Original Investigation

Association of Vitamin B_{12}, Folate, and Sulfur Amino Acids With Brain Magnetic Resonance Imaging Measures in Older Adults

A Longitudinal Population-Based Study

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IMPORTANCE Vitamin B_{12}, folate, and sulfur amino acids may be modifiable risk factors for structural brain changes that precede clinical dementia.

OBJECTIVE To investigate the association of circulating levels of vitamin B_{12}, red blood cell folate, and sulfur amino acids with the rate of total brain volume loss and the change in white matter hyperintensity volume as measured by fluid-attenuated inversion recovery in older adults.

DESIGN, SETTING, AND PARTICIPANTS The magnetic resonance imaging subsample of the Swedish National Study on Aging and Care in Kungsholmen, a population-based longitudinal study in Stockholm, Sweden, was conducted in 501 participants aged 60 years or older who were free of dementia at baseline. A total of 299 participants underwent repeated structural brain magnetic resonance imaging scans from September 17, 2001, to December 17, 2009.

MAIN OUTCOMES AND MEASURES The rate of brain tissue volume loss and the progression of total white matter hyperintensity volume.

RESULTS In the multi-adjusted linear mixed models, among 501 participants (300 women [59.9%]; mean [SD] age, 70.9 [9.1] years), higher baseline vitamin B_{12} and holotranscobalamin levels were associated with a decreased rate of total brain volume loss during the study period: for each increase of 1 SD, β (SE) was 0.048 (0.013) for vitamin B_{12} ($P < .001$) and 0.040 (0.013) for holotranscobalamin ($P = .002$). Increased total homocysteine levels were associated with faster rates of total brain volume loss in the whole sample (β [SE] per 1-SD increase, −0.035 [0.015]; $P = .02$) and with the progression of white matter hyperintensity among participants with systolic blood pressure greater than 140 mm Hg (β [SE] per 1-SD increase, 0.000019 [0.00001]; $P = .047$). No longitudinal associations were found for red blood cell folate and other sulfur amino acids.

CONCLUSIONS AND RELEVANCE This study suggests that both vitamin B_{12} and total homocysteine concentrations may be related to accelerated aging of the brain. Randomized clinical trials are needed to determine the importance of vitamin B_{12} supplementation on slowing brain aging in older adults.

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Vitamin B_{12} and folate are closely connected with the metabolism of homocysteine, a sulfur-containing nonessential amino acid. Inadequate levels of either vitamin can result in increased concentrations of total homocysteine (tHcy).[1] High levels of tHcy and low levels of vitamin B_{12} and folate are common in the elderly and are associated with a variety of disorders, including cardiovascular and cerebrovascular conditions.[2-5] In addition, they may influence brain structure through several mechanisms.[4] In older adults, substantial cerebral atrophy is associated with a diagnosis of dementia, which is maintained in very advanced age.[6,7] Whereas few longitudinal studies have linked low vitamin B_{12} or folate levels with structural brain changes,[8,9] others did not report such associations.[9-11] Higher blood levels of tHcy have also been associated with an increased rate of brain atrophy[10-14] and progression of white matter lesion volume,[15] although the evidence is inconsistent.[9,11]

Holotranscobalamin (holoTC), the biologically active fraction of vitamin B_{12}, may be a more sensitive marker of vitamin B_{12} levels.[16] However, to our knowledge, very few longitudinal studies have investigated the association of holoTC with brain volumes.[9] In addition, the effect of sulfur amino acids other than homocysteine on brain aging has rarely been investigated.[17]

The potential effect of vitamin B_{12}, folate, and sulfur amino acids on structural brain changes is of importance because they are modifiable factors and thus a potential target to be considered in preventive interventions. The aim of our study was to examine the associations of vitamin B_{12}, red blood cell (RBC) folate, and sulfur amino acids with changes over 6 years in brain tissue volumes and total white matter hyperintensity (WMH) volume as measured by fluid-attenuated inversion recovery in a population-based cohort of older adults without mandatory folic acid fortification.

Key Points

Question Are vitamin B_{12}, folate, and sulfur amino acids associated with progressive structural brain changes in older adults?

Findings In this population-based longitudinal study of 501 elderly individuals without dementia, higher blood levels of vitamin B_{12} and holotranscobalamin as well as lower homocysteine values were associated with a decreased rate of total brain tissue volume loss. Furthermore, elevated homocysteine values were associated with progression of white matter hyperintensities among participants with higher systolic blood pressure.

Meanings Vitamin B_{12} and homocysteine blood levels are associated with accelerated brain aging in older adults.

Methods

Study Population

The study population was derived from the Swedish National Study on Aging and Care in Kungsholmen (SNAC-K), a population-based prospective study conducted in the Kungsholmen area of central Stockholm, Sweden. SNAC-K involves a random sample of persons aged 60 years or older who live either at home or in an institution. Because of more rapid changes in health and a higher attrition rate among older age groups, sampling is stratified by age cohort. Assessments take place at 6-year intervals for younger cohorts (ages 60, 66, 72, and 78 years) and at 3-year intervals for older cohorts (ages 81, 84, 87, 90, 93, 96, and ≥99 years). From 2001 to 2004, of the 4590 living and eligible individuals randomly selected for SNAC-K, 3363 (73.3%) participated in the baseline examination.[18,19] The Ethics Committee at Karolinska Institutet and the Regional Ethical Review Board in Stockholm approved the protocols of each phase of SNAC-K and written informed consent was provided by all participants.

At baseline and each follow-up, the SNAC-K participants underwent a thorough clinical examination, interview, and assessments by a physician, a registered nurse, and a psychologist. Data on sociodemographic characteristics, medical history, drug use, and cognitive function were collected according to a structured protocol, and the diagnosis of dementia was made according to *DSM-IV* criteria.[20] Data on vitamin supplement use were collected from study participants and verified by inspecting drug prescriptions and containers. Systolic blood pressure (SBP) was measured twice using the participant's left arm after sitting for 5 minutes, and the mean of the measurements was calculated (eAppendix in the Supplement).

Brain Imaging Cohort

From September 17, 2001, to September 10, 2003, a total of 555 participants who were nondisabled, noninstitutionalized, and without dementia underwent a structural magnetic resonance imaging (MRI) scan; participants were followed up through December 17, 2009.[21] Participants whose MRI scan was of poor quality (n = 16) or who had a possible diagnosis of dementia (n = 3), Parkinson disease (n = 4), mood disorders (n = 3), evidence of brain infarctions on MRI scan (n = 13), arachnoid cysts (n = 3), or brain tumors (n = 1) were excluded from the current study, leaving 512 individuals. Blood drawn after clinical examination was routinely analyzed for RBC folate levels. Of the initial sample that underwent MRI scan, 11 indivudals did not take part in the blood drawing procedure, leaving 501 participants with available RBC folate values at baseline, which represent the baseline study population. Compared with the rest of the SNAC-K sample, the MRI subsample was younger (mean [SD] age, 70.9 [9.3] vs 75.4 [11.4] years; $P < .001$), had a better Mini-Mental State Examination total score (mean [SD], 29.1 [1.1] vs 26.8 [6.1]; $P < .001$), and higher educational level (mean [SD] years of schooling, 12.2 [4.1] vs 11.8 [4.0]; $P = .04$). Brain MRI scan was performed at baseline and thereafter every 3 years for the older cohort (ie, those ≥78 years at baseline; n = 92 at 3-year follow-up) and every 6 years for the whole cohort (n = 260; 53 in the older cohort and 207 in the younger cohort). Therefore, 299 participants had 1 or more MRI scan at follow-up (eAppendix and eFigure in the Supplement).

Brain Imaging

Participants were examined with a 1.5-T MRI scanner (eAppendix in the Supplement). Total gray matter volume (GMV)

and white matter volume (WMV) were calculated after automatic segmentation of the T1 images in native space using SPM12b software (Statistical Parametric Mapping, http://www.fil.ion.ucl.ac.uk/spm/), implemented in Matlab (The Mathworks Inc), using the unified segmentation approach.[22,23] Total brain tissue (TBT) volume was obtained by adding GMV and WMV. Total intracranial volume was finally calculated by adding the volumes of TBT and cerebrospinal fluid (CSF). Automatic volumetric segmentation of the hippocampus was performed using the Freesurfer image analysis suite, version 5.0.1 (Martinos Center for Biomedical Imaging, Harvard-Massachusetts Institute of Technology; http://surfer.nmr.mgh.harvard.edu/).[24] All segmentations were carefully checked visually. Total brain tissue volume, GMV, WMV, and hippocampal volume were expressed in proportion to total intracranial volume to correct for head size and multiplied by 100.

To measure global WMH volumes, all WMHs were manually drawn on fluid-attenuated inversion recovery images by a single rater (G.K.) and further interpolated on the corresponding T1-weighted images to compensate for the gap between slices in fluid-attenuated inversion recovery. Total WMH volumes were divided by TBT volumes before hypothesis testing.

Biochemical Analyses

At baseline, venous blood samples were taken while the participant was not fasting and routine analyses, including RBC folate assessment, were done within 2 hours using chemiluminescence microparticle folate binding protein assay at Sabbatsberg Hospital, Stockholm, Sweden (results available for 501 participants). The coefficient of variation was 4.8% and 6.1% at 147.4 and 238.4 ng/mL (to convert to nanomoles per liter, multiply by 2.266), respectively. Specimens were stored at -80° for 10 to 12 years. Batches were transferred thereafter on dry ice to the University of Oxford, Oxford, UK. Because our study was a substudy of the larger SNAC-K study, there was sufficient demand for blood samples to be used for a variety of other biochemical assays, and sufficient serum volumes were not available for 31 participants. Vitamin B_{12} and holoTC levels were measured by microbiological methods, as described previously.[9] The coefficient of variation for both assays was 5%. The levels of sulfur amino acids (tHcy, methionine, cystathionine, cysteine, and glutathione) were measured using tandem mass spectrometry after treatment of serum with a reducing agent, as described previously.[25] Interassay coefficients of variation were between 5% and 10%. One individual with a tHcy value of 98 µmol/L was excluded. Genotyping of *APOE* (OMIM 107741) was performed as described previously.[20]

Statistical Analysis

Baseline characteristics of individuals who participated at follow-up MRI scan were compared with those who did not participate using χ^2 tests for the proportions and *t* test or Mann-Whitney test for continuous variables, when appropriate. Linear mixed models for repeated measures were used to estimate β (SE) for the association of the levels of vitamin B_{12}, holoTC, RBC folate, and sulfur amino acids with repeated measures of brain volumes and WMH over 6 years. Models were adjusted for age and sex (model 1), and then additionally for other potential confounding or mediating factors, including educational level, *APOE*ε4, SBP, creatinine, use of vitamin supplements, smoking, treatment of hypertension, plasma total cholesterol level, obesity (ie, body mass index ≥30 [calculated as weight in kilograms divided by height in meters squared]), history of cardiovascular conditions (ie, atrial fibrillation, coronary heart disease, and heart failure), and plasma albumin level (model 2). The interaction between time and each covariate was also added in all models. In the linear mixed models, the β coefficient for vitamin B_{12}, folate, and sulfur amino acid values represent the cross-sectional association with the baseline brain volume. The β coefficient for the interaction between vitamin B_{12}, folate or sulfur amino acids and time represents the effect of these biomarkers on the rate of change in brain volume per year. A positive β coefficient indicates that an increase in these biomarkers was associated with decreased rate of brain volume loss over time. For the associations with WMH and CSF, a positive β coefficient indicates that an increase in these biomarkers was associated with increased volume of WMH or CSF.

Analyses were repeated after excluding participants with low levels of vitamin B_{12} (<148 pmol/L [n = 6] and <258 pmol/L [n = 110]),[26,27] holoTC (<35 pmol/L [n = 68]),[28] and RBC folate (<55.2 ng/mL [n = 46]). All analyses were also repeated after excluding 30 participants who developed dementia during follow-up. We analyzed the data using Stata, version 12 (StataCorp LP).

Results

The 501 study participants were a mean (SD) age of 70.9 (9.1) years and 300 (59.9%) were women. Selected characteristics are shown in **Table 1** of all participants at baseline and those who underwent a follow-up MRI scan compared with those who did not. Individuals who underwent a follow-up MRI scan were younger at baseline, were more educated, were less likely to have cardiovascular conditions, and had higher methionine levels compared with those who did not undergo a follow-up MRI scan. In addition, individuals who underwent a follow-up MRI scan had higher baseline TBT and lower WMH volume. Mean (SD) TBT volume declined from 74.3% (3.7%) of total intracranial volume at baseline to 71.6% (4.1%) at 6-year follow-up ($P < .001$). In contrast, mean (SD) WMH volume increased from 0.0004% (0.0007%) to 0.0007% (0.0009%) at 6-year follow-up ($P < .001$).

Vitamin B_{12}, Folate, and Sulfur Amino Acid Concentrations in Association With Brain Atrophy

Linear mixed models were used to examine the associations of vitamin B_{12}, holoTC, RBC folate, and sulfur amino acid values with the rate of loss of brain volume and WMH volume. There was no cross-sectional association between values for vitamin B_{12}, holoTC, and brain volumes. In the prospective analyses over 6 years, higher levels of vitamin B_{12} and holoTC were related to a decreased rate of TBT volume loss: for each 1-SD increase, β (SE) was 0.048 (0.013) for vitamin B_{12} level ($P < .001$) and 0.040 (0.13) for holoTC level ($P = .002$), after ad-

Table 1. Baseline Characteristics of the Study Population

Characteristic	All Participants (N = 501)	Participants With Follow-up MRI (n = 299)	Participants Without Follow-up MRI Scan (n = 202)	*P* Value
Age, mean (SD), y	70.9 (9.1)	70.0 (8.6)	72.3 (9.6)	.005
Women, No. (%)	300 (59.9)	179 (59.9)	121 (59.9)	.99
Educational level, mean (SD), y	12.6 (4.5)	13.1 (4.4)	11.9 (4.5)	.003
Use of vitamin supplements, No. (%)	100 (20.0)	60 (20.1)	40 (19.8)	.94
Systolic blood pressure, mean (SD), mm Hg	142.8 (19.6)	141.7 (19.8)	144.3 (19.3)	.14
*APOE*ε4 allele, No. (%)	150 (29.9)	89 (29.8)	61 (30.2)	.92
Ever smoked, No. (%)	280 (55.9)	166 (55.5)	114 (56.4)	.84
Obese, No. (%)[a]	70 (14.0)	43 (14.4)	27 (13.4)	.75
History of cardiovascular conditions, No. (%)	123 (24.6)	61 (20.4)	62 (30.7)	.009
Plasma creatinine, mean (SD), mg/dL	1.0 (0.17)	1.0 (0.16)	0.99 (0.17)	.67
RBC folate, median (IQR), ng/mL[b,c]	102.4 (83.8-134.2)	104.1 (85.6-134.2)	100.4 (80.8-132.2)	.33
Vitamin B_{12}, median (IQR), pmol/L[b]	339.0 (264.0-433.0)	340.0 (29.0-438.0)	338.5 (251.0-425.3)	.39
Holotranscobalamin, median (IQR), pmol/L[b]	65.0 (43.0-85.0)	64.0 (43.0-85.0)	65.5 (42.8-86.5)	.79
Homocysteine, median (IQR), μmol/L[b]	12.7 (10.6-15.7)	12.7 (10.7-15.4)	12.9 (10.7-15.8)	.52
Methionine, median (IQR), mg/dL[b]	0.35 (0.29-0.41)	0.36 (0.30-0.41)	0.34 (0.29-0.39)	.048
Cystathionine, median (IQR), nmol/L[b]	0.27 (0.20-0.38)	0.27 (0.20-0.38)	0.28 (0.20-0.39)	.45
Cysteine, mean (SD), μmol/L	323.6 (50.2)	321.8 (52.1)	326.5 (47.0)	.32
Glutathione, median (IQR), μmol/L[b]	3.6 (2.9-4.3)	3.6 (2.9-4.3)	3.7 (2.9-4.3)	.68
Total brain tissue volume, mean (SD)[d]	73.4 (4.2)	73.7 (4.1)	72.9 (4.3)	.03
White matter hyperintensity volume, mean (SD)[e]	0.0006 (0.0012)	0.0005 (0.001)	0.0008 (0.001)	.01

Abbreviations: IQR, interquartile range; MRI, magnetic resonance imaging; RBC, red blood cell.

SI conversion factors: to convert creatinine to micromoles per liter, multiply by 88.4; folate to nanomoles per liter, multiply by 2.266; methionine to micromoles per liter, multiply by 67.02.

[a] Defined as body mass index ≥30 (calculated as weight in kilograms divided by height in meters squared).

[b] Mann-Whitney test.

[c] Determination of RBC folate value is routinely performed for all participants (available for 501 individuals), but the additional markers are not. Thus, these values reflect those of the 470 participants clinically evaluated with available blood for further analysis. Of these 470 individuals, 283 participated at follow-up MRI scan, whereas 187 did not.

[d] Expressed in proportion to total intracranial volume to correct for head size and multiplied by 100.

[e] Expressed in proportion to total brain tissue volume and multiplied by 100.

justing for all study covariates (**Table 2**, model 2). These associations remained after excluding the participants who used vitamin supplements: β (SE) was 0.067 (0.023) for vitamin B_{12} levels (P = .004) and 0.167 (0.35) for holoTC levels (P < .001). Furthermore, increased levels of vitamin B_{12} and holoTC were associated with less progression in CSF volume and tended to have an association with decreased loss of WMV. In addition, increased levels of vitamin B_{12} had a borderline significant association with decreased loss of GMV and loss of hippocampal volume (eTable 1 and eTable 2 in the Supplement).

In addition to increased levels of vitamin B_{12}, increasing age and history of cardiovascular conditions were associated with loss of TBT volume: β (SE) was -0.008 (0.002) for age (P < .001) and -0.083 (0.035) for history of cardiovascular conditions (P = .02). The associations of vitamin B_{12} and holoTC levels with the rate of loss of TBT volume remained unchanged when excluding individuals whose levels of vitamin B_{12} were less than 148 pmol/L (β [SE], 0.048 [0.013]; P < .001) or less than 258 pmol/L (β [SE], 0.038 [0.014]; P = .006) or whose holoTC values were below 35 pmol/L (β [SE], 0.040 [0.013]; P = .002).

After adjusting for age and sex, elevated levels of tHcy had a significant cross-sectional association with TBT volume (β [SE], -0.574 (0.142); P < .001 for each 1-SD increase). Additional adjustment for other study covariates did not influence the results: β (SE) became -0.601 (0.153) (P < .001) (Table 2). In the longitudinal analysis over 6 years, levels of tHcy were associated with increased rate of loss of TBT volume: β (SE) was -0.035 (0.015) (P = .02) (Table 2). Further adjustment for estimated glomerular filtration rate did not change the associations. Increased levels of tHcy were also associated with higher CSF volume and increased rate of loss of GMV (eTables 1 and 2 in the Supplement). No significant cross-sectional or longitudinal associations were observed for RBC folate or other sulfur amino acid values.

Analyses were repeated after excluding 30 individuals with incident dementia at follow-up (eAppendix in the Supplement). After controlling for all study covariates (model 2), levels of tHcy remained associated with a faster rate of loss of TBT volume over 6 years: β (SE) -0.036 (0.015) (P = .01). In contrast, levels of vitamin B_{12} and holoTC were related to decreased rate of loss of TBT volume: β (SE) was 0.045 (0.013) for vitamin B_{12} (P = .001) and 0.039 (0.013) for holoTC (P = .003).

Vitamin B_{12}, Folate, and Sulfur Amino Acid Concentrations in Association With WMH Volume

No longitudinal associations were found between vitamin B_{12}, RBC folate, or sulfur amino acid levels and the change in WMH volume over 6 years in all participants (**Table 3**). However, levels of tHcy were significantly associated with the progression of WMH volume among individuals with SBP higher than 140

Table 2. Associations of Levels of Vitamin B_{12}, RBC Folate, and Sulfur Amino Acids With Change in Total Brain Tissue Volume Over 6 Years[a]

	Cross-sectional[b]		Vitamin × Time[c]	
Vitamin or Sulfur Amino Acid	β (SE)	*P* Value	β (SE)	*P* Value
RBC Folate				
Model 1[d]	0.041 (0.135)	.76	−0.002 (0.014)	.90
Model 2[e]	0.076 (0.143)	.59	0.001 (0.014)	.93
Vitamin B_{12}				
Model 1	0.003 (0.141)	.99	0.042 (0.012)	.001
Model 2	0.044 (0.152)	.77	0.048 (0.013)	<.001
Holotrascobalamin				
Model 1	−0.127 (0.141)	.37	0.034 (0.12)	.005
Model 2	−0.099 (0.155)	.52	0.040 (0.013)	.002
Homocysteine				
Model 1	−0.554 (0.143)	<.001	−0.031 (0.014)	.03
Model 2	−0.601 (0.254)	<.001	−0.035 (0.015)	.02
Methionine				
Model 1	0.138 (0.145)	.34	0.014 (0.012)	.25
Model 2	0.140 (0.145)	.30	0.015 (0.012)	.20
Cystathionine				
Model 1	−0.012 (0.079)	.88	−0.005 (0.006)	.38
Model 2	−0.0001 (0.085)	>.99	−0.005 (0.006)	.48
Cysteine				
Model 1	−0.107 (0.148)	.47	−0.006 (0.013)	.67
Model 2	−0.038 (0.157)	.81	−0.005 (0.013)	.71
Glutathione				
Model 1	0.106 (0.130)	.41	−0.012 (0.012)	.32
Model 2	0.078 (0.118)	.50	−0.016 (0.011)	.15

Abbreviations: β, coefficient for 1-SD change in each compound; MRI, magnetic resonance imaging; RBC, red blood cell.

[a] Associations were examined by linear mixed models. The term *cross-sectional* represents the cross-sectional association between levels of vitamin B_{12}, RBC folate, or sulfur amino acids and brain volumes at baseline. The term *vitamin × time* represents the effect of vitamin B_{12}, RBC folate, or sulfur amino acid levels on the rate of change in brain volumes per year. A positive coefficient for vitamin × time indicates that an increase in the value of the vitamin or sulfur amino acid value was associated with a decreased rate of brain atrophy over time. Average yearly change without including vitamins or sulfur amino acids in the model, −4.9449 (0.1444); $P < .0001$.

[b] For cross-sectional analysis, n = 501 for RBC folate and n = 470 for vitamin B_{12}, holotranscobalamin, and sulfur amino acids.

[c] For longitudinal analysis, n = 299 for those with available follow-up MRI scans and baseline RBC folate values and n = 281 for those with available follow-up MRI scans and available levels of vitamin B_{12}, holotransciobalamin, and sulfur amino acids.

[d] Model 1 was adjusted for age and sex and their interactions with time.

[e] Model 2 was additionally adjusted for educational level, creatinine, mean systolic blood pressure, *APOE*ε4 status, the use of vitamin supplements, smoking, treatment of hypertension, plasma total cholesterol level, and obesity and their interactions with time.

mm Hg at baseline: β (SE) was 0.000019 (0.00001) for each 1-SD increase in level of tHcy ($P = .047$), after controlling for all study covariates.

Discussion

In this longitudinal population-based study of older adults without dementia, higher levels of vitamin B_{12} and holoTC as well as lower levels of tHcy were associated with a decreased rate of loss of brain volume over 6 years. The observed associations were independent of common sociodemographic and vascular risk factors. The protective effect of vitamin B_{12} and holoTC appeared to be present across the whole distribution. No association between markers of the transsulfuration pathway and markers of brain aging were observed. This finding may suggest that markers of the methylation pathway may be more important than markers of the transsulfuration pathway in association with brain aging. In addition, elevated levels of tHcy were associated with increased WMH volume, but only among individuals with higher baseline SBP.

Relatively few longitudinal studies have investigated the associations of vitamin B_{12}, folate, and sulfur amino acids with the rate of loss of brain volume. Consistent with our findings, lower levels of vitamin B_{12} and holoTC, but not folate or tHcy, were associated with an increased rate of loss of brain volume over 5 years in the Oxford Project to Investigate Memory and Aging study.[9] Raised baseline levels of tHcy were associated with a faster rate of atrophy of the medial temporal lobe in patients with Alzheimer disease,[12] and with more rapid atrophy of the total brain volume in those with mild cognitive impairment.[13] Rates of brain atrophy were significantly correlated with levels of tHcy in the Study on Cognition and Prognosis in the Elderly (follow-up, 2 years),[11] but no associations

Table 3. Associations of Levels of Vitamin B_{12}, RBC Folate, and Sulfur Amino Acids With Change in White Matter Hyperintensity Volumes Over 6 Years[a]

	Cross-sectional[b]		Vitamin × Time[c]	
Vitamin or Sulfur Amino Acid	β (SE)	*P* Value	β (SE)	*P* Value
RBC Folate				
Model 1[d]	0.00002 (0.00005)	.69	-0.000004 (0.000005)	.45
Model 2[e]	-0.000007 (0.00005)	.89	-0.000005 (0.000005)	.38
Vitamin B_{12}				
Model 1	0.00006 (0.00005)	.23	-0.000003 (0.000005)	.53
Model 2	0.00003 (0.00006)	.64	-0.000001 (0.000005)	.86
Holotranscobalamin				
Model 1	0.00009 (0.00005)	.09	-0.000007 (0.000005)	.16
Model 2	0.00006 (0.00006)	.29	-0.000006 (0.000005)	.22
Homocysteine				
Model 1	0.00004 (0.00005)	.43	0.000003 (0.000005)	.59
Model 2	0.00006 (0.00006)	.30	0.000008 (0.000006)	.18
Methionine				
Model 1	0.00001 (0.00005)	.82	-0.000004 (0.000005)	.42
Model 2	0.00002 (0.00005)	.75	-0.000003 (0.000005)	.52
Cystathionine				
Model 1	0.00002 (0.00003)	.39	-0.000001 (0.000003)	.73
Model 2	0.00005 (0.00003)	.09	0.0000007 (0.000003)	.81
Cysteine				
Model 1	0.00004 (0.00005)	.48	0.000004 (0.000005)	.46
Model 2	0.00003 (0.00006)	.60	0.000007 (0.000006)	.16
Glutathione				
Model 1	-0.00004 (0.00005)	.45	-0.000002 (0.000005)	.63
Model 2	-0.00005 (0.00005)	.25	-0.000002 (0.000005)	.67

Abbreviations: β, coefficient for 1-SD change in each compound; MRI, magnetic resonance imaging; RBC, red blood cell.

[a] Associations were examined by linear mixed models. The term *cross-sectional* represents the cross-sectional association between levels of vitamin B_{12}, RBC folate, or sulfur amino acids and white matter hyperintensity volumes at baseline. The term *vitamin × time* represents the effect of vitamin B_{12}, folate, or sulfur amino acid levels on the rate of change in white matter hyperintensity volume per year. A positive coefficient for vitamin × time indicates that an increase in the value of the vitamin or sulfur amino acid was associated with an increase in white matter hyperintensity volume over time. Average yearly change without including vitamins or sulfur amino acids in the model: 0.0007 (0.0001); $P < .0001$.

[b] For cross-sectional analysis, n = 494 for levels of RBC folate and n = 464 for levels of vitamin B_{12}, holotranscobalamin, and sulfur amino acids.

[c] For longitudinal analysis, n = 295 for those with available follow-up MRI scans and baseline RBC folate values and n = 279 for those with available follow-up MRI scans and available levels of vitamin B_{12}, holotranscobalamin, and sulfur amino acids.

[d] Model 1 was adjusted for age and sex and their interactions with time.

[e] Model 2 was additionally adjusted for educational level, creatinine, mean systolic blood pressure, *APOE*ε4 status, the use of vitamin supplements, smoking, treatment of hypertension, plasma total cholesterol level, and obesity and their interactions with time.

with levels of folate and vitamin B_{12} were found.[11] In addition, higher levels of tHcy were associated with the progression of ventricular enlargement, another surrogate of brain atrophy, in the Second Manifestations of ARTerial Disease–Magnetic Resonance (SMART-MR) study (follow-up, 3.9 years).[14] Differences in follow-up periods, vitamin status, and other characteristics of the study populations can explain some of the discrepancies among the studies.

In our study, increased levels of tHcy were related to WMH progression among individuals with higher SBP. Hypertension is a major risk factor for WMH,[21] which is thought to reflect cerebral small-vessel disease, an important mediator in the association of hypertension with brain aging.[29] Our findings suggest that tHcy concentrations may exacerbate the deleterious effect of hypertension on WMH. Similar to our results, elevated levels of tHcy were associated with the progression of total WMH volume in the SMART-MR study, which included participants with symptomatic atherosclerotic disease.[15] However, no associations between levels of tHcy, folate, or vitamin B_{12} and progression of white matter lesions over 2 years were observed in the SCOPE study, which included 80 individuals with hypertension.[11]

High levels of tHcy have been associated with endothelial dysfunction, impaired nitric oxide activity, atherosclerosis, and subsequent increase in the risk of various cardiovascular or cerebrovascular events, which may increase the risk of brain aging and cognitive decline.[4,30] Furthermore, elevated levels of tHcy may potentiate generation of β-amyloid peptide and its neurotoxicity or promote formation of neurofibrillary tangles through several mechanisms, which may lead to increased rate of brain atrophy.[4,30,31] Alternatively, the protective effects of vitamin B_{12} may be mediated through S-adenosylmethionine, which is the primary methyl donor in many biochemical reactions involved in normal brain functions, including the production of cell membrane phospholipids, myelin, monoaminergic neurotransmitters, and nucleic

acids. Deficiency of S-adenosylmethionine may be linked to white matter damage and brain atrophy, factors associated with cognitive decline and dementia.[30]

In our study, high levels of tHcy were associated both cross-sectionally and longitudinally with loss of TBT volume, suggesting that tHcy may be involved in brain atrophy for a longer period. In contrast, we did not observe a cross-sectional association with vitamin B_{12} or holoTC. It may be possible that vitamin B_{12} needs a longer time to influence brain structure and that the effects first manifest after several years of follow-up. Our results showed an association between vitamin B_{12} and holoTC across the entire range with TBT volume change over 6 years, suggesting that individuals who are not classically deficient in vitamin B_{12} but have low-normal levels of vitamin B_{12} may benefit from vitamin B_{12} treatment, although this finding has to be determined in randomized clinical trials. A clinical trial (Homocysteine and B Vitamins in Cognitive Impairment) has shown that B vitamin treatment of individuals with mild cognitive impairment markedly slows whole[13] and regional brain atrophy[32] in those with elevated levels of tHcy and normal levels of vitamin B_{12}. Bayesian network analysis[32] indicated that the main factor in this protective effect was vitamin B_{12}. However, further trial evidence is needed to confirm that vitamin B_{12} supplementation will reduce the rate of loss of TBT volume in older adults with low-normal levels of vitamin B_{12}.[27,32]

The main strengths of this study are the relatively large number of community-dwelling older adults with available data on a large number of potential confounders, the availability of MRI scans on at least 2 to 3 occasions over 6 years, and the evaluation of vitamin B_{12}, folate, and sulfur amino acid levels simultaneously in association with the outcome. In addition, our results remained unchanged after excluding individuals with incident dementia. Stability of tHcy, vitamin B_{12}, and folate in samples stored for a long period at −70°C or more has been reported previously.[33,34]

The main limitations of our study include the measurement of levels of vitamin B_{12}, folate, or sulfur amino acids at only 1 time point, which may underestimate their associations owing to regression dilution.[30] Although participants at the 6-year follow-up MRI scan were younger and were less likely to have a history of cardiovascular conditions than did nonparticipants in the study, the effect of any nonresponse bias is to underestimate any associations with vitamin status.[35] Selective survival may also have contributed to underestimation of the associations, because low levels of vitamin B_{12} or folate and high levels of tHcy have been associated with increased mortality in previous studies.[1,4,36]

Conclusions

Vitamin B_{12} and tHcy might be independent predictors of markers of brain aging in elderly individuals without dementia. Because of the observational design, we caution against a causal interpretation of the findings. Future studies will need to investigate in more detail possible underlying mechanisms. However, if the association is causal, supplementation with B vitamins may be effective for prevention of brain damage due to increased levels of tHcy. Adequately timed and powered randomized clinical trials are needed to determine efficient treatment guidelines.

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