

Vitamin B₁₂ status and rate of brain volume loss in community-dwelling elderly

A. Vogiatzoglou, MSc
H. Refsum, MD, PhD
C. Johnston
S.M. Smith, DPhil
K.M. Bradley, FRCR,
FRCP
C. de Jager, PhD
M.M. Budge, MD
A.D. Smith, DPhil,
FMedSci

Address correspondence and reprint requests to Anna Vogiatzoglou, Department of Physiology, Anatomy and Genetics, University of Oxford, Le Gros Clark Building, South Parks Rd., Oxford OX1 3QX, UK
anna.vogiatzoglou@dpag.ox.ac.uk

ABSTRACT

Objectives: To investigate the relationship between markers of vitamin B₁₂ 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 B₁₂, 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 B₁₂ and holoTC levels and higher plasma tHcy and MMA levels at baseline. Linear regression analysis showed that associations with vitamin B₁₂ 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 B₁₂ 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 B₁₂ 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 AD² and is a marker of disease progression.³

An elevated total homocysteine (tHcy) concentration is an independent risk factor for AD⁴ 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 B₁₂ levels.⁸ Vitamin B₁₂ deficiency is a public

Supplemental data at
www.neurology.org

From OPTIMA (A.V., H.R., C.J., K.M.B., C.d.J., M.M.B., A.D.S.), Department of Physiology, Anatomy and Genetics, University of Oxford, UK; Institute of Basic Medical Sciences (A.V., H.R.), Department of Nutrition, University of Oslo, Norway; Oxford University Centre for Functional MRI of the Brain (S.M.S.), UK; and Department of Geriatric Medicine (M.M.B.), The Canberra Hospital and Australian National University Medical School, Australia.

Supported by Alzheimer's Research Trust (UK), the Medical Research Council, the Charles Wolfson Charitable Trust, the Norwegian Foundation for Health and Rehabilitation through the Norwegian Health Association, Axis-Shield plc, and the Johan Throne Holst Foundation for Nutrition Research.

Disclosure: The authors report no disclosures.

health problem, particularly among the elderly.^{9,10} Several studies have reported associations between impaired vitamin B₁₂ 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 B₁₂ status and brain atrophy in the elderly are limited^{17,18} and have used measurement of total plasma vitamin B₁₂, which has low diagnostic accuracy.⁸ Increased concentrations of methylmalonic acid (MMA) and tHcy are generally considered more sensitive metabolic markers of vitamin B₁₂ status.⁸ To improve the accuracy of diagnosis of vitamin B₁₂ 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 B₁₂ available for tissue uptake, and both have been proposed as more useful markers than total plasma vitamin B₁₂,^{19,21,22} especially for early changes in vitamin B₁₂ homeostasis.²³

In this study, we used longitudinal MRI to examine the relationship of baseline vitamin B₁₂ 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 de-

scribed.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 B₁₂ 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 × 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 B₁₂ were determined with microbiologic assays using a colistin sulfate-resistant strain of *Lactobacillus leichmannii*.²⁶ The vitamin B₁₂ 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 B₁₂ as above.²⁷ Plasma TC was measured after saturating TC with added vitamin B₁₂. The coefficient of variation (CV) for total plasma vitamin B₁₂ 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 non-linearity 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.

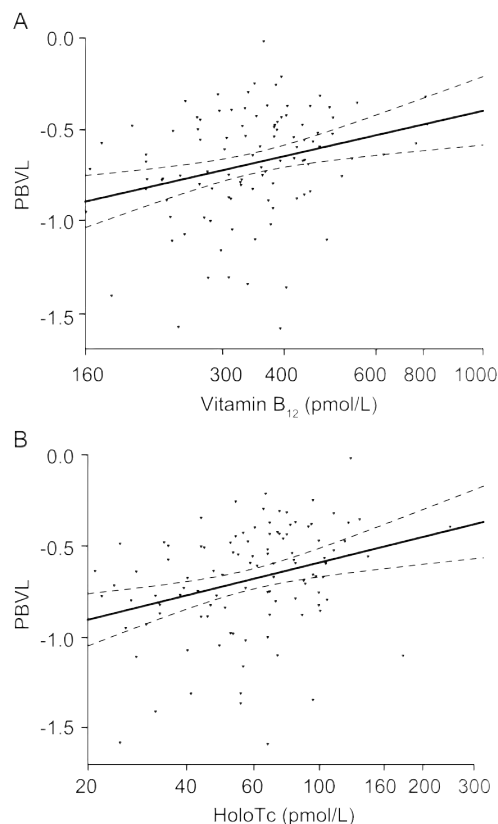
regression analyses, two models were routinely used. The first model was adjusted for age and sex; the second model was further adjusted for initial brain volume, creatinine, years of further education, CAMCOG score, systolic blood pressure, and ApoE ε4 status. Further adjustment was performed for additional baseline variables (history of diabetes, use of vitamin B₁₂ 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 B₁₂ was within normal range, with a geometric mean of 363 pmol/L, and no participant had a vitamin B₁₂ 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 B₁₂ 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 B₁₂ or (B) holoTC*



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

and had lower vitamin B₁₂ 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 B₁₂ 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 B₁₂ ($r = 0.33$) 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 B₁₂ status. After adjusting for age and sex, the association was slightly weakened (partial $r = 0.27$ [B₁₂] 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 $r = 0.25$ [B₁₂] 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 B₁₂, holoTC, and TC saturation divided into tertiles.

In the first model (adjustment for age and sex), individuals with plasma vitamin B₁₂ 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 $\epsilon 4$ 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 $\mu\text{mol/L}$) 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 $\mu\text{mol/L}$) 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 B₁₂ status in elderly subjects without dementia at baseline are associated with an increased rate of loss in brain volume. Our study adds low vitamin B₁₂ 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 B₁₂, holoTC, and TC saturation levels

Tertiles of dependent variable	PBVL over 5 y			
	Simple model*		Adjusted model 2*	
	OR (95% CI)	p Value	OR (95% CI)	p Value
Vitamin B₁₂				
>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 B₁₂ status seemed good, with higher vitamin B₁₂ and holoTC and lower tHcy and MMA than in previous studies. None had vitamin B₁₂ deficiency, defined as plasma vitamin B₁₂ < 150 pmol/L. Even though the sample consisted of subjects with relatively good vitamin B₁₂ status, the study was able to find a strong association of vitamin B₁₂ markers with brain volume loss.

The major finding of this study is that at baseline, vitamin B₁₂ status across the normal range is associated with brain atrophy at follow-up. In both linear and logistic regression analysis, plasma vitamin B₁₂ 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 prospec-

tive 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 B₁₂ status could cause cognitive impairment through its facilitation of brain atrophy. However, a number of studies have reported findings that low vitamin B₁₂ 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 holoTC³¹ or high MMA³² 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 B₁₂ 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 B₁₂ may influence brain function through methylation reactions in the brain. Vitamin B₁₂ 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 B₁₂ deficiency³⁷ and in subjects with inborn errors of vitamin B₁₂ 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 B₁₂ 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 B₁₂ 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 B₁₂ status may prevent further brain volume loss. These hypotheses provide plausible mechanisms through which vitamin B₁₂ 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 B₁₂ and holoTC can be markedly increased through oral supplementation of high-dose vitamin B₁₂,^{23,40} clinical trials could test whether supplementation with vitamin B₁₂ 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 B₁₂ status more accurately to characterize vitamin B₁₂ 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 B₁₂ status. Further-

more, 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 B₁₂ status at baseline is an important risk factor for loss of brain volume in older community-dwelling adults. These findings suggest that plasma vitamin B₁₂ 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 B₁₂ status markers are needed to support these conclusions. Moreover, intervention studies will help to define whether optimization of vitamin B₁₂ status contributes to the maintenance of cognitive performance with successful aging.

AUTHOR CONTRIBUTIONS

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

ACKNOWLEDGMENT

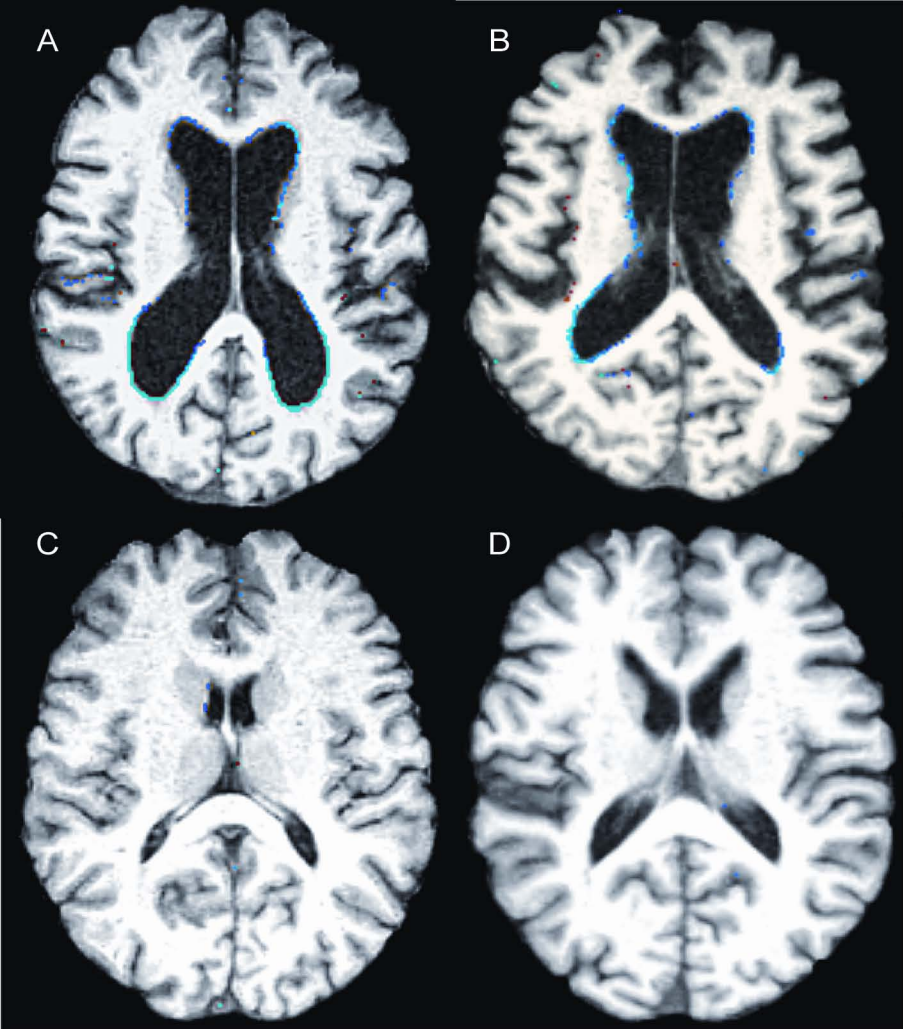
The authors thank all volunteers in the project, Cynthia Prendergast for her valuable assistance in the vitamin assays, and Donald Warden for determining the ApoE ϵ 4 genotypes.

Received December 12, 2007. Accepted in final form June 4, 2008.

REFERENCES

1. Smith AD. Imaging the progression of Alzheimer pathology through the brain. *Proc Natl Acad Sci USA* 2002;99:4135–4137.
2. Jobst KA, Smith AD, Szatmari M, et al. Detection in life of confirmed Alzheimer's disease using a simple measurement of medial temporal lobe atrophy by computed tomography. *Lancet* 1992;340:1179–1183.
3. Bradley KM, Bydder GM, Budge MM, et al. Serial brain MRI at 3-6 month intervals as a surrogate marker for Alzheimer's disease. *Br J Radiol* 2002;75:506–513.
4. Clarke R, Smith AD, Jobst KA, et al. Folate, vitamin B12, and serum total homocysteine levels in confirmed Alzheimer disease. *Arch Neurol* 1998;55:1449–1455.
5. Tucker KL, Qiao N, Scott T, et al. High homocysteine and low B vitamins predict cognitive decline in aging men: the Veterans Affairs Normative Aging Study. *Am J Clin Nutr* 2005;82:627–635.
6. den Heijer T, Vermeer SE, Clarke R, et al. Homocysteine and brain atrophy on MRI of non-demented elderly. *Brain* 2003;126:170–175.
7. 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.
8. Lindenbaum J, Rosenberg IH, Wilson PW, et al. Prevalence of cobalamin deficiency in the Framingham elderly population. *Am J Clin Nutr* 1994;60:2–11.
9. Baik HW, Russell RM. Vitamin B12 deficiency in the elderly. *Annu Rev Nutr* 1999;19:357–377.

10. Joosten E, van den Berg A, Riezler R, et al. Metabolic evidence that deficiencies of vitamin B-12 (cobalamin), folate, and vitamin B-6 occur commonly in elderly people. *Am J Clin Nutr* 1993;58:468–476.
11. Duthie SJ, Whalley LJ, Collins AR, et al. Homocysteine, B vitamin status, and cognitive function in the elderly. *Am J Clin Nutr* 2002;75:908–913.
12. Goodwin JS, Goodwin JM, Garry PJ. Association between nutritional status and cognitive functioning in a healthy elderly population. *JAMA* 1983;249:2917–2921.
13. Morris MS, Jacques PF, Rosenberg IH, Selhub J. Folate and vitamin B-12 status in relation to anemia, macrocytosis, and cognitive impairment in older Americans in the age of folic acid fortification. *Am J Clin Nutr* 2007;85:193–200.
14. Refsum H, Smith AD. Low vitamin B-12 status in confirmed Alzheimer's disease as revealed by serum holotranscobalamin. *J Neurol Neurosurg Psychiatry* 2003;74:959–961.
15. Kado DM, Karlamangla AS, Huang MH, et al. Homocysteine versus the vitamins folate, B6, and B12 as predictors of cognitive function and decline in older high-functioning adults: MacArthur Studies of Successful Aging. *Am J Med* 2005;118:161–167.
16. Kang JH, Irizarry MC, Grodstein F. Prospective study of plasma folate, vitamin B12, and cognitive function and decline. *Epidemiology* 2006;17:650–657.
17. Sachdev PS, Valenzuela M, Wang XL, et al. Relationship between plasma homocysteine levels and brain atrophy in healthy elderly individuals. *Neurology* 2002;58:1539–1541.
18. Scott TM, Tucker KL, Bhadelia A, et al. Homocysteine and B vitamins relate to brain volume and white-matter changes in geriatric patients with psychiatric disorders. *Am J Geriatr Psychiatry* 2004;12:631–638.
19. Miller JW, Garrod MG, Rockwood AL, et al. Measurement of total vitamin B12 and holotranscobalamin, singly and in combination, in screening for metabolic vitamin B12 deficiency. *Clin Chem* 2006;52:278–285.
20. Nexø E, Christensen AL, Hvas AM, et al. Quantification of holo-transcobalamin, a marker of vitamin B12 deficiency. *Clin Chem* 2002;48:561–562.
21. Bor MV, Nexø E, Hvas AM. Holo-transcobalamin concentration and transcobalamin saturation reflect recent vitamin B12 absorption better than does serum vitamin B12. *Clin Chem* 2004;50:1043–1049.
22. Hvas AM, Nexø E. Holotranscobalamin: A first choice assay for diagnosing early vitamin B deficiency? *J Intern Med* 2005;257:289–298.
23. Nexø E, Hvas AM, Bleie O, et al. Holo-transcobalamin is an early marker of changes in cobalamin homeostasis: a randomized placebo-controlled study. *Clin Chem* 2002;48:1768–1771.
24. de Jager CA, Milwain E, Budge M. Early detection of isolated memory deficits in the elderly: the need for more sensitive neuropsychological tests. *Psychol Med* 2002;32:483–491.
25. Smith SM, Zhang Y, Jenkinson M, et al. Accurate, robust, and automated longitudinal and cross-sectional brain change analysis. *Neuroimage* 2002;17:479–489.
26. Kelleher BP, Broin SD. Microbiological assay for vitamin B12 performed in 96-well microtitre plates. *J Clin Pathol* 1991;44:592–595.
27. Refsum H, Johnston C, Guttormsen AB, Nexø E. Holotranscobalamin and total transcobalamin in human plasma: determination, determinants, and reference values in healthy adults. *Clin Chem* 2006;52:129–137.
28. Husek P. Simultaneous profile analysis of plasma amino and organic acids by capillary gas chromatography. *J Chromatogr* 1995;669:352–357.
29. Clarke R, Grimley Evans J, Schneede J, et al. Vitamin B12 and folate deficiency in later life. *Age Ageing* 2004;33:34–41.
30. Clarke R, Refsum H, Birks J, et al. Screening for vitamin B-12 and folate deficiency in older persons. *Am J Clin Nutr* 2003;77:1241–1247.
31. Hin H, Clarke R, Sherliker P, et al. Clinical relevance of low serum vitamin B12 concentrations in older people: the Banbury B12 study. *Age Ageing* 2006;35:416–422.
32. McCracken C, Hudson P, Ellis R, McCaddon A. Methylmalonic acid and cognitive function in the Medical Research Council Cognitive Function and Ageing Study. *Am J Clin Nutr* 2006;84:1406–1411.
33. Snowdon DA, Tully CL, Smith CD, et al. Serum folate and the severity of atrophy of the neocortex in Alzheimer disease: findings from the Nun study. *Am J Clin Nutr* 2000;71:993–998.
34. Nurk E, Refsum H, Tell GS, et al. Plasma total homocysteine and memory in the elderly: the Hordaland Homocysteine Study. *Ann Neurol* 2005;58:847–857.
35. Seshadri S, Beiser A, Selhub J, et al. Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. *N Engl J Med* 2002;346:476–483.
36. Selhub J, Bagley LC, Miller J, Rosenberg IH. B vitamins, homocysteine, and neurocognitive function in the elderly. *Am J Clin Nutr* 2000;71:614S–620S.
37. Lovblad K, Ramelli G, Remonda L, et al. Retardation of myelination due to dietary vitamin B12 deficiency: cranial MRI findings. *Pediatr Radiol* 1997;27:155–158.
38. Rossi A, Cerone R, Biancheri R, et al. Early-onset combined methylmalonic aciduria and homocystinuria: neuro-radiologic findings. *Am J Neuroradiol* 2001;22:554–563.
39. Scalabrino G. Cobalamin (vitamin B(12)) in subacute combined degeneration and beyond: traditional interpretations and novel theories. *Exp Neurol* 2005;192:463–479.
40. Eussen SJ, de Groot LC, Clarke R, et al. Oral cyanocobalamin supplementation in older people with vitamin B12 deficiency: a dose-finding trial. *Arch Intern Med* 2005;165:1167–1172.



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.