have been evaluated 7 times; the eighth examination is under way. Plasma tHcy level was estimated at the fifth (1991-1995), sixth (1995-1999), and seventh (1998-2001) examinations. At the seventh examination, all participants (n=3359) were invited to undergo brain MRI. As of 2002, a total of 2047 participants had undergone MRI, whereas 1525 had a contraindication to MRI or declined or deferred the test. Participants who underwent MRI were excluded if they were known to have a neurologic illness that could affect MRI measurements, such as clinical stroke (n=29), dementia (n=2), or other relevant neurologic condition (multiple sclerosis, brain tumor, or head injury; n=18).

Our study sample consists of the remaining 1965 participants. Plasma tHcy levels were measured using high-performance liquid chromatography with fluorescence detection. Levels were measured at the fifth (initial or previous tHcy level, n=1663) or the seventh offspring examination (concurrent tHcy level, n=1923) in the 1965 eligible participants (1050 women). We did not relate tHcy levels measured at the sixth examination to brain MRI measurements because folate fortification was mandated halfway through this examination. Therefore, persons who underwent the sixth offspring examination before and after the initiation of folate fortification differed in their mean tHcy levels. At the fifth examination, the time of initial plasma tHcy measurement, mean (SD) participant age was 54 (10) years (age range, 26-81 years). The mean (SD) elapsed time between the fifth examination and MRI was 7.5 (1.0) years (range, 4.5-10.8 years), and between the concurrent plasma tHcy measurement and MRI was 0.6 (0.3) year (range, −2.3 to 3.0 years). The study protocol was approved by the Institutional Review Board of Boston University, Boston, Massachusetts, and informed consent was obtained from all participants.

**BRAIN IMAGING**

Magnetic resonance imaging acquisition and measurement techniques and interrater reliability have been described previously. The images were analyzed by operators (M.Y. and C.D.) blinded to the participant’s age, sex, identity, plasma tHcy levels, and exposure to stroke risk factors. Brain volume was determined by manual outlining of the intracranial vault to determine the total cranial volume and by subsequent mathematical modeling to determine total brain parenchymal volume. We computed TCBV as the ratio of total brain parenchymal volume to total cranial volume; thus, this is a measurement of brain parenchymal volume correcting for differences in head size.

Lobar volumes were computed by rotating the images into standard anatomical space followed by operator-defined outlining of the frontal, temporal, parietal, and occipital lobes using standard anatomical landmarks. The mean of the left and right lobar volumes was expressed as a ratio to total cranial volume. Hippocampal volume was estimated using operator-defined, manually traced boundaries to define the region of interest. Interrater and interrater reliability using this method was good, with coefficient of variation of 0.96. Hippocampal data were available for a subset of the population (n=661).

The volume of abnormal WMH was determined according to previously published methods, and participants were categorized as having extensive WMH if the log-WMH volume was more than 1 SD above the age-adjusted mean in this cohort. The presence or absence of SBIs was determined manually by the operator (M.Y. or C.D.), based on the size (≥ 3 mm), location, and imaging characteristics of the lesion. We chose SBI, WMH, and TCBV as our primary MRI measurements and the lobar volumes as secondary measurements.

**DEFINITIONS OF COVARIATES**

Educational achievement was dichotomized at high school graduation, and alcohol use as none or some drinks per day. Individuals were categorized according to the presence or absence of 1 or more apolipoprotein E (APOE) ε4 alleles. Serum creatinine concentration was estimated using the modified Jaffe method, and fasting plasma cholesterol concentration using standard enzymatic methods. Plasma folate concentration was estimated using a microbial (Lactobacillus casei) assay; cyanocobalamin (vitamin B12) levels were estimated using a radioassay kit (Magic; Ciba-Corning Diagnostics Corp, Medfield, Massachusetts); and pyridoxal-5’-phosphate (vitamin B6) was measured using the tyrosine decarboxylase apoenzyme method. We used log-normalized values of folate and pyridoxal-5’-phosphate in our analyses.

The Framingham Stroke Risk Profile (FSRP) has been previously described and validated for predicting stroke risk. The components include systolic blood pressure recorded as the mean of 2 physician-recorded measurements, use of antihypertensive therapy, diabetes mellitus (defined as a fasting blood glucose concentration >126 mg/dL [to convert to millimoles per liter, multiply by 0.0555], a previous diagnosis of diabetes mellitus, or the use of a hypoglycemic agent or insulin), current smoking status, presence or absence of atrial fibrillation, previous cardiovascular disease (a diagnosis of coronary heart disease, congestive heart failure, or peripheral vascular disease), and left ventricular hypertrophy at electrocardiography (based on a standard 12-lead electrocardiogram obtained at or before the initial examination).

**STATISTICAL ANALYSIS**

We used multivariate linear regression to analyze continuous outcomes and logistic regression to analyze binary outcomes to examine the association between plasma tHcy levels (predictor variable) and various primary and secondary brain MRI measurements (outcome variables). The level of plasma tHcy was categorized using sex- and age-specific quartiles defined within 10-year age groups at each examination. Inasmuch as we had previously shown that a plasma tHcy level in the highest quartile (quartile 4) was associated with increased risk of stroke, dementia, and Alzheimer disease, we decided a priori that our primary analysis would use threshold models to compare the various MRI measurements in participants with plasma tHcy levels in the highest quartile (quartile 4) with the rest of the sample (quartiles 1-3). In addition, we modeled plasma tHcy level as a continuous variable (after log-transformation to normalize the distribution) and also examined the trend across quartiles. All analyses were adjusted for sex, age at MRI examination, time elapsed for each subject between the baseline examination and the date of brain MRI; the TCBV and lobar volume analyses were additionally adjusted for age squared. Because our sample was overwhelmingly white, the analyses were not adjusted for race/ethnicity. This constituted our basic model A. We found no effect modification by sex, and, therefore, all analyses were sex-pooled but sex-adjusted. We conducted age-stratified analyses categorizing participants as younger than 55 years or 55 years or older at the time of MRI, based on our pre-
vicious observations that risk factor relationships to brain MRI measurements may be stronger in the older age group.

Vascular risk factors have been independently associated in the Framingham Study with the examined MRI variables and may lie along the causal pathway; therefore, in secondary analyses, we reexamined the relationships between plasma tHcy levels and MRI measurements after accounting for the FSRP score at the time of plasma tHcy level estimation. We also adjusted for covariates that influence plasma tHcy levels (serum folate, pyridoxal-5'-phosphate, cyanocobalamin, body mass index, and serum creatinine concentration) or have been postulated to influence brain MRI measurements (APOE ε4 genotype, serum cholesterol concentration, alcohol consumption, and educational achievement).

We chose plasma tHcy levels at the fifth offspring examination as our primary predictor variable because we believed that prolonged exposure to vascular risk factors was more likely to be reflected in MRI changes. Furthermore, we have shown that after mandated folic acid fortification of all enriched grain products, which began in 1997, mean plasma tHcy levels have declined in the Framingham cohort; therefore, plasma tHcy levels at the seventh offspring examination might not accurately reflect long-term plasma tHcy levels in individuals.

We then declined in the Framingham cohort; therefore, plasma tHcy levels have to be reflected in MRI changes. Furthermore, we have shown that prolonged exposure to vascular risk factors was more likely to have higher mean systolic blood pressure, to be receiving antihypertensive medication, to have diabetes, to be currently smokers, and to have a history of cardiovascular disease. The mean FSRP score and mean body mass index were higher and mean plasma levels of folic acid, pyridoxal-5’-phosphate, and cyanocobalamin were lower in this group.

Table 1 gives the results of analyses relating various measurements of plasma tHcy, as a continuous variable and examining the trend across quartiles, to the primary MRI variables (SBI, WMH, and TCBV). The results of our primary analyses relating plasma tHcy concentrations (quartile 4 vs quartiles 1-3) with our primary MRI variables using sex-pooled models adjusted for age, sex, and time elapsed between plasma tHcy measurement and brain MRI are given. Table 4 gives results of subgroup analyses relating plasma tHcy to SBI and TCBV among persons aged 55 years or older and the results of secondary analyses adjusting for vascular and other covariates. Table 5 gives the effect of initial, concurrent, and sustained hyperhomocysteinemia on these brain MRI measurements, defining hyperhomocysteinemia as a level in the highest age- and sex-specific quartile.

Table 1. Age and Plasma tHcy Levels in Framingham Offspring Undergoing MRI

<table>
<thead>
<tr>
<th>Age, y</th>
<th>No. of Subjects at Fifth and Seventh Examinations, Respectively</th>
<th>Plasma tHcy Level, Mean (Range), µmol/L b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Finland examination</td>
<td>Seventh Offspring Examination</td>
</tr>
<tr>
<td></td>
<td>Q1 Q2 Q3 Q4</td>
<td>Q1 Q2 Q3 Q4</td>
</tr>
<tr>
<td>&lt; 40</td>
<td>101 and 0</td>
<td>5.9 (4.0-7.6)</td>
</tr>
<tr>
<td>40-49</td>
<td>481 and 219</td>
<td>6.3 (4.0-8.3)</td>
</tr>
<tr>
<td>50-59</td>
<td>543 and 708</td>
<td>6.4 (3.9-8.5)</td>
</tr>
<tr>
<td>60-69</td>
<td>464 and 590</td>
<td>6.8 (4.3-8.3)</td>
</tr>
<tr>
<td>≥ 70</td>
<td>74 and 406</td>
<td>7.4 (5.4-8.9)</td>
</tr>
<tr>
<td>All ages</td>
<td>1683 and 1923</td>
<td>6.5 (3.9-8.9)</td>
</tr>
</tbody>
</table>

Abbreviations: MRI, magnetic resonance imaging; Q, quartile; tHcy, total homocysteine.

a Total homocysteine values in adjacent quartiles overlap because the range of values within each quartile differed in men and women.

b Number of subjects in this age range at MRI who had plasma tHcy levels available at fifth and seventh offspring examinations, respectively.

c Age younger than 50 years at MRI.

RESULTS

Mean plasma tHcy levels for the entire group were 9.8 µmol/L (range, 3.9-97 µmol/L) at the fifth offspring examination and 8.3 µmol/L (range, 3.3-93 µmol/L) at the seventh examination. Mean plasma tHcy levels and the range of values within each age- and sex-specific quartile are given in Table 1. At the fifth and seventh offspring examinations, mean (SD) plasma tHcy was 14.3 (5.9) and 11.8 (5.3) µmol/L, respectively, for participants in the highest age- and sex-adjusted quartile (quartile 4) and 8.4 (1.8) and 7.2 (1.5) µmol/L, respectively, for participants with plasma tHcy levels in the lower 3 quartiles.

The distribution of demographic and vascular risk factors across the quartiles of plasma tHcy levels are given in Table 2. Participants in the highest quartile of plasma tHcy levels were more likely to have higher mean systolic blood pressure, to be receiving antihypertensive medication, to have diabetes, to be currently smokers, and to have a history of cardiovascular disease. The mean FSRP score and mean body mass index were higher and mean plasma levels of folic acid, pyridoxal-5’-phosphate, and cyanocobalamin were lower in this group.
years in age for persons in the overall study sample and was equivalent to 3 years of aging in persons older than 55 years at the time of MRI. The effect of concurrent plasma tHcy level was greater than that of the previous tHcy level ($P < .05$) and was greatest in subjects who had a mean (SD) plasma tHcy level in the highest quartile at both the fifth and seventh offspring examinations ($-0.43 [0.20]; P = .03$ compared with those who had a plasma tHcy level in the first to third quartile at either the fifth or the seventh offspring examination or at both; see also Table 5).

Two hundred eighteen participants had an SBI. An elevated initial plasma tHcy level was associated with an increased risk of SBI, and in models evaluating multiple tHcy thresholds, participants with a plasma tHcy level above the median had a greater prevalence of SBI (Table 3).

### Table 2. Description of Stroke Risk Factors (Prevalence and Levels) and Other Covariates in Subjects Grouped by Quartile of Baseline Plasma tHcy Level

<table>
<thead>
<tr>
<th>Variable</th>
<th>Fifth Offspring Examination</th>
<th>Seventh Offspring Examination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Framingham Stroke Risk Profile variables</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, mean ± SD, y</td>
<td>54 ± 10</td>
<td>55 ± 10</td>
</tr>
<tr>
<td>Systolic blood pressure, mean ± SD, mm Hg</td>
<td>124 ± 18</td>
<td>125 ± 19</td>
</tr>
<tr>
<td>Treatment with antihypertensive medication, %</td>
<td>14.3</td>
<td>19.1</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>5.1</td>
<td>4.6</td>
</tr>
<tr>
<td>Current smoker, %</td>
<td>13.1</td>
<td>25.8</td>
</tr>
<tr>
<td>History of cardiovascular disease, %</td>
<td>5.5</td>
<td>7.5</td>
</tr>
<tr>
<td>Atrial fibrillation, %</td>
<td>1.0</td>
<td>1.2</td>
</tr>
<tr>
<td>ECG-LVH, %</td>
<td>1.7</td>
<td>2.7</td>
</tr>
<tr>
<td>FSRP score, mean ± SD</td>
<td>4.0 ± 4.7</td>
<td>4.5 ± 5.5</td>
</tr>
<tr>
<td>Other covariates</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Educational achievement, high school graduate, %</td>
<td>96.5</td>
<td>96.0</td>
</tr>
<tr>
<td>Folic acid level, mean ± SD, ng/mL</td>
<td>9 ± 6</td>
<td>5 ± 4</td>
</tr>
<tr>
<td>Cyanocobalamin level, mean ± SD, pg/mL</td>
<td>470 ± 241</td>
<td>390 ± 234</td>
</tr>
<tr>
<td>Cyanocobalamin level &lt; 150 pg/mL, %</td>
<td>4.5</td>
<td>6.4</td>
</tr>
<tr>
<td>Pyridoxal-5′-phosphate level, mean ± SD, nmol/mL</td>
<td>80 ± 65</td>
<td>61 ± 48</td>
</tr>
<tr>
<td>Serum creatinine concentration, mean ± SD, mg/dL</td>
<td>10.0 ± 0.2</td>
<td>11.0 ± 0.2</td>
</tr>
<tr>
<td>BMI</td>
<td>27.5</td>
<td>28.5</td>
</tr>
<tr>
<td>Plasma cholesterol concentration, mean ± SD, mg/dL</td>
<td>203 ± 36</td>
<td>207 ± 39</td>
</tr>
<tr>
<td>APOE ε4 genotype having ≥ 1 APOE ε4 allele, %</td>
<td>23.6</td>
<td>20.9</td>
</tr>
<tr>
<td>Any alcohol use, %</td>
<td>72.3</td>
<td>68.7</td>
</tr>
</tbody>
</table>

#### Table 3. Results of Multivariate Regression Analysis of Plasma tHcy Levels at the Fifth and Seventh Offspring Examinations on Primary MRI Variables Using Various Measurements of tHcy

<table>
<thead>
<tr>
<th>Outcome Variable</th>
<th>Observed Value in Entire Group</th>
<th>Per Log Elevation of tHcy</th>
<th>Quartile of Homocysteine, Age-Specific</th>
<th>P Trend Across Quartiles</th>
<th>Change in tHcy Level at Fifth Offspring Examination (n=1663)</th>
<th>Plasma tHcy Measurement at Seventh Offspring Examination (n=1923)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCBV, mean ± SD</td>
<td>77.8 ± 3.2</td>
<td>$\beta = -0.40$ .05</td>
<td>77.9</td>
<td>77.9</td>
<td>77.9</td>
<td>77.9</td>
</tr>
<tr>
<td>Extensive WMH, %</td>
<td>12.2</td>
<td>OR, 1.06 .81</td>
<td>1 Reference</td>
<td>1.23</td>
<td>1.05</td>
<td>0.98</td>
</tr>
<tr>
<td>SBI, %</td>
<td>11.2</td>
<td>OR, 1.72 .03</td>
<td>1 Reference</td>
<td>0.98</td>
<td>1.25</td>
<td>1.60</td>
</tr>
<tr>
<td>TCBV, mean ± SD</td>
<td>77.9 ± 3.2</td>
<td>$\beta = -0.68$ .001</td>
<td>78.0</td>
<td>78.2</td>
<td>78.0</td>
<td>77.6</td>
</tr>
<tr>
<td>Extensive WMH, %</td>
<td>12.4</td>
<td>OR, 0.98 .92</td>
<td>1 Reference</td>
<td>0.73</td>
<td>0.80</td>
<td>0.87</td>
</tr>
<tr>
<td>SBI, %</td>
<td>11.2</td>
<td>OR, 1.42 .16</td>
<td>1 Reference</td>
<td>1.34</td>
<td>1.29</td>
<td>1.51</td>
</tr>
</tbody>
</table>

Abbreviations: APOE, apolipoprotein E; BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); ECG-LVH, left ventricular hypertrophy at electrocardiography; FSRP, Framingham Stroke Risk Profile; Q, quartile; tHcy, total homocysteine.

**SI conversion factors:** To convert cholesterol to millimoles per liter, multiply by 0.0259; creatinine to micromoles per liter, multiply by 88.4; and folic acid to nanomoles per liter, multiply by 2.266.

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was also associated with an increased prevalence of SBI (Table 4). Initial hyperhomocysteinemia had a more powerful effect than concurrent hyperhomocysteinemia (Table 5), which is not surprising because we were examining prevalent rather than incident SBI.

Extensive WMH was noted in 250 participants. However, the prevalence of extensive WMH did not differ across quartiles of initial or concurrent plasma tHcy (odds ratio for quartile 4 vs quartiles 1-3, 1.01; 95% confidence interval, 0.72-1.42; P=.96) (Table 3). We also failed to find any effect in the subgroup aged 55 years or older (data not shown) and in persons with sustained hyperhomocysteinemia (odds ratio, 1.2; 95% confidence interval, 0.75-1.85).

Elevated plasma tHcy level (quartile 4) at the seventh examination, but not at the fifth examination, was associated with smaller mean frontal and temporal lobe volume. However, we failed to find an association between elevated plasma tHcy level at either examination and parietal or occipital lobar volume (Table 6).

**COMMENT**

We found a strong, independent, cross-sectional association between higher plasma tHcy levels and lower MRI total brain volume using volumetric brain MRI in our community-based sample of middle-aged adults. This intriguing observation affirms data from some previous, smaller studies that used semiquantitative MRI techniques and extends their observations to younger participants. We also found an increased risk of subclinical (covert) infarcts in these participants, again in accord with previous studies that found an association between plasma tHcy levels and clinical stroke and SBI. We observed a greater effect in older adults, consistent with our previous observations that the relation between elevated plasma tHcy levels and poorer scores on cognitive testing is stronger in older adults.

We observed that concurrent, but not initial, elevated plasma tHcy levels were related to smaller frontal and temporal brain volume. To our knowledge, this has not been previously reported. Although our results require replication, they are interesting in that these areas are maximally affected by Alzheimer disease. Previous reports have described a cross-sectional association of plasma tHcy levels with smaller hippocampal volume, and we observed a similar trend, although the results failed to reach statistical significance; perhaps because our sample was younger (mean age, 61 [9] years vs 73 [8] years in a previous article) and healthier (persons with clinical stroke were included in the previous report). While hippocampal data were available for only

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**Table 4. Results of Age-Stratified Multivariate Regression Analysis of Plasma tHcy Levels on Primary MRI Measurements Related to tHcy in Overall Analysis**

<table>
<thead>
<tr>
<th>Dependent Variable and Covariates in Model</th>
<th>Entire Group (n=1663)</th>
<th>Age ≥ 55 y at MRI (n=1229)</th>
<th>Entire Group (n=1923)</th>
<th>Age ≥ 55 y at MRI (n=1408)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCBV Model A: age, age squared, sex, interval between examination and MRI</td>
<td>-0.37 ± 0.15</td>
<td>0.01</td>
<td>-0.56 ± 0.18</td>
<td>0.001</td>
</tr>
<tr>
<td>TCBV Model A and FSRP score</td>
<td>-0.34 ± 0.15</td>
<td>0.02</td>
<td>-0.53 ± 0.18</td>
<td>0.003</td>
</tr>
<tr>
<td>TCBV Model A and vitamin levels, BMI, and creatinine concentration</td>
<td>-0.38 ± 0.17</td>
<td>&lt;0.001</td>
<td>-0.59 ± 0.21</td>
<td>0.005</td>
</tr>
<tr>
<td>TCBV Model A and alcohol intake, educational achievement level, plasma cholesterol concentration, and presence of APOE ε4 allele</td>
<td>-0.36 ± 0.15</td>
<td>0.02</td>
<td>-0.54 ± 0.18</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Abbreviations: APOE, apolipoprotein; BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); CI, confidence interval; FSRP, Framingham Stroke Risk Profile; MRI, magnetic resonance imaging; OR, odds ratio; SBI, silent brain infarct; SE, standard error of beta; TCBV, total cerebral brain volume; tHcy, total homocysteine.
a subset of our sample, to our knowledge, our sample size was larger than in any previous reported sample. The association of lobar brain volumes with concurrent rather than initial plasma tHcy levels may reflect the identification of a subgroup of participants resistant to correction of plasma tHcy levels with increased dietary folate.

We did not find an association between plasma tHcy levels and volume of WMH, and this is consistent with the results of some previous studies that described a relation between plasma tHcy levels, or the MTHFR TT genotype, and the risk of SBI or the combined risk of SBI and WMH, but not with WMH alone. The different relations of plasma tHcy levels to SBI vs WMH may reflect differences in the pathophysiology of these 2 conditions. While SBI may be due in part to similar vascular mechanisms as for clinical infarcts, the pathologic correlates of WMH include ependymal and matrix changes, evidence of inflammation, fluid accumulation, and de-myelination, in addition to ischemic changes.

It is also possible that we failed to observe an association between plasma tHcy levels and extensive WMH in our relatively young and healthy population, but such a relationship may exist in older subjects, in those with a greater cardiovascular risk factor burden, and in those with clinical stroke or dementia. Previous epidemiological studies that observed a relationship between plasma tHcy levels and WMH included older populations with a greater prevalence of cardiovascular risk factors.
than was noted in our population\textsuperscript{12,13}, furthermore, 2 of the studies did not exclude participants having clinical stroke.\textsuperscript{7,13} However, we failed to find an association between plasma tHcy levels and extensive WMH, even in a subset of older participants (>70 years at MRI) within our population. Other reasons for the differences between these results and our results may include residual confounding by age in previous studies (none defined age-specific quartiles of plasma tHcy levels), differences in the techniques used to measure WMH,\textsuperscript{12,13} and racial/ethnic differences between the study populations.\textsuperscript{12}

Our results support data from tissue and animal studies that both cellular and vascular pathways, or their combination, mediate the observed association of elevated plasma tHcy levels with brain aging.

The strengths of our study are the inclusion of younger participants than previously studied, the use of volumetric brain MRI techniques, and the availability of both concurrent and previous plasma tHcy levels. Limitations include the predominantly white population and the current and previous plasma tHcy levels. Limitations in-include the availability of only a single MRI, such that we are un-

able to relate initial plasma tHcy levels to changes in brain volume or to incident (rather than prevalent) SIBs. The subset of participants who underwent brain MRI were healthier than the entire group of surviving Framing-
hompspring.\textsuperscript{4} This bias may be inevitable in epidemi-

ological studies, and our enrollment of participants older than 25 years before MRI may have minimized the healthy volunteer bias as compared with other studies in which MRI was performed at enrollment.

In previous studies, we demonstrated an effect of elevated plasma tHcy levels on cognitive function in participants not having dementia or stroke.\textsuperscript{30} Overall, our findings suggest that plasma tHcy levels may have a sus-
tained role in the changes of brain aging and dementia, affecting not only the incidence of clinically overt stroke and dementia, as we have previously demonstrated, but also the prevalence of subclinical brain MRI changes in an apparently healthy population.

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**Announcement**

**Trial Registration Required.** In concert with the International Committee of Medical Journal Editors (ICMJE), Archives of Neurology will require, as a condition of consideration for publication, registration of all trials in a public trials registry (such as http://ClinicalTrials.gov). Trials must be registered at or before the onset of patient enrollment. This policy applies to any clinical trial starting enrollment after July 1, 2005. For trials that began enrollment before this date, registration will be required by September 13, 2005, before considering the trial for publication. The trial registration number should be supplied at the time of submission.

For details about this new policy, and for information on how the ICMJE defines a clinical trial, see the editorial by DeAngelis et al in the January issue of Archives of Dermatology (2005;141:76-77). Also see the Instructions to Authors on our Web site: www.archneurol.com.