Association of vitamin B_{12}, folate, and sulfur amino acids with brain magnetic resonance imaging measures in older adults: a longitudinal population-based study

Subtitle: Vitamin B12, homocysteine, and brain volumes

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Authors' contributions

BH, FM, AS, DS, HR, LF, and MK conceived and designed the study. BH, FM, AS, DS, HR, RW, LB, LF, and MK performed the literature search. GK did the imaging analysis (white matter hyperintensities, Segmentation of the T1-weighted images). BH and IK did the data analysis. BH, FM, AS, GK, IK, DS, HR, RW, MM, BEW, EJL, LB, LF, and MK interpreted the results and drafted the report. BH, AS, DS, HR, LF, and MK obtained funding. All authors received the article and critically revised the Article for important intellectual content. BH is the guarantor.

Confliction of interest disclosures

We declare no competing interests.

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Abstract

Importance: Vitamin B12, folate, and sulfur amino-acids may be modifiable risk factors for structural brain changes which precede clinical dementia.

Objective: To investigate the association of circulating levels of vitamin B12, folate, and sulfur amino-acids with the rate of total brain volume loss and the change in FLAIR white matter hyperintensity (WMH) volume in older adults.

Design: Population-based longitudinal study.

Setting: The Swedish National Study on Aging and Care in Kungsholmen (SNAC-K), Stockholm, Sweden.

Participants: A dementia free sample of 501 subjects at baseline, aged 60-97 years, of which 299 subjects underwent repeated structural brain magnetic resonance imaging (MRI) scans over 6 years.

Main outcomes: The rate of brain tissue volume loss and the progression of total WMH volume.

Results: In the multi-adjusted linear mixed models, higher baseline vitamin B12 and holotranscobalamin levels were associated with decreased rate of total brain volume loss over 6 years: β coefficient and standard error (SE) for each increase of 1 standard deviation (SD) were 0.048 (0.013); $p < 0.001$ for B12 and 0.040 (0.013); $p = 0.002$ for holotranscobalamin. Increased total homocysteine (tHcy) levels were related to faster rates of total brain volume loss in the whole sample [β(SE) per 1 SD increase: -0.35 (0.15); $p = 0.019$], and were also associated with the progression of WMH among subjects with systolic blood pressure > 140 mmHg [β(SE) per 1 SD increase: 0.000019 (0.00001); $p = 0.047$]. No longitudinal associations were found for RBC folate levels and other sulfur amino acids.

Conclusions and relevance: This study suggests that both vitamin B12 and tHcy may be related to acceleratde brain aging. Randomized controlled trials are needed to determine the importance of vitaminB12 supplementation on slowing brain aging in older adults.

Introduction

Vitamin B12 and folate are closely connected with the metabolism of homocysteine, a sulfur-containing non-essential amino-acid. Inadequate levels of either vitamin can result in increased concentrations of total homocysteine (tHcy)[1]. High levels of tHcy and low B12 and folate status are common conditions 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 dementia diagnosis, and the association is maintained also in very advanced age[6,7]. Whereas few longitudinal studies have linked low B12 or folate status with structural brain changes[8,9], others did not report such associations[9-11]. Higher blood levels of tHcy have also been related to 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 B12, may be a more sensitive marker of B12 status[16]. However, very few longitudinal studies have investigated its association with brain volumes[9]. In addition, the impact of sulfur amino-acids other than homocysteine on brain aging has rarely been investigated[17].

The potential impact of B12, 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 the current study was to examine the associations of B12, red blood cell (RBC) folate, and sulfur amino-acids with 6-year changes in brain tissue volumes and total fluid attenuated inversion recovery (FLAIR) white matter hyperintensity (WMH) volume in a population-based cohort of older adults without mandatory folic acid fortification.

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 who live either at home or in institution. Because of more rapid changes in health and a higher attrition rate among older age groups, the sampling is stratified by age cohort. Assessments take place at 6-year interval for younger cohorts (60, 66, 72, and 78 years) and at 3-year intervals for older cohorts (81, 84, 87, 90, 93, 96, and 99+ years). In 2001-2004, of the 4590 alive and eligible subjects randomly selected for SNAC-K, 3363 (73.7%) participated at the baseline examination[18,19].

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 socio-demographic characteristics, medical history, drug use and cognitive function were collected according to a structured protocol and the diagnosis of dementia was made according to the 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 from the left arm of the subject twice after sitting for 5 minutes, and the mean of the measurements was calculated (see details in the Supplementary Material).

The Ethics Committee at Karolinska Institutet and the Regional Ethical Review Board in Stockholm, approved the protocols of each phase of the SNAC-K project, and informed consent was collected from all participants.

Brain imaging cohort

During September 2001 to October 2003, participants who were nondisabled, noninstitutionalized, and without dementia were invited to undergo a structural MRI scan (n=2204); 555 participants underwent MR-imaging[21]. Participants with poor MRI quality (n=16), possible dementia diagnosis (n=3), Parkinson's disease (n=4), mood disorders (n=3), MRI evidence of brain infarctions (n=13) or arachnoid cysts (n=3) or brain tumors (n=1) were excluded from the current study, leaving 512 subjects. Blood drawn after clinical examination was routinely analyzed for RBC-folate levels. Of the initial MRI sample, 11 subjects did not take part in the blood drawing procedure, leaving 501 subjects with available RBC-folate values at baseline, which represent the baseline study population. Compared to the rest of the SNAC-K sample, the MRI sub-sample was on average younger [age, mean (SD): 70.9 (9.3) versus 75.4 (11.4) years, p<0·001)], had better Mini-Mental State Examination total score [mean (SD): 29.1 (1.1) versus 26.8 (6.1), p<0.001] and higher education [years of schooling, mean (SD): 12.2 (4.1) versus 11.8 (4.0) years, p=045]. Brain MR-imaging was performed at baseline, and thereafter three years later for the older cohort (i.e., those ≥78y at baseline, n=92 at 3-year follow-up) and six years later for the whole cohort (n=260; 53 subjects belonging to the older cohort and 207 subjects belonging to the younger cohort). Therefore, 299 subjects had ≥1 MRI scans at follow-up (see further details in the Supplementary Material and Supplementary Figure).

Brain imaging

Participants were examined on a 1.5T MR scanner (see the protocol in the Supplementary Material). 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, implemented in Matlab, using the unified segmentation approach[22,23]. Total brain

tissue volume (TBT) was obtained by adding GMV and WMV. Total intracranial volume (TIV) was finally calculated by adding the volumes of TBT and cerebrospinal fluid (CSF). Automatic volumetric segmentation of hippocampus was performed using the Freesurfer image analysis suite[24]. All segmentations were carefully checked visually. TBT, GMV, WMV, and hippocampal volume were expressed in proportion to TIV to correct for headsize and multiplied by 100.

To measure global WMH volumes, all white matter hyperintensities were manually drawn on FLAIR images by a single rater (GK) and further interpolated on the corresponding T1-weighted images to compensate for the gap between slices in FLAIR. Total WMH volumes were divided by subjects' TBT prior to hypothesis testing.

Biochemical analyses

At baseline, non-fasting venous blood samples were taken, and routine analyses including RBC-folate assessment, were done within two hours using chemiluminescence microparticle folate binding protein assay at Sabbatsberg hospital, Stockholm (available in 501 subjects). The coefficient of variation (CV) was 4.8% and 6.1% at 334.0 and 540.2 nmol/L, respectively. Specimens were stored at -80º for 10-12 years. Batches were transferred thereafter on dry ice to the University of Oxford. Because the present study was a sub-study 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 was not available in 31 subjects. Vitamin B12 and holoTC were measured by microbiological methods, as described previously[9]. The CV for both assays was 5%. The 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 CVs

were between 5 and 10%. One subject with tHcy value of 98 μmol/L was excluded. *APOE* Genotyping was performed as described previously[20].

Statistical analyses

Baseline characteristics of subjects who participated at follow-up MRI with those who did not were compared using χ^2 for the proportions and student t-test or Mann-Whitney U test for continuous variables, when appropriate. Linear mixed models for repeated measures were used to estimate β and standard error (SE) for the association of B12, 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 education, *APOE*ε4, SBP, creatinine, vitamin supplements, smoking, treatment of hypertension, cholesterol, obesity (i.e. BMI≥30), history of cardiovascular conditions (i.e. atrial fibrillation, coronary heart disease, and heart failure), and plasma albumin (model 2). The interaction between time and each covariate was also added in all models. In the linear mixed models, the β coefficient for B12, folate, and sulfur amino-acids represents the cross-sectional association with the baseline brain volume. The β coefficient for the B12, folate or sulfur amino-acids × time interaction 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 WMH or CSF volume.

Analyses were repeated after excluding subjects with low levels of B12 (B12<148 pmol/L, n=6 and B12<258 pmol/L, n=110)[26,27], holoTC (<35 pmol/L, n=68)[28] and RBC-folate

(<125nmol/L, n=46). All analyses were also repeated after excluding 30 subjects who developed dementia during follow-up. We analyzed the data using Stata version 12.

Results

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 ($p<0.001$). In contrast, mean WMH volume increased from 0.0004% (0.0007) to 0.0007% (0.0009) at 6-year follow-up ($p<0.001$).

Selected characteristics are shown in table 1 of all participants at baseline, of those who participated at follow-up MRI compared with those who did not. Individuals who participated at follow-up MRI were younger at baseline, were more educated, were less likely to have cardiovascular conditions, and had higher methionine levels compared to those who did not. In addition, they had higher baseline TBT and lower WMH volume.

Vitamin B12, folate, and sulfur amino-acids in relation to brain atrophy

Linear mixed models were used to examine the associations of B12, holoTC, RBC-folate, and sulfur amino-acids with the rate of brain volume loss and WMH volume. There was no cross-sectional association between B12, holoTC and brain volumes. In the prospective analyses over 6 years, higher B12 and holoTC were related to a decreased rate of TBT volume loss: for each increase in 1 SD, β (SE) was 0.048 (0.013); $p<0.001$ for B12 and 0.040 (0.13); $p=0.002$ for holoTC, after adjusting for all study covariates (table 2, model 2). These associations remained after excluding the vitamin users: β (SE) was 0.067 (0.023); $p=0.004$ for B12 and 0.167 (0.35); $p<0.001$ for holoTC. Furthermore, increased B12 and holoTC were associated with less progression in CSF volume and tended to relate to decreased WMV loss. In addition, B12 had a borderline significant association with decreased GMV and hippocampal volume loss (Supplementary Tables 1 and 2).

In addition to B12, increasing age and history of cardiovascular conditions were associated with TBT volume loss: β (SE) was -0.008 (0.002); p<0.001 for age and -0.083 (0.035); p=0.017 for history of cardiovascular conditions. The associations of B12 and holoTC with the rate of TBT volume loss remained unchanged when excluding subjects with B12<148 pmol/L (β (SE): 0.048 (0.013); p<0.001 or B12<258 pmol/L (β (SE): 0.038 (0.014); p=0.006) or holoTC values below 35 pmol/L (β (SE): 0.040 (0.013); p=0.002.

After adjusting for age and sex, tHcy had a significant cross-sectional association with TBT volume (β (SE): -0.574 (0.142); p<0.001 for each increase in 1 SD). Additional adjustment for other study covariates did not influence the results: β (SE) became -0.601 (0.153); p<0.001 (table2, model 2). In the longitudinal analysis over 6 years, tHcy was associated with increased rate of TBT volume loss: β (SE) was -0.035 (0.015); p=0.019 (table 2, model 2). Further adjustment for eGFR did not change the associations. Increased tHcy values were also associated with higher CSF volume and increased rate of GMV loss (Supplementary tables 1 and 2).

No significant cross-sectional or longitudinal associations were observed for RBC-folate or other sulfur amino-acids.

Analyses were repeated after excluding 30 subjects with incident dementia at follow-up (details presented in Supplementary Material). After controlling for all study covariates (model 2), tHcy remained associated with faster rate of TBT volume loss over 6 years: β (SE -0.036 (0.15); p=0.013. In contrast, B12 and holoTC were related to decreased rate of TBT volume loss: β (SE) was 0.045 (0.013); p=0.001 for B12 and 0.039 (0.013); p=0.003 for holoTC.

Vitamin B12, folate, and sulfur amino-acids in relation to WMH volume

No longitudinal associations were found between B12, RBC-folate, or sulfur amino-acids and the change in WMH volume over 6 years in all subjects (table 3). However, tHcy was significantly associated with the progression of WMH volume among subjects with SBP>140 mmHg at baseline: β (SE) was 0.000019 (0.00001); p=0.047 for each increase in 1 SD in tHcy, after controlling for all study covariates.

Discussion

In this longitudinal population-based study of non-demented older adults, higher vitamin B12 and holoTC concentrations as well as lower tHcy values were related to decreased rate of brain volume loss over six years. The observed associations were independent of common socio-demographic and vascular risk factors. The protective effect of B12 and holoTC appeared to be present over the whole distribution. No association between markers of transsulfuration pathway and markers of brain aging were observed. This may suggest that markers of methylation pathway may be more important than the markers of the transsulfuration pathway in relation to brain aging. In addition, elevated tHcy was associated with increased WMH volume, but only among subjects with higher baseline SBP.

Relatively few longitudinal studies have investigated the associations of B12, folate, and sulfur amino-acids with the rate of brain volume loss. Consistent with our findings, lower B12 and holoTC values but not folate or tHcy were associated with an increased rate of brain volume loss over 5 years in the OPTIMA study[9]. Raised baseline tHcy concentrations were associated with a faster rate of the medial temporal lobe atrophy in subjects with Alzheimer's disease[12], and with more rapid total brain atrophy in subjects with mild cognitive impairment (MCI)[13]. Brain atrophy rates were significantly correlated with tHcy in the SCOPE study

(follow-up 2 years)[11], but no associations with folate and B12 levels were found[11]. In addition, higher tHcy values were related to the progression of ventricular enlargement in the SMART-MR study (follow-up 3.9 years), another surrogate of brain atrophy[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, tHcy was 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 relation of hypertension with brain aging[29]. Our findings suggest that tHcy may exacerbate the deleterious effect of hypertension on WMH. Similar to our results, elevated tHcy was associated with the progression of total WMH volume in the SMART-MR study including subjects with symptomatic atherosclerotic disease[15]. However, no associations between tHcy, folate or B12 and progression of white matter lesions over 2 years were observed in the SCOPE study, including 80 hypertensive individuals[11].

High tHcy levels have been related to 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 tHcy may potentiate β-amyloid peptide generation and its neurotoxicity or promote neurofibrillary tangle formation through several mechanisms, which may lead to increased rate of brain atrophy[4,30,31]. Alternatively, the protective effects of B12 may be mediated through S-adenosylmethionine (SAM). SAM 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 SAM may be linked to white matter damage and brain atrophy, factors associated with cognitive decline and dementia[30].

In our study, high tHcy levels were associated both cross-sectionally and longitudinally with total brain tissue volume loss, suggesting that tHcy may be involved in brain atrophy over a longer period. In contrast, we did not observe a cross-sectional association with B12 or holoTC. It may be possible that B12 needs longer time to influence brain structure and the effects become first manifest after several years of follow-up. Our results showed a relationship between B12 and holoTC across the entire range with TBT volume change over 6 years, suggesting that individuals who are not classically deficient in B12 but are at low-normal B12 status may benefit from B12 treatment, although this has to be determined in randomized clinical trials. A clinical trial (VITACOG) has shown that treatment of subjects with MCI with B-vitamins markedly slows whole[13] and regional brain atrophy[32] in subjects with elevated tHcy concentrations and B12 in normal range. It is noteworthy that Bayesian network analysis indicated that the main factor in this protective effect was B12[32]. However, further trial evidence is needed to confirm that B12 supplementation will reduce the rate of total brain tissue volume loss in older adults with low-normal B12 status[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 2-3 occasions over six years, and the evaluation of B12, folate, and sulfur amino-acids simultaneously in relation to the outcome. In addition, our results remained unchanged after excluding subjects with incident dementia. Stability of tHcy, B12, and folate in longtime stored samples at -70ºC≥ has been reported previously[33,34]. The main limitations include the availability of B12, folate, or sulfur amino-acids at only one time point, which may underestimate their associations due to regression dilution[30]. Although participants at 6-year follow-up MRI were younger and were less likely to have a history of cardiovascular conditions than did non-participants in the study, the effect of any non-response bias is to underestimate any associations with vitamin status[35]. Selective survival may also have

contributed to underestimation of the associations, because low B12 or folate and high tHcy status have been related to increased mortality in previous studies[1,4,36].

In conclusion, we suggest that B12 and tHcy might be independent predictors of markers of brain aging in non-demented elderly individuals. Because of the observational design, we must 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 raised tHcy. Adequately timed and powered randomized controlled trials are needed to determine efficient treatment guidelines.

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Conflict of interest

We declare no competing interests.

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Table 1. Characteristics of the study population at baseline

Characteristic	All (n = 501)	Participants with follow-up MRI (n = 299)	Participants without follow-up MRI (n = 202)	P-value
Age (years)	70.9 (9.1)	70.0 (8.6)	72.3 (9.6)	0.005
Women, n (%)	300 (59.9)	179 (59.9)	121 (59.9)	0.994
Education (years)	12.6 (4.5)	13.1 (4.4)	11.9 (4.5)	0.003
Use of vitamins, n (%)	100 (20.0%)	60 (20.1)	40 (19.8)	0.942
Systolic blood pressure (mmHg)	142.8 (19.6)	141.7 (19.8)	144.3 (19.3)	0.140
*APOE*ε4 allele, n (%)	150 (29.9%)	89 (29.8)	61 (30.2)	0.917
Ever smoked, n (%)	280 (55.9)	166 (55.5)	114 (56.4)	0.839
Obese, n (%)[1]	70 (14.0)	43 (14.4)	27 (13.4)	0.753
History of cardiovascular conditions, n (%)	123 (24.6%)	61 (20.4)	62 (30.7)	0.009
Plasma creatinine (μmol/L)	88.1 (14.6)	88.3 (14.3)	87.8 (15.0)	0.674
RBC folate (nmol/L)[2]	232.0 (190.0 – 304.0)	236.0 (194.0 – 304.0)	227.5 (183.0 – 299.5)	0.334
Vitamin B12 (pmol/L)[2]	339.0 (264.0 – 433.0)	340.0 (29.0 – 438.0)	338.5 (251.0 – 425.3)	0.393
Holotranscobalamin (pmol/L)[2]	65.0 (43.0 – 85.0)	64.0 (43.0 – 85.0)	65.5 (42.8 – 86.5)	0.794
Homocysteine (μmol/L)[2]	12.7 (10.6 – 15.7)	12.7 (10.7 – 15.4)	12.9 (10.7 – 15.8)	0.515
Methionine (μmol/L)[2]	23.5 (19.7 – 27.3)	24.2 (19.8 – 27.6)	22.9 (19.7 – 26.4)	0.048
Cystathionine (nmol/L)[2]	0.27 (0.20 – 0.38)	0.27 (0.20 – 0.38)	0.28 (0.20 – 0.39)	0.447
Cysteine (μmol/L)	323.6 (50.2)	321.8 (52.1)	326.5 (47.0)	0.316
Glutathione (μmol/L)[2]	3.6 (2.9 – 4.3)	3.6 (2.9 – 4.3)	3.7 (2.9 – 4.3)	0.677
Total brain tissue volume[4]	73.4 (4.2)	73.7 (4.1)	72.9 (4.3)	0.030
White matter hyperintensity volume[5]	0.0006 (0.0012)	0.0005 (0.001)	0.0008 (0.001)	0.014

Values are mean (standard deviation) or n (%) unless otherwise stated

[1]Defined as BMI ≥ 30.

[2]Median (interquartile range), Mann-Whitney U test was used.

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

[4]Expressed in proportion to total intracranial volume to correct for headsize and multiplied by 100.

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

Abbreviations: MMSE = Mini-Mental State Examination; RBC = red blood cell.

Table 2. Associations of vitamin B12, RBC folate and sulfur amino-acids levels with change in total brain tissue volume over six years[1]

		Cross-sectional[2] β (SE); p-value	Vitamin × time[3] β (SE); p-value
Folate	Model 1	0.041 (0.135); 0.764	-0.002 (0.014); 0·899
	Model 2	0.076 (0.143); 0·593	0.001 (0.014); 0.932
B12	Model 1	0.003 (0.141); 0·985	0.042 (0.012); **0·001**
	Model 2	0.044 (0.152); 0.772	0.048 (0.013); **<0·001**
Holotrascobalamin	Model 1	-0.127 (0.141); 0.368	0.034 (0.12); **0.005**
	Model 2	-0·099 (0.155); 0.524	0.040 (0.013); **0.002**
Homocysteine	Model 1	-0·554 (0.143); **<0·001**	-0·031 (0·014); **0.028**
	Model 2	-0.601 (0.254); **<0·001**	-0.035 (0.015); **0·019**
Methionine	Model 1	0.138 (0.145); 0·344	0.014 (0.012); 0·249
	Model 2	0.140 (0.145); 0·300	0.015 (0.012); 0·202
Cystathionine	Model 1	-0.012 (0.079); 0.882	-0.005 (0.006); 0.381
	Model 2	-0.0001 (0.085); 0·999	-0.005 (0.006); 0.478
Cysteine	Model 1	-0.107 (0.148); 0.469	-0.006 (0.013); 0.671
	Model 2	-0.038 (0.157); 0.811	-0.005 (0.013); 0.705
Glutathione	Model 1	0.106 (0.130); 0·414	-0.012 (0.012); 0.318
	Model 2	0.078 (0.118); 0.503	-0.016 (0.011); 0.149

β represents the coefficient for one standard deviation change in each compound and SE represents the standard error.
[1]Associations were examined by linear mixed models. The term *cross-sectional* represents the cross-sectional association between B12, folate or sulfur amino-acids and brain volumes at baseline. The term *Vitamin/sulfur amino-acid × time* represents the effect of B12 or folate or sulfur amino-acids on the rate of change in brain volumes per year. A positive coefficient for Vitamin/Sulfur amino-acid × time indicates that an increase in the vitamin/sulfur amino-acid value was associated with a decreased rate of brain atrophy over time.
Average yearly change without including vitamin/sulfur amino-acids in the model: -4·9449 (0·1444), p<0·0001
[2]For cross sectional analysis: n=501 for folate, n= 470 for vitamin B12, holotranscobalamin, and sulfur amino-acids
[3]For longitudinal analysis, n=299 for those with available follow-up MRI scans and baseline folate values; n=281 for those with available follow-up MRI and available vitamin B12, holotransciobalamin and sulfur amino-acids.
Model 1: adjusted for age and sex and their interactions with time.
Model 2: additionally adjusted for education, creatinine, mean systolic blood pressure, *APOE*ε4 status, the use of vitamin supplements, smoking, treatment of hypertension, plasma cholesterol, obesity and their interactions with time.

Table 3. Associations of vitamin B12, RBC folate and sulfur amino-acids levels with change in white matter hyperintensities volumes over six years[1]

		Cross-sectional[2] β (SE); p-value	Vitamin × time[3] β (SE); p-value
Folate	Model 1	0.00002 (0.00005); 0.686	-0.000004 (0.000005); 0.451
	Model 2	-0.000007 (0.00005); 0.885	-0.000005 (0.000005); 0.378
B12	Model 1	0.00006 (0.00005); 0·225	-0.000003 (0.000005); 0·528
	Model 2	0.00003 (0.00006); 0·640	-0.000001 (0.000005); 0·856
Holotranscobalamin	Model 1	0.00009 (0.00005); 0·088	-0.000007 (0.000005); 0·163
	Model 2	0.00006 (0.00006); 0.288	-0.000006 (0.000005); 0.220
Homocysteine	Model 1	0.00004 (0.00005); 0.425	0.000003 (0.000005); 0·593
	Model 2	0.00006 (0.00006); 0.303	0.000008 (0.000006); 0.177
Methionine	Model 1	0.00001 (0.00005); 0.824	-0.000004 (0.000005); 0.422
	Model 2	0.00002 (0.00005); 0.753	-0.000003 (0.000005); 0·521
Cystathionine	Model 1	0.00002 (0.00003); 0·394	-0.000001 (0.000003); 0·732
	Model 2	0.00005 (0.00003); 0.087	0.0000007 (0.000003); 0·806
Cysteine	Model 1	0.00004 (0.00005); 0.475	0.000004 (0.000005); 0.462
	Model 2	0.00003 (0.00006); 0.597	0.000007 (0.000006); 0.164
Glutathione	Model 1	-0.00004 (0.00005); 0.452	-0.000002 (0.000005); 0.630
	Model 2	-0.00005 (0.00005); 0.249	-0.000002 (0.000005); 0.669

β represents the coefficient for one standard deviation change in each compound and SE represents the standard error.

[1]Associations were examined by linear mixed models. The term *cross-sectional* represents the cross-sectional association between B12, folate or sulfur amino-acid and white matter hyperintensity volumes at baseline. The term *Vitamin/sulfur amino-acid × time* represents the effect of B12 or folate or sulfur amino-acids on the rate of change in white matter hyperintensity volume per year. A positive coefficient for Vitamin/sulfur amino-acid × time indicates that an increase in the vitamin/sulfur amino-acid value was associated with an increase in white matter hyperintensity volume over time.

Average yearly change without including vitamin/sulfur amino-acids in the model: 0·0007 (0·0001), p<0·0001

[2]For cross sectional analysis: n=494 for folate, n= 464 for vitamin B12, holotranscobalamin, and sulfur amino-acids

[3]For longitudinal analysis, n=295 for those with available follow-up MRI scans and baseline folate values; n=279 for those with available follow-up MRI and available vitamin B12, holotransciobalamin and sulfur amino-acids.

Model 1: adjusted for age and sex and their interactions with time.

Model 2: additionally adjusted for education, creatinine, mean systolic blood pressure, *APOE*ε4 status, the use of vitamin supplements, smoking, treatment of hypertension, plasma cholesterol, obesity, and their interactions with time