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Association of Plasma Homocysteine Levels with Subclinical Brain Injury: Cerebral Volumes, White Matter Hyperintensity and Silent Brain Infarcts on Volumetric MRI in the Framingham Offspring Study

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Abstract

Objective—To evaluate the relation between plasma homocysteine (tHcy) and brain MRI in a community-based sample.

Background—Elevated tHcy levels have been associated with an increased risk of dementia and stroke, but it is uncertain if the mediating mechanisms are predominantly cellular, vascular or both.

Design—Our sample comprised 1965 Framingham Offspring participants (1050 women; age 62±9 yrs) who were free of clinical stroke, dementia, or other neurological disease affecting brain MRI and who had at least one measurement of plasma tHcy (1991-2001) and a brain MRI (1999-2002). We used multivariable regressions to relate initial (1991-95) and concurrent (1998-2001) plasma tHcy concentrations to total cerebral brain volume (TCBV) and lobar volumes as measures of neuronal loss and atrophy; and to the presence or absence of silent brain infarcts (SBI) and extensive white matter hyperintensity (log-WMH ≥1 SD above the age-adjusted mean) as separate measures of vascular injury.

Results—Mean TCBV was 78%. 218 participants had SBI; 250 had extensive WMH. Participants with a plasma tHcy level in the highest age-, sex-specific quartile had a smaller TCBV (-0.37% and -0.48%; p=0.01 and <0.001 respectively), compared to participants with lower levels. Initial tHcy levels were associated with an increased prevalence of SBI (RR: 1.5; 95% CI: 1.1-2.1; p=0.02) and concurrent tHcy levels with smaller frontal and temporal lobar volumes (-0.14% and -0.10%; p=0.001 and 0.04 respectively). Prevalence of extensive WMH did not differ according to initial or concurrent plasma tHcy levels (RR: both 1.0, 95% CIs: 0.7-1.4 and 0.8-1.4, respectively).

Conclusion—Higher plasma tHcy levels are associated with smaller brain volumes and presence of silent infarcts on MRI, even in healthy, middle-aged adults. Thus, both cellular and vascular

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mechanisms may underlie the association of plasma tHcy with brain aging, as reflected by the effects on subclinical as well as overt disease.

Keywords

magnetic resonance imaging; homocysteine; brain volume; silent brain infarcts; white matter hyperintensity; epidemiology

Elevated plasma total homocysteine (tHcy) levels have been associated with an increased risk of clinical stroke,[1] dementia and Alzheimer's disease (AD).[2] The mechanisms underlying the association with clinical dementia are uncertain and may involve both vascular and neuronal pathways. MRI brain imaging provides subclinical markers that may reflect vascular or nonvascular brain injury. Thus, the presence or absence of silent brain infarcts (SBI) and extensive white matter hyperintensity (WMH) are considered indicators of subclinical macro- and microvascular injury respectively, whereas the total cerebral brain volume (TCBV), hippocampal volume (HV) and lobar volumes are accepted as measures of neuronal loss and other generalized brain changes, although such loss could be secondary to vascular changes. These MRI measures have been associated with the risk of clinical stroke (SBI and WMH)[3] and with impaired cognitive function and the risk of AD (TCBV, HV).[4] Prior studies have suggested that an elevated plasma tHcy level may be associated with MRI changes in each of these measures[5-15] but the techniques used to assess WMH and brain volume were, with two exceptions,[7,12] qualitative rather than quantitative. Further several of these studies were hospital-based series of patients with stroke or psychiatric illness.[10,13] In the present investigation, we related both prior and concurrent plasma tHcy levels to these various MRI measures in a large community-based, stroke- and dementia-free cohort of middle-aged adults (younger than those described in previous studies).

METHODS

Study participants

The Framingham Offspring cohort comprises 5124 participants who were enrolled in 1971 and have been evaluated 7 times; the 8th examination is currently underway.[16] Plasma tHcy was estimated at the 5th (1991-95), 6th (1995-99) and 7th (1998-2001) examinations. At the 7th examination, all participants (n=3,539) were invited to undergo brain MRI. As of 2002, n=2,014 participants had completed the MRI, while 1525 had a contraindication to MRI or had declined or deferred the test. Participants with an MRI were excluded if they were known to have a neurological illness that could affect MRI measurements such as a clinical stroke (n=29), dementia (n=2), or other relevant neurological condition (multiple sclerosis, brain tumor or head injury; n= 18). Our study sample consists of the remaining n=1,965 participants.

Plasma tHcy levels were measured, using high performance liquid chromatography with fluorescence detection.[17] Levels were measured at the 5th (initial or prior tHcy level, n=1663) and/or the 7th Offspring examinations (concurrent tHcy level, n=1923) in all 1965 eligible participants (1050 women).[18] We did not relate tHcy levels measured at the 6th examination to brain MRI measures because folate fortification has been mandated since half-way through this examination. Hence, persons who underwent the 6th Offspring examination before and after the initiation of folate fortification differed in their mean tHcy levels.[19]

Participant age at the 5th examination, the time of initial plasma tHcy measurement was mean (± SD) 54 ± 10 years (age range: 26-81 years). The interval between this examination and MRI was 7.5 years (SD 1.0, range 4.5-10.8 years), while the interval between the concurrent plasma tHcy measurement and MRI was 0.6 years (SD 0.5, range -2.3 – 3.0). The study protocol was

approved by the Institutional Review Board of Boston University and informed consent was obtained from all participants.

Brain Imaging

MRI acquisition and measurement techniques and inter-rater reliability have been described previously.[4,20-22] The images were analyzed by operators blinded to the participant's identity, age, sex, 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 (TCV) and subsequent mathematical modeling to determine total brain parenchymal volume (TCB).[20] We computed the Total Cerebral Brain Volume (TCBV) as the ratio of TCB to TCV; thus this is a measure of brain parenchymal volume correcting for differences in head size.

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

The volume of abnormal white matter hyperintensity (WMH) was determined according to previously published methods[22] 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 silent brain infarcts (SBI) was determined manually by the operator, based on the size (≥3 mms), location and imaging characteristics of the lesion.[23] We chose TCBV, SBI and WMH as our primary MRI measures and the lobar volumes as secondary measures.

Definitions of covariates

Education was dichotomized at high school graduation and alcohol use as 0 or >0. Persons were categorized according to the presence or absence of ≥1 apolipoprotein E ε4 allele. Serum creatinine was estimated using the modified Jaffe method and fasting plasma cholesterol using standard enzymatic methods. Plasma folate was estimated by a microbial (*Lactobacillus casei*) assay; cyanocobalamin (vitamin B_{12}) levels were estimated using a radioassay kit (Magic, Ciba—Corning, Medfield, MA); and pyridoxal-5′-phosphate (vitamin B6) was measured by 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.[24,25] The components include systolic blood pressure (SBP) recorded as the average of two physician-recorded measurements, use of antihypertensive therapy, diabetes mellitus (defined by a fasting blood glucose >126 mg/dl or 7 mmol/L, a previous diagnosis of diabetes mellitus, or the use of a hypoglycemic agent or insulin), current smoking status, presence or absence of prior cardiovascualr disease (a diagnosis of coronary heart disease, congestive heart failure or peripheral vascular disease), atrial fibrillation and ECG-LVH (based on a standard 12-lead EKG obtained at, or prior to, the initial examination).

Statistical analysis

We used multivariable linear (for continuous outcomes) and logistic (for binary outcomes) regression models to examine the association between plasma tHcy levels (the predictor variable) and various primary and secondary brain MRI measures (outcome variables). Plasma tHcy was categorized using sex- and age-specific quartiles, defined within 10-year age groups

at each examination. Since we had previously shown that a plasma tHcy in the highest quartile (Q4) was associated with an increased risk of stroke, dementia and AD, we decided *a priori* that our primary analysis would utilize threshold models to compare the various MRI parameters in participants with plasma tHcy in the top quartile (Q4) with the rest of the sample (Q1-3). However, we additionally modeled plasma tHcy as a continuous variable (after log-transformation to normalize the distribution) and also examined the trend across quartiles. All analyses were adjusted for age (at MRI examination), sex, the time elapsed in each subject between the baseline examination and the date of brain MRI; the TCBV and lobar volume analyses were additionally adjusted for age squared. Since our sample is overwhelmingly Caucasian, the analyses were not adjusted for race. 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 age <55 years or ≥55 years at the time of MRI, based on our prior observations that risk factor relations to brain MRI measures may be stronger in the older age group.[4]

Vascular risk factors have been independently associated in the Framingham study with the examined MRI variables, and may lie along the causal pathway, hence in secondary analyses, we re-examined the relations between plasma tHcy and MRI parameters after accounting for the FSRP score at the time of plasma tHcy estimation. We also adjusted for covariates that influence plasma tHcy levels (serum folate, vitamins B_6 and B_{12}, body mass index [BMI], and serum creatinine) or have been postulated to influence brain MRI measures (education, APOE ε4 genotype, serum cholesterol and alcohol consumption).

We chose plasma tHcy levels at the 5th Offspring examination as our primary predictor variable, since we believed that a prolonged exposure to vascular risk factors was more likely to be reflected in MRI changes. Further we have shown that following mandated folic acid fortification of all enriched grain products which began in 1997, mean plasma tHcy levels have declined in the Framingham cohort; hence plasma tHcy levels at the 7th Offspring examination might not accurately reflect long-term plasma tHcy levels in individuals.[19] We then examined the relations between plasma tHcy levels at the 7th examination and MRI parameters in the same manner to see if the patterns observed with the original analysis were also seen when relating concurrent plasma tHcy levels to MRI parameters. The adjustment for vitamin and serum creatinine levels was not made in these analyses as they were not available at the 7th examination. All analyses were performed using Statistical Analyses System (SAS® Institute, Cary, North Carolina)©

RESULTS

Mean plasma tHcy levels for the entire group were 9.8 μmol/L (range 3.9 to 97) at the 5th Offspring examination and 8.3 μmol/L (range 3.3 to 93) at the 7th examination. Mean plasma tHcy levels and the range of values within each age-, sex-specific quartile are shown in Table 1. Mean plasma tHcy was 14.3±5.9 μmol/L and 11.8±5.3 for participants in the top age-, sex-adjusted quartile (Q4) and was 8.4±1.8 μmol/L and 7.2±1.5 for participants with plasma tHcy in the lower 3 quartiles at the 5th and 7th Offspring examinations respectively.

The distributions of demographic and vascular risk factors across the quartiles of plasma tHcy are summarized in Table 2. Participants in the highest quartile of plasma tHcy were more likely to have a higher mean SBP, to be on antihypertensive medication, to be diabetic, currently smoking and to have a history of prior CVD. The mean FSRP score and mean BMI were higher and mean plasma levels of folate, vitamins B12 and B6 were lower in this group.

Table 3 presents the results of analyses relating various measures of plasma tHcy, as a continuous variable and examining the trend across quartiles, to the primary MRI variables

(TCBV, WMH and SBI). The results of our primary analyses relating the plasma tHcy concentrations (Q4 versus Q1-3) with our primary MRI variables using sex-pooled models adjusted for age, sex and time-interval between plasma tHcy measurement and brain MRI are shown. Table 4 presents results of subgroup analyses relating plasma tHcy to TCBV and SBI among persons age ≥55 years and the results of secondary analyses adjusting for vascular and other covariates. In Table 5 we examine the effect of initial, concurrent and sustained hyperhomocysteinemia on these brain MRI measures defining hyperhomocysteinemia as a level in the highest age- and sex-specific quartile.

Mean TCBV in the 1965 participants was 78%. Elevated plasma tHcy at either the 5th or the 7th Offspring examination was associated with a smaller TCBV and quartile specific analyses showed a threshold effect; participants with a plasma tHcy in the highest quartile (Q4) at each examination had a lower TCBV (Table 3). We observed a stronger association in persons aged ≥55 years (Table 4). This association between elevated plasma tHcy and TCBV persisted after adjusting for multiple covariates, FSRP score *and the presence or absence of SBI*. The decrease in TCBV in participants with an initial plasma tHcy in the highest quartile was equivalent to that associated with an increase of 2 years in age for persons in the overall study sample and was equivalent to 3 years of .aging among persons aged >55 years at the time of MRI. The impact of concurrent plasma tHcy was greater than that of prior tHcy level ($p<0.05$) and the effect was greatest in subjects who had a plasma tHcy in the highest quartile at both the 5th and the 7th Offspring examinations (-0.43±0.20, p= 0.029 compared to all others, also data in Table 5).

Two hundred and eighteen participants had a SBI.. An elevated *initial* plasma tHcy was associated with an increased risk of SBI and in models evaluating multiple tHcy thresholds, participants with a plasma tHcy above the median had a greater prevalence of SBI (Table 3). The association was strongest in persons aged ≥55 years and in this subgroup *concurren*t plasma tHcy was also associated with an increased prevalence of SBI (Table 4). Initial hyperhomocysteinemia had a more powerful effect than concurrent hyperhomocysteinemia (Table 5). ; this is not surprising since we were looking at *prevalent* rather than incident SCI.

Extensive WMH was seen in 250 participants. However, the prevalence of extensive WMH did not differ across quartiles of initial or concurrent plasma tHcy (odds-ratio, OR for Q4 vs. Q1, 2, 3: 1.01, 95% CI 0.72-1.42, p= 0.96) (Table 3). We also failed to find any effect in the subgroup aged ≥55 years (data not presented) and in persons with sustained hyperhomocysteinemia (OR: 1.2, 95% CI:0.75-1.85).

Elevated plasma tHcy (Q4) at the 7th (but not the 5th) examination was associated with a lower mean frontal and temporal lobe volume but we failed to find an association between elevated plasma tHcy at either examination and parietal or occipital lobar volumes (Table 6).

DISCUSSION

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 reaffirms data from some previous, smaller studies that used semi-quantitative MRI techniques[8,11,14] and extends their observations to younger participants. We also found an increased risk of subclinical (or covert) infarcts in these participants, again in accordance with prior studies showing an association between plasma tHcy levels and clinical stroke[1,26-29] as well as SBI. [6] We observed a greater impact in older adults, consistent with our prior observations that the relation between elevation in plasma tHcy and poorer scores on cognitive testing was stronger in older adults.[30]

We observed that concurrent (but not initial) elevated plasma tHcy concentrations were related to a smaller frontal and temporal brain volume. To our knowledge this has not been previously reported. While our results require replication, they are interesting since these areas are maximally affected by Alzheimer pathology. Prior reports have described a cross-sectional association of plasma tHcy levels with smaller hippocampal volumes,[14,31,32] and we observed a similar trend although the result failed to reach statistical significance, perhaps because of our lower mean age (61±9 years in our sample versus 73±8 years in a prior report) [14] and the healthy sample (persons with clinical stroke were not excluded in the prior report). [14] While we had hippocampal data available for only a subset of our sample, our sample size was larger than in any previous report. 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 prior 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.[5,33] The different relations of plasma tHcy levels to SBI versus WMH may reflect differences in the pathophysiology of these two conditions. While SBI may be partly due to similar vascular mechanisms as clinical infarcts, the pathological correlates of WMH include ependymal and matrix changes, evidence of inflammation, fluid accumulation and demyelination in addition to ischemic changes.[34]

It is also possible that we failed to observe an association between plasma tHcy and extensive WMH in our relatively young and healthy population, but that such a relationship may exist in older subjects, in those with a greater cardiovascular risk factor burden, or in subjects with clinical stroke or dementia.[35] Prior epidemiological studies that observed a relationship between plasma tHcy and WMHI[7,12,15] have studied older populations with a greater prevalence of cardiovascular risk factors than was seen in our population;[12,15] further two of the studies did not exclude participants with clinical stroke.[7,15] However, we failed to find an association between plasma tHcy levels and extensive WMH, even in a subset of older participants (age >70 yrs at time of MRI) within our population. Other reasons for the differences between these and our results may include residual confounding by age in previous studies (none of them defined age-specific quartiles of plasma tHcy), differences in the techniques used to measure WMH[12,15] and ethnic differences between the study populations. [12] Our results support data from tissue and animal studies suggesting 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 remote plasma tHcy levels. Limitations include the predominantly Caucasian population and the availability of only a single MRI so that we are unable to relate initial plasma tHcy levels to changes in brain volume or to incident (rather than prevalent) silent infarcts. The subset of participants who underwent brain MRI were healthier than the entire group of surviving Framingham Offspring.[4] This bias may be inevitable in epidemiological studies and in fact our enrollment of participants over 25 years prior to the MRI may have minimized the healthy volunteer bias as compared to other studies that undertook MRI at enrollment.

In prior studies we have also demonstrated an effect of elevated plasma tHcy levels on cognitive function in dementia- and stroke-free persons.[30] Overall our findings suggest that plasma tHcy levels may play a sustained 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.

Reference List

(1). Bostom AG, Rosenberg IH, Silbershatz H, et al. Nonfasting plasma total homocysteine levels and stroke incidence in elderly persons: The Framingham Study. Annals of Internal Medicine 1999;131:352–355. [PubMed: 10475888]

(2). Seshadri S, Beiser A, Selhub J, et al. Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. New England Journal of Medicine 2002;346:476–483. [PubMed: 11844848]

(3). Vermeer SE, den Heijer T, Koudstaal PJ, Oudkerk M, Hofman A, Breteler MMB. Incidence and risk factors of silent brain infarcts in the population-based Rotterdam Scan Study. Stroke 2003;34:392–396. [PubMed: 12574548]

(4). Seshadri S, Wolf PA, Beiser A, et al. Stroke risk profile, brain volume, and cognitive function: the Framingham Offspring Study. Neurology 2004;63:1591–1599. [PubMed: 15534241]

(5). Longstreth WT, Katz R, Olson J, et al. Plasma total homocysteine levels and cranial magnetic resonance imaging findings in elderly persons - The Cardiovascular Health Study. Archives of Neurology 2004;61:67–72. [PubMed: 14732622]

(6). Matsui T, Arai H, Yuzuriha T, et al. Elevated plasma homocysteine levels and risk of silent brain infarction in elderly people. Stroke 2001;32:1116–1119. [PubMed: 11340219]

(7). Sachdev P, Parslow R, Salonikas C, et al. Homocysteine and the brain in midadult life - Evidence for an increased risk of leukoaraiosis in men. Archives of Neurology 2004;61:1369–1376. [PubMed: 15364682]

(8). Sachdev P. Homocysteine, cerebrovascular disease and brain atrophy. J Neurol Sci 2004;226:25–29. [PubMed: 15537514]

(9). Sachdev PS, Valenzuela M, Wang XL, Looi JCL, Brodaty H. Relationship between plasma homocysteine levels and brain atrophy in healthy elderly individuals. Neurology 2002;58:1539–1541. [PubMed: 12034795]

(10). Scott TM, Tucker KL, Bbadelia A, et al. Homocysteine and B vitamins relate to brain volume and white-matter changes in geriatric patients with psychiatric disorders. American Journal of Geriatric Psychiatry 2004;12:631–638. [PubMed: 15545331]

(11). Whalley LJ, Staff RT, Murray AD, et al. Plasma vitamin C, cholesterol and homocysteine are associated with grey matter volume determined by MRI in non-demented old people. Neuroscience Letters 2003;341:173–176. [PubMed: 12697276]

(12). Wright CB, Paik MC, Brown TR, et al. Total homocysteine is associated with white matter hyperintensity volume: the Northern Manhattan Study. Stroke 2005;36:1207–1211. [PubMed: 15879345]

(13). Wong A, Mok V, Fan YH, Lam WW, Liang KS, Wong KS. Hyperhomocysteinemia is associated with volumetric white matter change in patients with small vessel disease. J Neurol 2006;253:441–447. [PubMed: 16267639]

(14). Den HT, Vermeer SE, Clarke R, et al. Homocysteine and brain atrophy on MRI of non-demented elderly. Brain 2003;126:170–175. [PubMed: 12477704]

(15). Vermeer SE, van Dijk EJ, Koudstaal PJ, et al. Homocysteine, silent brain infarcts, and white matter lesions: The Rotterdam Scan Study. Ann Neurol 2002;51:285–289. [PubMed: 11891822]

(16). Feinleib M, Garrison RJ, Stallones L, Kannel WB, Castelli WP, McNamara PM. A comparison of blood pressure, total cholesterol and cigarette smoking in parents in 1950 and their children in 1970. Am J Epidemiol 1979;110:291–303. [PubMed: 474566]

(17). Araki A, Sako Y. Determination of free and total homocysteine in human plasma by high-performance liquid chromatography with fluorescence detection. J Chromatogr 1987;422:43–52. [PubMed: 3437026]

(18). Selhub J, Jacques PF, Wilson PWF, Rush D, Rosenberg IH. Vitamin Status and Intake As Primary Determinants of Homocysteinemia in An Elderly Population. Jama-Journal of the American Medical Association 1993;270:2693–2698.

(19). Jacques PF, Selhub J, Bostom AG, Wilson PWF, Rosenberg IH. The effect of folic acid fortification on plasma folate and total homocysteine concentrations. New England Journal of Medicine 1999;340:1449–1454. [PubMed: 10320382]

(20). DeCarli C, Maisog J, Murphy DG, Teichberg D, Rapoport SI, Horwitz B. Method for quantification of brain, ventricular, and subarachnoid CSF volumes from MR images. J Comput Assist Tomogr 1992;16:274–284. [PubMed: 1545026]

(21). DeCarli C, Massaro J, Harvey D, et al. Measures of brain morphology and infarction in the framingham heart study: establishing what is normal. Neurobiol Aging 2005;26:491–510. [PubMed: 15653178]

(22). Jeerakathil T, Wolf PA, Beiser A, et al. Stroke risk profile predicts white matter hyperintensity volume: the Framingham Study. Stroke 2004;35:1857–1861. [PubMed: 15218158]

(23). DeCarli C, Miller BL, Swan GE, et al. Predictors of brain morphology for the men of the NHLBI twin study. Stroke 1999;30:529–536. [PubMed: 10066847]

(24). D'Agostino RB, Wolf PA, Belanger AJ, Kannel WB. Stroke risk profile: adjustment for antihypertensive medication. The Framingham Study. Stroke 1994;25:40–43. [PubMed: 8266381]

(25). Wolf PA, D'Agostino RB, Belanger AJ, Kannel WB. Probability of stroke: a risk profile from the Framingham Study. Stroke 1991;22:312–318. [PubMed: 2003301]

(26). Verhoef P, Hennekens CH, Malinow MR, Kok FJ, Willett WC, Stampfer MJ. A prospective study of plasma homocyst(e)ine and risk of ischemic stroke. Stroke 1994;25:1924–1930. [PubMed: 8091435]

(27). Sacco RL, Anand K, Lee HS, et al. Homocysteine and the risk of ischemic stroke in a triethnic cohort: the NOrthern MAnhattan Study. Stroke 2004;35:2263–2269. [PubMed: 15345803]

(28). Kittner SJ, Giles WH, Macko RF, et al. Homocyst(e)ine and risk of cerebral infarction in a biracial population : the stroke prevention in young women study. Stroke 1999;30:1554–1560. [PubMed: 10436100]

(29). Eikelboom JW, Hankey GJ, Anand SS, Lofthouse 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;31:1069–1075. [PubMed: 10797167]

(30). Elias MF, Sullivan LM, D'Agostino RB, et al. Homocysteine and cognitive performance in the Framingham offspring study: age is important. Am J Epidemiol 2005;162:644–653. [PubMed: 16107567]

(31). Bleich S, Sperling W, Degner D, et al. Lack of association between hippocampal volume reduction and first-onset alcohol withdrawal seizure. A volumetric MRI study. Alcohol and Alcoholism 2003;38:40–44. [PubMed: 12554606]

(32). Williams JH, Pereira EA, Budge MM, Bradley KM. Minimal hippocampal width relates to plasma homocysteine in community-dwelling older people. Age Ageing 2002;31:440–444. [PubMed: 12446289]

(33). Kohara K, Fujisawa M, Ando F, et al. MTHFR gene polymorphism as a risk factor for silent brain infarcts and white matter lesions in the Japanese general population - The NILS-LSA Study. Stroke 2003;34:1130–1135. [PubMed: 12690212]

(34). Fazekas F, Schmidt R, Scheltens P. Pathophysiologic mechanisms in the development of age-related white matter changes of the brain. Dement Geriatr Cogn Disord 1998;9(Suppl 1):2–5. [PubMed: 9716237]

(35). Hogervorst E, Ribeiro HM, Molyneux A, Budge M, Smith AD. Plasma homocysteine levels, cerebrovascular risk factors, and cerebral white matter changes (leukoaraiosis) in patients with Alzheimer disease. Arch Neurol 2002;59:787–793. [PubMed: 12020261]

Table 1

Age and Plasma Homocysteine Levels in Framingham Offspring Undergoing MRI.

Age at Exam (years)	Plasma tHcy at 5th Offspring Examination (mean and range in µmol/L)				Plasma tHcy at 7th Offspring Examination (mean and range in µmol/L)			
N at exams 5 and 7	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
<40 (n= 101 and 0)	5.9 4.0-7.6	7.6 5.8-8.8	9.5 8.1-11.0	13.6 10.9-26.4				
40-49 (n= 481 and 219)	6.3 4.0-8.3	8.2 6.6-10.0	9.8 8.3-11.4	13.4 9.1-45.0	5.3 3.6-6.7*	6.6 5.7-7.7*	7.7 6.6-9.0*	11.8 7.7-92.6*
50-59 (n= 543 and 708)	6.4 3.9-8.5	8.4 7.0-10.1	10.1 8.3-11.9	14.8 10.6-97.1	5.6 3.3-7.3	7.0 5.8-8.3	8.1 6.8-9.8	11.0 8.1-65.2
60-69 (n= 464 and 590)	6.8 4.3-8.3	8.7 7.4-10.2	10.6 8.9-12.4	14.8 11.1-35.0	5.8 3.3-7.2	7.4 6.2-8.8	8.9 7.6-10.7	11.9 9.2-28.9
70+ (n= 74 and 406)	7.4 5.4-8.9	9.6 8.6-11.2	11.4 10.2-12.5	13.7 11.9-16.8	6.2 4.0-7.7	7.8 6.7-9.2	9.3 8.0-10.9	12.9 10.-37.2
All ages (n= 1663 and 1923)	6.5 3.9-8.9	8.4 5.8-11.2	10.2 8.1-12.5	14.3 9.9-97.1	5.8 3.3-7.7	7.2 5.7-9.2	8.6 6.6-92.6	11.8 7.7-92.6

* age group <50 years at the time of MRI

† tHcy values in adjacent quartiles overlap since the range of values with in each quartile differed in men and women.

≠ Number of subjects at this age range at time of MRI who had plasma tHcy measurements available at 5th and 7th Offspring examinations respectively

Table 2

Description of Stroke Risk Factors (Prevalence and Levels) and Other Covariates in Subjects Grouped By Quartile Of Baseline Plasma Homocysteine Level.

	5th Offspring Examination			7th Offspring Examination		
	Q1-Q3	Q4	P value	Q1-Q3	Q4	P value
Framingham Stroke Risk Profile Variables	**N-1247**	**N=416**		**N=1442**	**N=481**	
Age (in years)*	54 ± 10	55 ± 10	0.598	61 ± 9	61 ± 9	0.705
Systolic Blood Pressure (mmHg)*	124 ± 18	125 ± 19	0.375	125 ± 18	127 ± 20	0.046
Treatment with antihypertensive medication (%)	14.3%	19.1%	0.020	28.0%	37.6%	<0.001
Diabetes (%)	5.1%	4.6%	0.647.	7.7%	11.7%	0.008
Current Smoker (%)	13.1%	25.8%	<0.001	10.8%	15.2%	0.010
History of cardiovascular disease (%)	5.5%	7.5%	0.136	8.0%	12.9%	0.001
Atrial fibrillation (%)	1.0%	1.2%	0.786	2.8%	3.7%	0.322
ECG-LVH (%)	1.7%	2.7%	0.209	1.8%	2.3%	0.435
FSRP Score*	4.0 ± 4.7	4.5 ± 5.5	0.069	6.3 ± 7.3	7.6 ± 8.9	0.005
Other Covariates						
Education: High school graduate (%)	96.5%	96.0%	0.654	96.9%	95.3%	0.106
Folate level (ng/ml)	9 ± 6	5 ± 4	<0.001			
Vitamin B12 level (pg/ml)*	470 ± 241	390 ± 234	<0.001			
Vitamin B12 < 150	4.5%	6.4%	0.151			
Pyridoxal 5′-phosphate level (nmol/ml)*	80 ± 65	61 ± 48	<0.001			
Serum creatinine (mg/dl)*	1.0 ± 0.2	1.1 ± 0.2	0.004			
Body Mass Index (kg/m^2)*	27 ± 5	28 ± 5	0.091	28 ± 5	29 ± 5	0.001
Plasma cholesterol (mg/dl)*	203 ± 36	207 ± 39	0.069	200 ± 36	202 ± 39	0.475
Apolipoprotein genotype: ε4 +ve (%)	23.6%	20.9%	0.256	22.6%	22.0%	0.796
Any Alcohol Use	72.3%	68.7%	0.158	67.0%	66.2%	0.744

*: Mean ± SD

Table 3

Results of Multivariable Regression Analyses of Plasma Homocysteine (tHcy) levels at the 5th and 7th Offspring Examination on Primary MRIV ariables: Total Cerebral Brain Volume (TCBV), Risk Of Extensive White Matter Hyperintensity On Brain MRI (WMH) And Risk Of Silent Cerebral Infarcts (SCI) — analyses using different measures of tHcy

Outcome variable*	Observed value in entire group	per log elevation of tHcy		Quartiles of homocysteine (age-specific)				p (trend across quartiles)	Q4 vs. Q1-3; change and p value
		Change	p	Q1	Q2	Q3	Q4		
Plasma homocysteine (tHcy) measure at 5th Offspring Examination (N=1663)									
TCBV	Mean ± SD: 77.8 ± 3.2	β= -0.40	0.05	77.9	77.9	77.9	77.5	0.08	β= -0.38; p= 0.01
				(adjusted means ± SE)					
Extensive WMHI (%)	12.2%	OR: 1.06	0.81	1.00	1.23	1.05	1.10	0.85	OR: 1.01; p= 0.96
SCI (%)	11.2%	OR: 1.72	0.03	1.00	0.98	1.25	1.60	0.02	OR: 1.49; p= 0.02
Plasma homocysteine (tHcy) measure at 7th Offspring Examination (N =1923)									
TCBV	Mean ± SD: 77.9 ± 3.2	β= -0.68	0.001	78.0	78.2	78.0	77.6	0.004	β= -0.489; p<0.001
				(adjusted means ± SE)					
Extensive WMHI (%)	12.4%	OR: 0.98	0.92	1.00	0.73	0.80	0.87	0.58	OR: 1.04; p=0.82
SCI (%)	11.2%	OR: 1.42	0.16	1.00	1.34	1.29	1.51	0.08	OR; 1.25 ; p=0.17

*All analyses are adjusted for age, gender, and interval between the baseline examination and date of brain MRI; TCBV is addit ionally adjusted for age-squared.

Table 4

Results Of Age-Stratified Multivariable Regression Analyses of Plasma Homocysteine (tHcy) levels on Primary MRI Parameters Related to tHcy in overall analyses : Total Cerebral Brain Volume (TCBV) And Risk Of Silent Cerebral Infarcts (SCI) - Results expressed as Q4 versus Q1-3

	Initial (5th) Offspring Examination		Final (7th) Offspring Examination	
Dependent Variable (and covariates in model)	**Entire Group**	**Age ≥55 years at time of MRI**	**Entire Group**	**Age ≥55 years at time of MRI**
	N=1663	**N=1229**	**N=1923**	**N=1408**
	TCBV expressed as β±SE and p value			
TCBV (Model A: age, age squared, sex, interval between exam and MRI)	-.37 ±.15, p= 0.011	-.56 ±.18, p= 0.001	-.48 ±.14, p<0.001	-.67 ±.16, p<0.001
TCBV (Model A and FSRP score)	-.34 ± .15, p=0.021	-.53 ± .18, p=0.003	-.44 ± .14, p=0.001	-.63 ± .17, p<0.001
TCBV (Model A, FSRP score and presence of SCI)	-.34 ± .15, p=0.023	-.53 ± .18, p=0.003	-.44 ± .14, p=0.001	-.63 ± .17, p<0.001
TCBV (Model A and vitamin levels, body mass index and creatinine)	-.38 ± .17, p= 0.027	-.59 ± .21, p= 0.005		
TCBV (Model A and alcohol intake, education, plasma cholesterol, presence of APOE ε4 allele)	-.36 ± .15, p=0.017	-.54 ± .18, p=0.003	-.46 ± .14, p=0.001	-.63 ± .17, p<0.001
	SCI expressed as OR, 95% CI			
SCI (Model A: age, sex, interval between exam and MRI)	1.49, 1.07-2.08	1.56, 1.08-2.24	1.25, 0.91-1.72	1.44, 1.02-2.03
SCI (Model A and FSRP score)	1.48, 1.06-2.07	1.54, 1.06-2.23	1.22, 0.88-1.69	1.39, 0.98-1.98
SCI (Model A and vitamin levels, body mass index and creatinine)	1.39, 0.93-2.08	1.42, 0.90-2.22		
SCI (Model A and alcohol, education, plasma cholesterol, presence of APOE ε4 allele)	1.61, 1.14-2.25	1.68, 1.16-2.44	1.26, 0.91-1.74	1.43, 1.00-2.04

β=Regression coefficient- unstandardized beta weights adjusted for specified predictor variables; each row represents a separate multivariable model. S.E. refers to the standard error of beta.

Table 5

Results of Multivariable Regression Analyses of Plasma Homocysteine (tHcy) levels at Both Initial and Concurrent Examinations with Total Cerebral Brain Volume Ratio (TCBV), And Risk Of Silent Cerebral Infarcts (SCI) - adjusted for age, gender, and interval between exam 5 and MRI (TCBV analyses additionally adjusted for age squared)

Dependent Variable (and covariates in model)	Entire Group N =1634	Age ≥55 years at time of MRI N =1210
TCBV (β±SE [*†])		
Plasma tHcy Q1-3 at both initial and concurrent examinations (Low-Low)	0	0
Plasma tHcy Q1-3 at initial and Q4 at concurrent examination (Low-High)	-0.57±.19, p=0.003	-0.74±.23, p=0.001
Plasma tHcy Q4 at initial and Q1-3 at concurrent examinations (High-Low)	-0.34±.20, p=0.08	-0.48±.23, p=0.039
Plasma tHcy Q4 at both initial and concurrent examinations (High-high)	-0.56±.20, p=0.005	-0.85±.24, p=<0.001
SCI (OR and 95% CI)		
Plasma tHcy Q1-3 at both initialand concurrent examinations (Low-Low)	1.00	1.00
Plasma tHcy Q1-3 at initial and Q4 at concurrent examination (Low-High)	1.35, 0.86-2.13	1.41, 0.86-2.33
Plasma tHcy Q4 at initial and Q1-3 at concurrent examinations (High-Low)	1.73, 1.13-2.66	1.65, 1.02-2.66
Plasma tHcy Q4 at both initial and concurrent examinations (High-high)	1.36, 0.85-2.18	1.61, 0.97-2.67

[*] Regression coefficient- unstandardized beta weights adjusted for specified predictor variables; each row represents a separate multivariable model.

[†] S.E. refers to the standard error of beta. P values are given for all significant results.

Table 6

Results Of Multivariable Regression Analyses Of Plasma homocysteine (tHcy) levels on Secondary MRI Measures

	5th Offspring Examination			7th Offspring Examination		
Predictor variable*	**Mean ± SD**	**Q4 vs. Q1-3 Change**	**P value**	**Mean ± SD**	**Q4 vs. Q1-3 Change**	**P value**
Frontal Brain Volume	35.6 ± 3.3	-0.07	0.160	35.6 ± 3.3	-0.14	0.001
Occipital Brain Volume	10.4 ± 2.8	-0.02	0.731	10.4 ± 2.7	0.01	0.901
Parietal Brain Volume	21.5 ± 3.5	-0.03	0.631	21.6 ± 3.5	0.02	0.722
Temporal Brain Volume	10.4 ± 0.9	-0.01	0.924	10.4 ± 0.9	-0.10	0.037
Hippocampal Volume (n=661)	0.31 ± 0.04	-0.11	0.192	0.31 ± 0.04	-0.15	0.065

* All analyses are adjusted for age, age squared, gender, and tim e between Offspring examination and MRI; Frontal, occipital, parietal, temporal and hippocampal brain volumes are all expressed in standard deviation units.