Vitamin B_{12} status and rate of brain volume loss in community-dwelling elderly

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ABSTRACT

Objectives: To investigate the relationship between markers of vitamin B_{12} status and brain volume loss per year over a 5-year period in an elderly population.

Methods: A prospective study of 107 community-dwelling volunteers aged 61 to 87 years without cognitive impairment at enrollment. Volunteers were assessed yearly by clinical examination, MRI scans, and cognitive tests. Blood was collected at baseline for measurement of plasma vitamin B_{12}, transcobalamin (TC), holotranscobalamin (holoTC), methylmalonic acid (MMA), total homocysteine (tHcy), and serum folate.

Results: The decrease in brain volume was greater among those with lower vitamin B_{12} and holoTC levels and higher plasma tHcy and MMA levels at baseline. Linear regression analysis showed that associations with vitamin B_{12} and holoTC remained significant after adjustment for age, sex, creatinine, education, initial brain volume, cognitive test scores, systolic blood pressure, ApoE ε4 status, tHcy, and folate. Using the upper (for the vitamins) or lower tertile (for the metabolites) as reference in logistic regression analysis and adjusting for the above covariates, vitamin B_{12} in the bottom tertile (<308 pmol/L) was associated with increased rate of brain volume loss (odds ratio 6.17, 95% CI 1.25–30.47). The association was similar for low levels of holoTC (<54 pmol/L) (odds ratio 5.99, 95% CI 1.21–29.81) and for low TC saturation. High levels of MMA or tHcy or low levels of folate were not associated with brain volume loss.

Conclusion: Low vitamin B_{12} status should be further investigated as a modifiable cause of brain atrophy and of likely subsequent cognitive impairment in the elderly. ***Neurology***® **2008;71:826–832**

GLOSSARY

AD = Alzheimer disease; **CAMCOG** = Cambridge Mental Disorders of the Elderly Examination; **CV** = coefficient of variation; **holoTC** = holotranscobalamin; **MMA** = methylmalonic acid; **NS** = not significant; **OPTIMA** = Oxford Project to Investigate Memory and Aging; **OR** = odds ratio; **PBVL** = percentage of brain volume loss; **SIENA** = structural image evaluation using normalization of atrophy; **TC** = transcobalamin; **tHcy** = total homocysteine.

Cognitive impairment and dementia are common disorders among the elderly and represent a major public health concern in aging populations. Subtle neuropathologic and cognitive changes in the brain and risk factors for cognitive impairment may be present years before clinical dementia and Alzheimer disease (AD) can be diagnosed.[1] Therefore, it is important to identify individuals at risk of developing dementia. Brain atrophy is associated with confirmed AD[2] and is a marker of disease progression.[3]

An elevated total homocysteine (tHcy) concentration is an independent risk factor for AD[4] and cognitive decline.[5] Furthermore, elevated levels of tHcy are associated with a smaller hippocampus[6,7] and with a greater rate of atrophy of the medial temporal lobe in AD.[4] A common cause of elevated tHcy is low vitamin B_{12} levels.[8] Vitamin B_{12} deficiency is a public

Supplemental data at www.neurology.org

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health problem, particularly among the elderly.[9,10] Several studies have reported associations between impaired vitamin B_{12} status and cognitive deficit in community-dwelling elderly subjects[5,11-13] and neuropsychiatric patients[4,14]; however, others have found null results.[15,16] Studies on the association between vitamin B_{12} status and brain atrophy in the elderly are limited[17,18] and have used measurement of total plasma vitamin B_{12}, which has low diagnostic accuracy.[8] Increased concentrations of methylmalonic acid (MMA) and tHcy are generally considered more sensitive metabolic markers of vitamin B_{12} status.[8] To improve the accuracy of diagnosis of vitamin B_{12} deficiency, two new markers, holotranscobalamin (holoTC) and transcobalamin (TC) saturation (the fraction of total TC present as holoTC), have been introduced.[19,20] HoloTC and TC saturation reflect the biologically active fraction of total vitamin B_{12} available for tissue uptake, and both have been proposed as more useful markers than total plasma vitamin B_{12},[19,21,22] especially for early changes in vitamin B_{12} homeostasis.[23]

In this study, we used longitudinal MRI to examine the relationship of baseline vitamin B_{12} status, assessed by several different markers, with the rate of atrophy of the brain over a 5-year period in a sample of community-dwelling individuals. Relationships to cognitive impairment will not be reported.

METHODS

Subjects. Community-dwelling, self-caring volunteers older than 60 years were recruited through talks and radio advertising for those who thought that their health, memory, and thinking were good compared with that of their peers.[24] A total of 160 participants were referred to the Oxford Project to Investigate Memory and Aging (OPTIMA) for a longitudinal study with annual visits that aimed to define early biochemical and radiologic markers or predictors of cognitive impairment. All volunteers were cognitively screened at baseline to exclude those who were impaired using the Cambridge Mental Disorders of the Elderly Examination (CAMCOG); all subjects had a CAMCOG score $>$ 80 and a Mini-Mental State Examination score $>$ 24. Among these subjects, 148 agreed to undergo annual MRI scans. This study is confined to the 107 subjects who had MRI scans both at baseline and 5 years later, whereas 41 were excluded because they did not complete the follow-up MRI scans, because of death or withdrawal. The study was approved by the Central Oxford Research Ethics Committee. Written informed consent was obtained for all procedures, including the initial cognitive screening.

Assessment. All volunteers underwent at baseline a detailed medical history and physical examination, as previously described.[24] Subjects with concomitant physical disease, such as diabetes mellitus, coronary artery disease, hypertension, prior TIA, or a minor cerebrovascular accident ($>$1 year previously), and subjects receiving vitamin B_{12} supplements or injections were not excluded. Cognitive assessment, brain MRI and CT scans, and blood sample collection were performed at baseline and thereafter on an annual basis. The neuropsychological test battery included tests in the domains of episodic, working, and semantic memory, executive function, and processing speed.[24]

MRI scans. The same Siemens 1.5-tesla Magnetom Vision MRI scanner was used throughout the study. Participants had a T1-weighted brain scan (magnetization-prepared rapid gradient echo, 2-mm axial slices, in-slice resolution 0.86 $\times$ 0.86 mm, three repeats) at each visit. A fully automated, quantitative method, structural image evaluation using normalization of atrophy (SIENA), was used to derive percentage of whole brain volume loss (PBVL) per year. SIENA is accurate (around 0.2% brain volume change error) and achieves high robustness.[25] The rate of change is estimated from two magnetic resonance images taken at different time points. SIENA automatically segments brain from nonbrain in each image and estimates the external surface of the skull in each image. The two brain images are registered while using the skull images to constrain scaling and skew; this corrects for changes in imaging geometry over time. Brain surface points (including ventricle surfaces) are found using the registered brain images to subvoxel accuracy, and the surface motion estimated on the basis of these points. The mean perpendicular edge motion across the entire brain surface produces a change image and can be converted into estimates of PBVL. PBVL reflects loss of brain (gray and white) matter only.[25]

Blood sample collection and analyses. Plasma concentrations of vitamin B_{12} were determined with microbiologic assays using a colistin sulfate–resistant strain of *Lactobacillus leichmanii*.[26] The vitamin B_{12} assays were adapted to a microtiter plate format and performed by a robotic workstation (Perkin Elmer MultiProbe11). Plasma holoTC and TC concentrations were measured using magnetic beads (microspheres) with immobilized monoclonal antibody specific for human TC to isolate TC, followed by a conventional microbiologic assay for vitamin B_{12} as above.[27] Plasma TC was measured after saturating TC with added vitamin B_{12}. The coefficient of variation (CV) for total plasma vitamin B_{12} was 5%. The CV for holoTC and TC was 5% to 8%. Plasma MMA was analyzed by a modified gas chromatography–mass spectrometry method based on ethylchloroformate derivatizations.[28] The CV for MMA was $<$5%. Plasma tHcy was measured by fluorescence polarization immunoassay using the Abbott IMx fully automated immunoassay analyzer (CV approximately 3%). Serum folate was measured by the Abbott Architect binding assay (CV $<$ 10%). ApoE ε4 genotypes were determined by a one-stage PCR assay.

Statistical analysis. Results are expressed as mean or geometric mean with 95% CI. Skewed variables were log transformed before analyses. Differences between mean values were examined using the Student t test, whereas differences in proportions were tested with a χ^2 test. Pearson correlations were performed to assess simple correlations, and linear regression models were used to adjust for multiple variables. The relations of tertiles of vitamins and metabolites to the risk of increased PBVL (defined as PBVL in the upper tertile vs the two lower tertiles) were assessed by logistic regression analyses. Covariates were categorized and are represented in the model as indicator variables to assess nonlinearity in the dose–response relation. In both linear and logistic

Table 1 Baseline characteristics of the study population who were community-living elderly without cognitive impairment at baseline

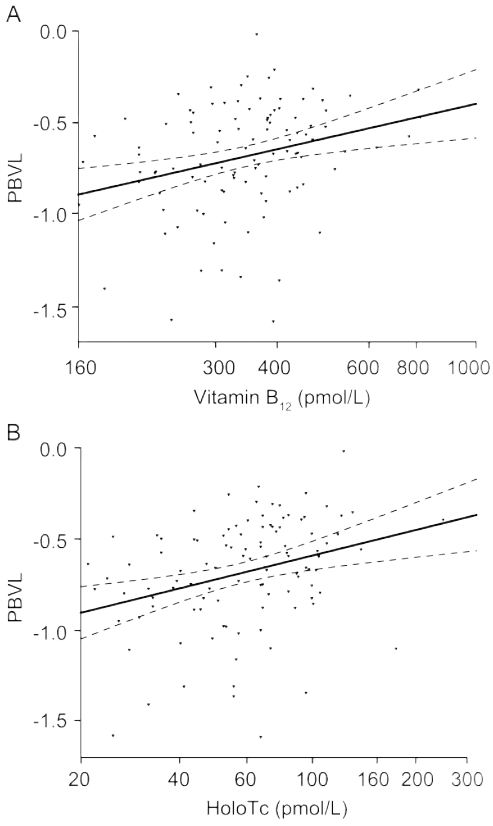
Variable	All subjects	PBVL after 5 y		p Value*
		1st and 2nd tertiles	3rd tertile (most loss)	
n	107	71	36	
Age, y	73.2 (72.0–74.4)	71.3 (69.9–72.8)	76.7 (74.5–78.9)	<0.001
Sex, n (%) women	54 (50.5)	35 (49.3)	19 (52.8)	NS†
Years of further education	2.7 (2.1–3.2)	2.2 (1.6–2.8)	3.2 (1.9–4.5)	NS
Smoking status, n (%)	5 (4.7)	4 (5.6)	1 (2.8)	NS†
Diabetes, n (%)	3 (2.8)	2 (2.8)	1 (2.8)	NS†
Vitamin B_{12} therapy (oral/injection), n (%)	6 (5.6)	4 (5.6)	2 (5.6)	NS†
Systolic blood pressure, mm Hg	152 (148–156)	150 (144–155)	155 (149–160)	NS
CAMCOG score	98.3 (97.5–99.1)	98.5 (97.5–99.5)	97.4 (95.9–98.8)	NS
ApoE ε4 polymorphism, n (%)	17.8	14 (19.7)	5 (13.9)	NS†
Initial brain volume, cm^3	1,458 (1,441–1,475)	1,477 (1,456–1,499)	1,423 (1,391–1,455)	0.001
PBVL per year over the 5-y period	0.69 (0.63–0.75)	0.51 (0.47–0.55)	1.05 (0.96–1.14)	<0.001
Cognitively impaired after 5 y, n (%)	34 (31.8)	19 (26.8)	15 (41.7)	NS†
Triglycerides, mmol/L	2.1 (1.9–2.4)	2.1 (1.9–2.4)	2.1 (1.6–2.5)	NS
Creatinine, μmol/L	104 (101–107)	106 (102–110)	101 (95–106)	0.096
tHcy, μmol/L‡	12.2 (11.5–12.9)	11.2 (10.5–11.9)	12.9 (11.7–14.2)	0.015
MMA, μmol/L‡	0.22 (0.20–0.24)	0.20 (0.18–0.21)	0.23 (0.20–0.26)	0.022
Vitamin B_{12}, pmol/L‡	363 (336–391)	368 (339–400)	300 (275–329)	0.003
HoloTC, pmol/L‡	68.7 (61.9–75.6)	67.8 (60.9–75.5)	50.8 (42.7–60.6)	0.004
TC, pmol/L‡	970 (871–1,079)	927 (885–970)	914 (846–988)	NS
TC saturation, %	7.4 (6.7–8.2)	8.1 (7.2–9.0)	6.3 (5.1–7.4)	0.016
Folate, nmol/L‡	12.6 (11.9–13.3)	12.5 (11.7–13.4)	11.4 (10.1–12.7)	NS

Data are mean (95% CI) or percent.
*Student *t* test.
†χ^2 test.
‡Geometric mean.
PBVL = percentage of brain volume loss; NS = not significant; CAMCOG = Cambridge Mental Disorders of the Elderly Examination; tHcy = total homocysteine; MMA = methylmalonic acid; holoTC = holotranscobalamin; TC = total transcobalamin.

regression analyses, two models were routinely used. The first model was adjusted for age and sex; the second model was further adjusted for initial brain volume, creatinine, years of further education, CAMCOG score, systolic blood pressure, and ApoE ε4 status. Further adjustment was performed for additional baseline variables (history of diabetes, use of vitamin B_{12} supplements, blood levels of triglycerides, tHcy, and folate) as well as the development of cognitive impairment during follow-up. Sex, the use of supplements, ApoE ε4 status, diabetes at baseline, and cognitive impairment at follow-up were entered as dichotomous variables, whereas age and years of further education above 12 years were entered as three categories. Serum creatinine, initial brain volume, CAMCOG score, systolic blood pressure, and serum triglycerides were entered as categorical variables (quartiles). A p value < 0.05 was considered significant. SPSS for Windows (15th ed.; Chicago, IL) was used for all statistical analyses.

RESULTS **Baseline characteristics of the subjects.** The baseline characteristics of the study population are listed in table 1. The mean age of the 107 participants was 73.2 years, and 54% were women. In general, the educational level was relatively high because 60% of subjects had more than 12 years of education, and few were current smokers. At baseline, systolic blood pressure and serum creatinine were in the high normal range, probably reflecting the advanced age of the participants. Plasma vitamin B_{12} was within normal range, with a geometric mean of 363 pmol/L, and no participant had a vitamin B_{12} level below 150 pmol/L. Age and systolic blood pressure at baseline were higher in the 41 subjects who were excluded from the study because they did not have a final MRI scan, but there were no significant differences between these subjects and the 107 subjects who completed the study in terms of CAMCOG score, education, ApoE ε4 status, diabetes mellitus, vitamin B_{12} level, or initial brain volumes (data not shown). During the 5 years of follow-up, the mean PBVL per year in the 107 subjects in the study was 0.69. The PBVL did not differ between men and women or according to ApoE ε4 status. At baseline, subjects with the greatest PBVL at follow-up were older, had higher tHcy and MMA concentrations,

Figure Linear regression plots (mean and 95% CI) showing correlations between PBVL per year and levels of (A) vitamin B_{12} or (B) holoTC*



*Unadjusted models, logarithmic scales; $p < 0.001$ for both. PBVL = percentage of brain volume loss; holoTC = holotranscobalamin.

and had lower vitamin B_{12} and holoTC concentrations (table 1).

Correlations with brain volume loss. In simple, unadjusted correlations, increasing age ($r = 0.37$, $p < 0.001$), initial brain volume ($r = -0.34$, $p < 0.001$), and baseline CAMCOG score ($r = -0.21$, $p = 0.034$) were all associated with greater PBVL. Systolic blood pressure, educational level, and serum concentrations of folate, creatinine, and triglycerides at baseline were not significantly associated with PBVL.

In the figure, linear regression plots for PBVL and vitamin B_{12} and holoTC are presented, and figure e-1 on the *Neurology*® Web site at www.neurology.org shows MRI scans from subjects with contrasting holoTC levels. There is a linear association between vitamin B_{12} ($r = 0.33$) and holoTC ($r = 0.36$) vs PBVL without an apparent threshold effect. Based on the R^2 of the linear regression, up to 13% of the variance in PBVL is explained by vitamin B_{12} status. After adjusting for age and sex, the association was slightly weakened (partial $r = 0.27$ [B_{12}] and 0.26 [holoTC], $p < 0.01$ for both). Even after further adjustment to include a variety of covariates (model 2), the associations remained significant (partial $r = 0.25$ [B_{12}] and 0.22 [holoTC]). Additional adjustments for folate, tHcy, triglycerides, the use of supplements, or the presence of diabetes at baseline or cognitive impairment at follow-up only modestly changed the associations.

MMA ($r = 0.27$) and TC saturation ($r = -0.29$) were only associated with PBVL in unadjusted analyses ($p = 0.002$ for both), not after further adjustments. Plasma tHcy was also only correlated with PBVL in the unadjusted analyses ($r = 0.28$, $p = 0.004$). Folate was not associated with PBVL either before or after adjustments.

Risk of increased brain volume loss. We defined increased loss as having a PBVL in the highest tertile. Table 2 shows adjusted odds ratios (ORs) with 95% CIs for increased PBVL according to baseline concentrations of plasma vitamin B_{12}, holoTC, and TC saturation divided into tertiles.

In the first model (adjustment for age and sex), individuals with plasma vitamin B_{12} in the bottom tertile (<308 pmol/L) compared with those in the top tertile (>386 pmol/L) had a more than threefold risk of increased PBVL ($p = 0.047$). When initial brain volume, creatinine, years of further education, CAMCOG scores, systolic blood pressure, and ApoE $\varepsilon 4$ status were added to the model, there was a sixfold increased risk of high PBVL ($p = 0.026$). Similar patterns were found for holoTC and for TC saturation, i.e., there was an approximate sixfold increased risk of high PBVL for those in the lowest tertile in the more extensively adjusted model (table 2). Addition of folate, tHcy, triglycerides, use of supplements, history diabetes at baseline, and cognitive impairment at the end of the study to the model did not alter these associations (data not shown).

In contrast, individuals with MMA in the top tertile (>0.22 μmol/L) compared with those in the bottom tertile showed no association with increased PBVL (OR 1.99, 95% CI 0.42–9.54). Similarly, tHcy in the top tertile (>12.8 μmol/L) was only associated with PBVL in the crude model (OR 2.87, 95% CI 1.09–7.59) (data not shown). After adjustments according to model 2, neither MMA nor tHcy was related to PBVL. We observed no significant change in risk of high PBVL across tertiles of serum folate (data not shown).

DISCUSSION We have shown that markers of low vitamin B_{12} status in elderly subjects without dementia at baseline are associated with an increased rate of loss in brain volume. Our study adds low vitamin B_{12} status to factors that may be modifiable causes of brain atrophy in the elderly.

Table 2 Odds ratios for PBVL per year over 5 years for loss in the highest tertile vs the other two tertiles by plasma vitamin B_{12}, holoTC, and TC saturation levels

	PBVL over 5 y			
	Simple model*		Adjusted model 2†	
Tertiles of dependent variable	OR (95% CI)	*p* Value	OR (95% CI)	*p* Value
Vitamin B_{12}				
>386 pmol/L	1.00 (reference)		1.00 (reference)	
308–386 pmol/L	2.89 (0.90–9.33)	0.076	4.39 (1.01–19.03)	0.048
<308 pmol/L	3.35 (1.02–11.00)	0.047	6.17 (1.25–30.47)	0.026
p Trend		0.053		0.028
HoloTC				
>78 pmol/L	1.00 (reference)		1.00 (reference)	
54–78 pmol/L	1.55 (0.50–4.86)	0.451	2.63 (0.63–10.92)	0.184
<54 pmol/L	2.61 (0.83–8.20)	0.100	5.99 (1.21–29.81)	0.029
p Trend		0.097		0.029
TC saturation				
>8.4%	1.00 (reference)		1.00 (reference)	
5.7–8.4%	2.83 (0.87–9.20)	0.085	6.64 (1.31–33.73)	0.022
<5.7%	2.46 (0.73–8.27)	0.145	6.63 (1.22–36.05)	0.029
p Trend		0.152		0.041

*Adjusted for age and sex.
†As simple model plus adjustment for initial Cambridge Mental Disorders of the Elderly Examination (CAMCOG) score, serum creatinine, years of further education, systolic blood pressure, initial brain volume, ApoE ε4 polymorphism.
PBVL = percentage of brain volume loss; holoTC = holotranscobalamin; TC = transcobalamin; OR = odds ratio.

Our population had general good health and overall a high education level. Furthermore, in contrast to other reports from UK population studies of older people,[29-32] vitamin B_{12} status seemed good, with higher vitamin B_{12} and holoTC and lower tHcy and MMA than in previous studies. None had vitamin B_{12} deficiency, defined as plasma vitamin $B_{12} < 150$ pmol/L. Even though the sample consisted of subjects with relatively good vitamin B_{12} status, the study was able to find a strong association of vitamin B_{12} markers with brain volume loss.

The major finding of this study is that at baseline, vitamin B_{12} status across the normal range is associated with brain atrophy at follow-up. In both linear and logistic regression analysis, plasma vitamin B_{12} and holoTC were equally strong and significant predictors of brain volume loss after adjusting for several potential confounding factors. In contrast, there was no association for high levels of MMA. Moreover, in adjusted analyses, we did not observe an association between tHcy and brain atrophy, which is in agreement with one recent study.[18] However, tHcy has been suggested as a risk factor for brain atrophy in other studies (see introduction).

Our study showed no significant association of folate with brain volume loss. In contrast, a prospective study observed that low serum folate was associated with atrophy of the cerebral cortex at autopsy,[33] whereas a cross-sectional study showed that serum folate is associated with hippocampal and amygdala volumes.[18]

Our limited sample size including relatively healthy elderly did not allow us investigate the hypothesis that low vitamin B_{12} status could cause cognitive impairment through its facilitation of brain atrophy. However, a number of studies have reported findings that low vitamin B_{12} status is associated with impaired cognition.[5,11-13] Elevated tHcy is correlated with cognitive decline and predicts the development of dementia in case–control and cross-sectional studies and in prospective studies of community-dwelling elderly.[34,35] Associations between cognition and MMA, or holoTC, are less well documented. In a case–control study of confirmed AD patients,[14] it was found that low holoTC or high MMA was associated with autopsy-confirmed AD. In community studies, low holoTC[31] or high MMA[32] was associated with cognitive impairment.

Although the mechanisms underlying the observed associations remain to be established, certain hypotheses can be considered. Besides age, risk factors for brain atrophy include hypertension, diabetes mellitus, hyperlipidemia, and elevated plasma tHcy.

It has been proposed that vitamin B_{12} exerts an indirect and longer-term effect on the brain through its effect on tHcy.[4] However, in our study, tHcy was not related to brain atrophy. Low concentrations of vitamin B_{12} may influence brain function through methylation reactions in the brain. Vitamin B_{12} is required for the methylation of homocysteine and the subsequent formation of *S*-adenosylmethionine, a crucial methyl donor in the brain.[36] Evidence suggests that deficiency of *S*-adenosylmethionine is related to white matter demyelination. In infants with vitamin B_{12} deficiency[37] and in subjects with inborn errors of vitamin B_{12} metabolism,[38] deficiency of *S*-adenosylmethionine is associated with brain atrophy, which is reversible if treatment is initiated. Another possibility is that our finding of brain atrophy could be related to the lesions observed in subacute combined degeneration after vitamin B_{12} deficiency. These neuropathologic lesions have been associated with overproduction of the myelinolytic tumor necrosis factor α and the reduced synthesis of the two neurotrophic agents, epidermal growth factor and interleukin 6.[39] Taken as a whole, our data suggest that subclinical low vitamin B_{12} status, within what is usually considered to be the normal range, can affect brain volume even in the early stages of cognitive decline possibly by disturbing the integrity of brain myelin or through inflammation. Thus, early treatment of low vitamin B_{12} status may prevent further brain volume loss. These hypotheses provide plausible mechanisms through which vitamin B_{12} can affect brain function and volume. However, further work is required to establish whether these associations are causal. Because it has been established that blood levels of vitamin B_{12} and holoTC can be markedly increased through oral supplementation of high-dose vitamin B_{12},[23,40] clinical trials could test whether supplementation with vitamin B_{12} can slow or prevent atrophy of the brain in the elderly.

Strengths of our investigation include its prospective design with a relatively long follow-up period and its population-based setting. Another advantage is the use of various metabolic markers of vitamin B_{12} status more accurately to characterize vitamin B_{12} status in subjects. The longitudinal assessment of brain atrophy with MRI scans at yearly intervals overcomes the inaccuracies related to short follow-up time. Limitations of the study include the relatively small sample size and that we did not investigate whether the loss in brain volume is focal or diffuse. Forty-one subjects of 148 did not have a second brain scan. These subjects had higher age and systolic blood pressure but did not differ in other measured parameters, most importantly neither in baseline brain volume nor in vitamin B_{12} status. Furthermore, adjustment for relevant covariates (including systolic blood pressure and age) did not alter the main result. However, as with all cohort studies in contrast to randomized trials, we cannot exclude that residual confounding, due to unknown factors, might account for the findings.

Our study shows that low vitamin B_{12} status at baseline is an important risk factor for loss of brain volume in older community-dwelling adults. These findings suggest that plasma vitamin B_{12} status may be an early marker of brain atrophy and thus a potentially important modifiable risk factor for cognitive decline in the elderly. Larger studies in different population settings using sensitive vitamin B_{12} status markers are needed to support these conclusions. Moreover, intervention studies will help to define whether optimization of vitamin B_{12} status contributes to the maintenance of cognitive performance with successful aging.

AUTHOR CONTRIBUTIONS

The statistical analysis was conducted by H.R. and A.V.

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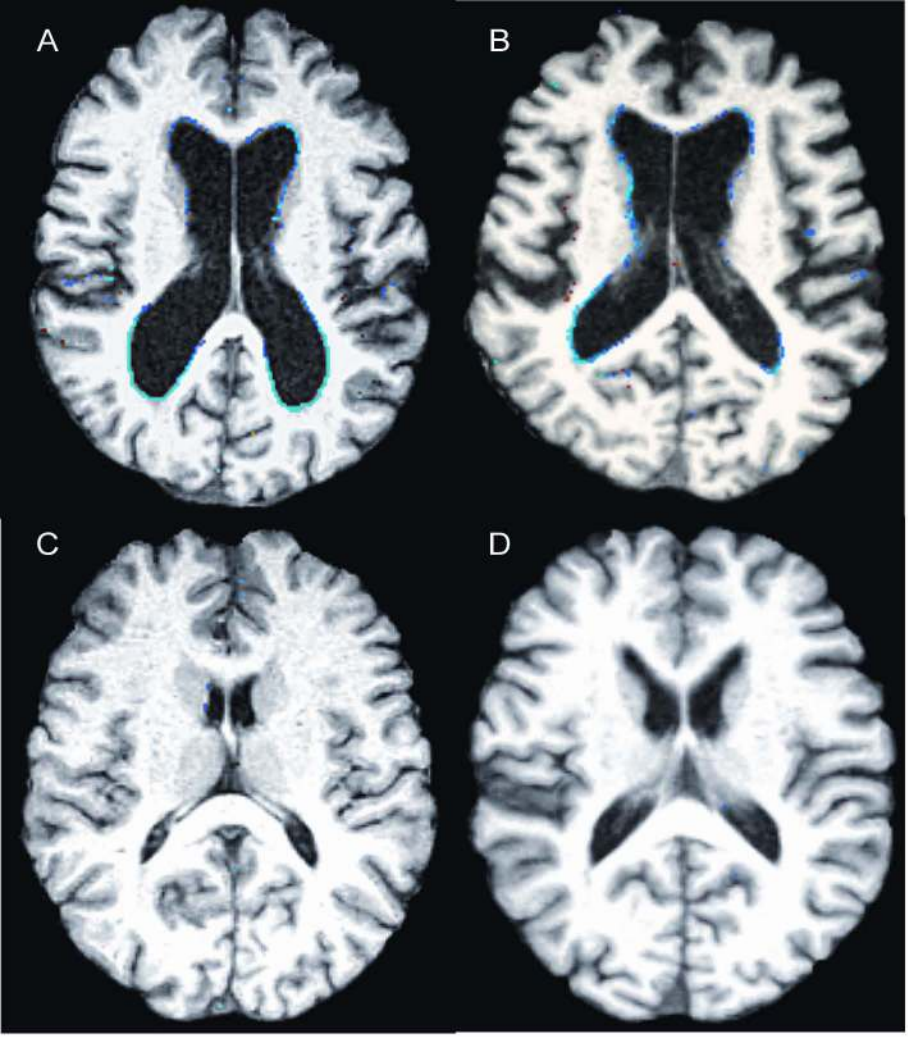

E-Figure 1 (Supplemental data on web). MRI subtraction scans of four subjects with contrasting holoTC values and brain volume changes. Subjects A and B had a high rate of brain volume loss per year and low levels of baseline plasma holoTC while subjects C and D had a low rate of brain volume loss per year and high levels of baseline plasma holoTC. The colored parts of the brain-tissue boundaries show where the boundary has moved more than 1mm between scans; blue means that the boundary has moved towards brain tissue (atrophy) and red shows the opposite.