Low vitamin B-12 status and risk of cognitive decline in older adults[1–3]

Robert Clarke, Jacqueline Birks, Ebba Nexo, Per M Ueland, Joern Schneede, John Scott, Anne Molloy, and John Grimley Evans

ABSTRACT

Background: Elevated total homocysteine (tHcy) concentrations have been associated with cognitive impairment, but it is unclear whether low vitamin B-12 or folate status is responsible for cognitive decline.

Objective: We examined the associations of cognitive decline with vitamin B-12 and folate status in a longitudinal cohort study performed from 1993 to 2003 in Oxford, United Kingdom.

Design: Cognitive function was assessed with the Mini-Mental State Examination on ≥3 occasions during 10 y and related to serum concentrations of vitamin B-12, holotranscobalamin (holoTC), tHcy, methylmalonic acid (MMA), and folate with the use of linear mixed models in 1648 participants who provided blood in 1995.

Results: Cognitive function declined abruptly at younger ages in some participants but remained intact in others until very old age. In multivariate regression analyses after adjustment for established risk factors, concentrations of holoTC (a marker of reduced vitamin B-12 status), tHcy, and MMA predicted cognitive decline, but folate did not. A doubling in holoTC concentrations (from 50 to 100 pmol/L) was associated with a 30% slower rate of cognitive decline (−0.137 to −0.083), whereas a doubling in tHcy (from 10 to 20 μmol/L) or MMA (from 0.25 to 0.50 μmol/L) was associated with >50% more rapid cognitive decline (−0.090 to −0.169) and (−0.104 to −0.169), respectively. After adjustment for all vitamin markers simultaneously, the associations of cognitive decline with holoTC and MMA remained significant.

Conclusions: Low vitamin B-12 status was associated with more rapid cognitive decline. Randomized trials are required to determine the relevance of vitamin B-12 supplementation for prevention of dementia. *Am J Clin Nutr* 2007;86:1384–91.

KEY WORDS Vitamin B-12, holotranscobalamin, folate, cognitive decline

INTRODUCTION

Elevated concentrations of serum total homocysteine (tHcy) have been linked with Alzheimer disease, but it is unclear whether this reflects underlying vascular disease that may have contributed to the dementia or insufficient folate or vitamin B-12 status (1, 2). The tHcy hypothesis of dementia has attracted considerable interest because tHcy concentrations are easily lowered by dietary supplementation with folic acid and vitamin B-12 (3), raising the prospect that these vitamins might prevent the onset of dementia. The initial epidemiologic evidence in support of this hypothesis came from retrospective case-control studies that reported elevated tHcy concentrations were associated with Alzheimer disease (1, 4, 5) or with cognitive impairment (6–15). Some prospective cohort studies (16, 17), but not all (18), have also reported associations of dementia with elevated tHcy concentrations. Vitamin B-12 deficiency is particularly common in older adults, and the prevalence increases with age (19). The introduction of mandatory folic acid fortification has prompted concerns about the safety for older adults with vitamin B-12 deficiency, with some reports indicating that persons with low vitamin B-12 status had a more rapid deterioration in cognitive function in a setting of high intakes of folic acid (20, 21).

The aim of this study was to assess the longitudinal associations of cognitive decline during 10 y with serum tHcy and related markers of vitamin B-12 and folate status in an elderly population without mandatory folic acid fortification. Cognitive function assessed with the use of the Mini-Mental State Examination (MMSE) on ≥3 occasions during 10 y was related to vitamin status after taking into account the established risk factors for cognitive impairment. In view of the poor predictive value of the standard vitamin B-12 assays (22), holotranscobalamin (holoTC; the biologically active fraction of vitamin B-12) and methylmalonic acid (MMA; an indicator

[1] From the Clinical Trial Service Unit, University of Oxford, (RC), Division of Clinical Geratology, Nuffield Department of Clinical Medicine (JB and JGE), University of Oxford, Oxford, United Kingdom; Department of Clinical Biochemistry, AS, Aarhus University Hospital, Aarhus, Denmark (EN); Section for Pharmacology, Institute of Medicine, University of Bergen and Auckland University Hospital, Bergen, Norway (PMU); Department of Clinical Chemistry, Umeå University Hospital, Umeå, Sweden (J Schneede); and Department of Biochemistry, Trinity College, Dublin, Ireland (AM and J Scott).

[2] Supported by the Medical Research Council and the Department of Health (MRC CFAS programme). The blood collection and analysis in the OHAP component of the MRC CFAS study was supported independently of CFAS by grants from the European Union (QLK3-CT-2002-01775), Health Foundation (554/1236), Food Standards Agency. and the Cloth Workers Foundation. JB was also supported by the Joan Dawkins Fellowship of the British Medical Association. All the analyses were performed independent of the sources of support.

[3] Reprints not available. Address correspondence to R Clarke, Clinical Trial Service Unit, University of Oxford, Richard Doll Building, Old Road Campus, Roosevelt Drive, Oxford, OX3 7LF, United Kingdom. E-mail: robert.clarke@ctsu.ox.ac.uk.

Received January 19, 2007.

Accepted for publication July 9, 2007.

The American Journal of Clinical Nutrition

of vitamin B-12 function) were used as markers of vitamin B-12 status.

SUBJECTS AND METHODS

Study population

The Oxford Healthy Aging Project is a longitudinal cohort study of 2741 randomly selected persons aged ≥65 y who resided in the city of Oxford, United Kingdom. The Oxford Healthy Aging Project study is a component part of the Medical Research Council Cognitive Function and Aging Study (MRC CFAS) that was designed as a UK population-based cohort study of the causes of dementia and cognitive decline (23, 24). In 1993, we randomly selected the population sample from general practice registers for people living in Oxford city to provide equal numbers of persons aged 65–74 y and ≥75 y (19). Research nurses visited all study participants in their own homes and performed a structured interview. The collected data asked about medical history, smoking, and use of medication. A history of vascular disease was defined as being present if persons gave positive answers to the Rose questionnaire for angina or peripheral vascular disease (25) or reported a history of a heart attack or stroke or transient ischemic attack. The design and characteristics of the MRC CFAS study were described in previous publications (http://www.cfas.ac.uk) (23, 24). Blood pressure was measured at the beginning and again at the end of the interview with the use of automated aneroid machines calibrated against a mercury sphygmomanometer, and the mean of the 2 readings was used for analysis.

Cognitive function

Measurement of cognitive function included the MMSE (26), and for most participants this was presented on ≥3 occasions. All participants underwent cognitive function assessment at enrollment in 1993, and most participants had a repeat MMSE in 1995. Some participants with suspected cognitive impairment, together with a sample of persons without cognitive impairment, were interviewed on >3 occasions (maximum 8 times) At 10 y in 2003, all participants who had not previously refused to be interviewed were invited for a further assessment of cognitive function. The version of MMSE used by MRC CFAS includes serial sevens, and the words to repeat and recall were "apple, table, penny" at enrollment and at year 2 and "tree, clock, boat" at all other interviews. If persons were unable to answer a particular question, they were classified as having a zero value for that question.

Blood collection

All surviving participants, who had not previously refused to be interviewed, were invited to provide a blood sample in 1995 (baseline) and again in 2003. The relation between each biochemical variable at baseline and at 10 y was investigated to estimate the extent to which a single measurement at 2 y could accurately classify persons during a 10-y period. Nonfasting blood samples were collected into plain evacuated tubes that were allowed to clot at room temperature, and the serum was separated within 2 h. Serum samples were stored at −80 °C until shipped on dry ice or thawed for analysis. All participants provided signed consent to participate in the study, which was approved by the Central Oxford Research Ethics Committee (COREC 97206) and the Multicenter Research Ethics Committee (05/MRE05/37).

Laboratory methods

Frozen blood samples were thawed for measurements of serum concentrations of creatinine, holoTC, tHcy, MMA, vitamin B-12, and folate. Serum holoTC measurements were performed at Aarhus University Hospital, Aarhus, Denmark, with the use of an enzyme-linked immunoabsorbent assay method modified for use on an automated analyzer (27). Serum tHcy measurements were measured on an Abbott IMx autoanalyzer (FPIA; Axis-Shield, Oslo, Norway) in Oxford, United Kingdom, by a fluorescence polarization immunoassay (18). Serum folate measurements were performed with the use of a microbiological method at the University of Dublin, Ireland (28), and serum vitamin B-12 measurements were performed on an ACS Centaur with an automated chemiluminescence system (Bayer A/S, Leverkusen, Germany), with the use of a competitive protein binding assay at Aarhus University Hospital, Aarhus, Denmark. MMA assays were done at the University of Bergen, Bergen, Norway, by gas chromatography–mass spectrometry (29). DNA was extracted from blood samples with the use of the DNeasy blood kit (Qiagen, West Sussex, United Kingdom) according to the manufacturer's protocol, and genotyping for *APOE* (apolipoprotein E gene) polymorphisms was performed with standard methods.

Statistical methods

Continuous variables were summarized as means and SDs or medians if more appropriate. Measurements of vitamin status were log transformed for all analyses because the distributions were extremely skewed. Participants ($n = 9$) with extreme elevations of vitamin B-12 (>1000 pmol/L) or holoTC (>400 pmol/L) or who reported use of vitamin B-12 injections or any B-vitamin supplements ($n = 22$) were excluded from all analyses of vitamin status. Data on some variables were missing either because the variable was not assessed or the participants were lost to follow-up. Nonresponse bias for blood sampling was examined by comparing the baseline characteristics of participants who provided a blood sample in 1995 with characteristics of the survivors who did not. Similarly, nonresponse bias for cognitive function at the end of the study was examined by comparing the baseline characteristics of participants who underwent cognitive testing in 2003 with the survivors who did not. One-factor random-effects analysis of variance models were used to estimate between- and within-person variability and, hence, reliability coefficients for concentrations of vitamin status in persons with replicate measurements. Provided that, conditional on baseline measurements, such data are "missing at random" for baseline measurements, a statistical model that includes both the follow-up and baseline measurements should provide unbiased estimates of reliability coefficients estimated as previously described (30). One-factor analysis of variance models were also used to examine the cross-sectional associations of cognitive function at year 0 and year 10 with status of established risk factors for cognitive impairment separately and with tertiles of vitamin status at baseline.

Longitudinal predictors of cognitive decline were assessed by linear mixed models relating MMSE scores to age at measurement with the use of the PROC MIXED procedure in SAS (SAS Institute Inc, Cary, NC). Differences in the rate of cognitive

The American Journal of Clinical Nutrition

TABLE 1
Distribution of characteristics at enrollment in all participants and in survivors with or without blood samples available in 1995 and in survivors with or without cognitive function scores available in 2003[1]

Baseline characteristic	All (n = 2741)	With blood samples (n = 1648)	Without blood samples (n = 1093)	Survivors examined at 10 y (n = 691)	Survivors not examined at 10 y (n = 653)
Age (y)	75.7 ± 7.0[2]	74.7 ± 6.5	77.3 ± 7.6[3]	71.9 ± 5.2	73.6 ± 5.7[4]
Men (%)	38.5	41.2	34.4[3]	39.6	32.0[5]
Current smoker (%)	16.8	16.1	18.0	13.5	16.4
Duration of education (y)	10.6 ± 2.9	10.8 ± 3.0	10.1 ± 2.5[3]	11.2 ± 3.2	10.5 ± 3.1[4]
Medical history					
Heart attack (%)	9.9	10.0	9.8	6.5	7.1
Angina (%)	16.6	16.5	16.7	13.2	13.4
Stroke (%)	6.4	5.6	7.8[6]	3.2	3.0
Peripheral vascular disease (%)	3.1	2.8	3.7	2.2	1.7
Any vascular disease (%)	34.6	33.9	35.7	27.6	25.6
Diabetes mellitus (%)	5.8	5.4	6.5	4.4	4.0
Systolic blood pressure (mm Hg)	155.6 ± 24.9	154.9 ± 23.8	157.0 ± 26.8	152.2 ± 22.6	156.7 ± 23.6[5]
Diastolic blood pressure (mm Hg)	86.5 ± 14.4	86.2 ± 14.0	87.1 ± 14.9	85.7 ± 13.0	87.5 ± 13.0[5]
MMSE (score; maximum: 30)	24.8 ± 5.6	26.2 ± 3.6	22.7 ± 7.2[3]	27.3 ± 2.7	25.6 ± 3.9[4]

[1] Data from some participants were missing for each characteristic. MMSE, Mini-Mental State Examination.
[2] $\bar{x}$ ± SD (all such values).
[3,6] Significantly different from subjects with blood samples: [3]$P < 0.001$, [6]$P < 0.05$.
[4,5] Significantly different from survivors examined at 10 y: [4]$P < 0.001$, [5]$P < 0.05$.

decline associated with differences in the concentrations of markers of vitamin status were tested by an age by vitamin concentration interaction term in the regression models. The random effects were the coefficients for the intercept and slopes of the regression of MMSE on age at measurement for each participant. Adjustment for covariates at baseline was performed with the use of fixed-effect terms for sex, smoking, history of vascular disease, systolic blood pressure, level of education, and *APOE* genotypes. Initially, the effect of differences in the concentrations of each vitamin marker was assessed separately in the mixed models ("single-vitamin" models). The estimate for the interaction between age and vitamin status indicates the additional annual deterioration in MMSE for a unit difference in the log-transformed values of vitamin status. Subsequently, the independent relevance of each marker of vitamin status was assessed in models, which included all markers of vitamin status simultaneously ("multiple-vitamin" models). All analyses were performed with the use of SAS version 9 (SAS Institute Inc).

RESULTS

Characteristics of study participants

Selected characteristics are shown in **Table 1** of all participants in 1993, of those who provided a blood sample in 1995, those who did not, and those who had a repeat assessment of cognitive function in 2003 compared with the survivors who did not. Among all participants (n = 2741), the mean (±SD) age was 75.7 ± 7 y, and mean MMSE score was 24.8 ± 5.6 (maximum score: 30). Blood samples were obtained from 1648 participants in 1995 and from 472 of the 691 survivors in 2003. Data in Table 1 show that participants who provided a blood sample in 1995 were 2.6 y younger ($P < 0.001$) and had a mean MMSE score that was 3.5 points (maximum score: 30) higher ($P < 0.001$) than those who did not do so. Surviving participants who had their cognitive function assessed at 10 y were 2 y younger and had an ≈2-point higher mean MMSE score at baseline than survivors who did not do so.

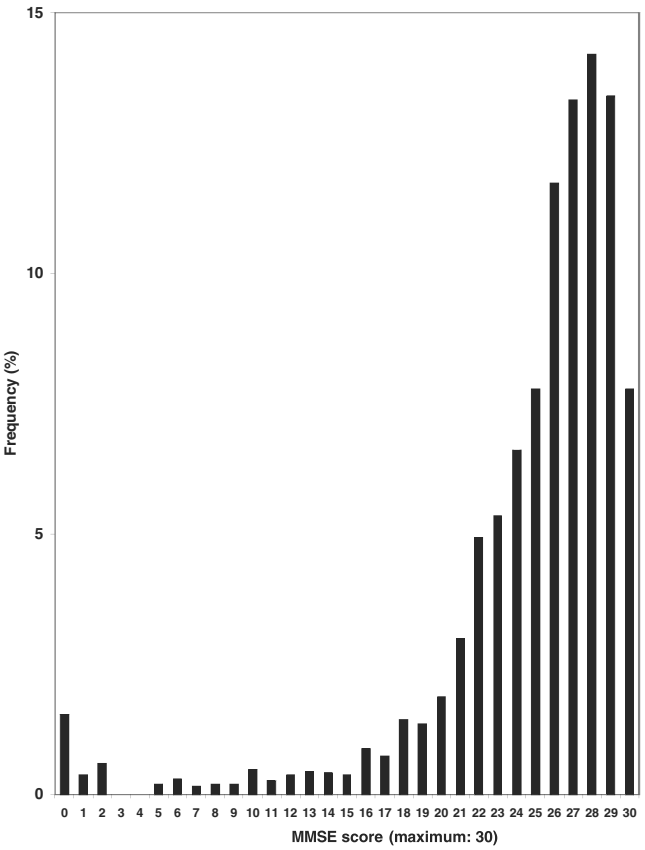

FIGURE 1. Distribution of Mini-Mental State Examination (MMSE) scores in all participants (n = 2741) at enrollment.

The American Journal of Clinical Nutrition

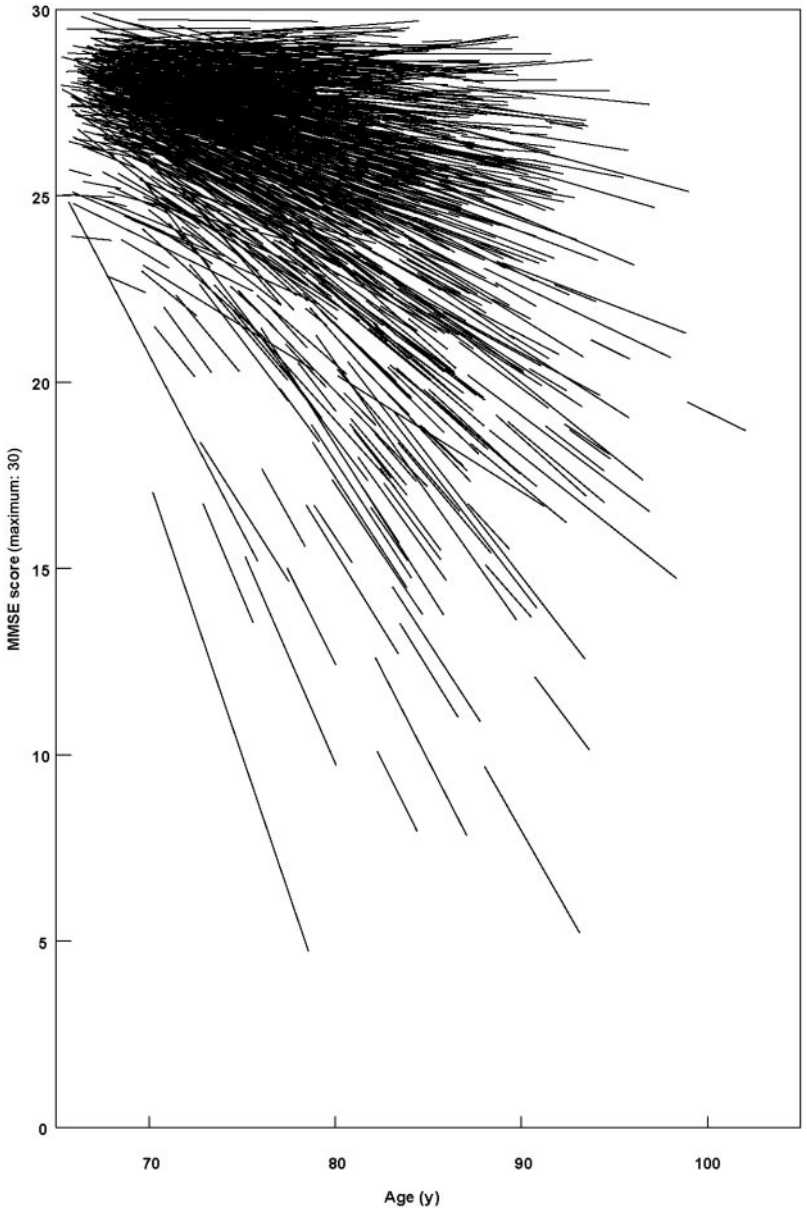

FIGURE 2. Trajectories of cognitive decline for all participants during 10 y ($n = 2741$). The fitted lines were obtained from linear mixed models adjusted for all covariates.

Variability in cognitive function and cognitive decline

The distribution of cognitive function in all participants at enrollment was positively skewed with a continuum of severity, including a small proportion with extreme cognitive impairment, consistent with a diagnosis of dementia (**Figure 1**). The variation in age-related cognitive declined within and between participants during the 10-y period (**Figure 2**). The lines presented in Figure 2 are the predicted lines obtained from a linear mixed model adjusted for baseline covariates and show that cognitive function declined abruptly and irreversibly at relatively younger ages in some participants, but remained intact in others until very old age.

Variability in vitamin status

The distribution of vitamin status at baseline in 1995 and in the subset with repeat blood samples in 2003 is shown in **Table 2**. Median serum concentrations of holoTC and vitamin B-12 were largely unchanged during the 8 y between measurements, but the concentrations of tHcy and MMA increased. Allowing for the attrition in data on vitamin status between 1995 and 2003, the intraclass correlation coefficients were 0.53 for holoTC and for MMA, and 0.27 for tHcy, 0.26 for vitamin B-12, and 0.20 for folate. At baseline, vitamin B-12 was inversely correlated with tHcy (Spearman correlation coefficient $= -0.24$; $P < 0.0001$) and with MMA ($r = -0.28$; $P < 0.0001$). HoloTC was also inversely associated with tHcy ($r = -0.28$; $P < 0.0001$) and with MMA ($r = -0.36$; $P < 0.0001$). In contrast, folate concentrations were inversely associated with tHcy concentrations ($r = -0.26$; $P < 0.0001$) but not with MMA concentrations ($r = -0.06$; NA), indicating that elevated MMA was a marker of low vitamin B-12 status and elevated tHcy was a marker of low status of both folate and vitamin B-12.

The American Journal of Clinical Nutrition

TABLE 2
Distribution of serum concentrations of holotranscobalamin (holoTC), vitamin B-12, folate, and related metabolites in 1995 and in 2003 and the intraclass correlation coefficients during an 8-y period between replicate measurements[1]

	holoTC	tHcy	MMA	Vitamin B-12	Folate
	pmol/L	*μmol/L*	*μmol/L*	*pmol/L*	*nmol/L*
Baseline blood measurements, 1995 (n = 1562)[2]					
Mean ± SD (untransformed)	73 ± 43	14.5 ± 6.3	0.35 ± 0.30	280 ± 106	15.8 ± 14.6
Median (25th–75th percentiles)	65 (45–89)	13.2 (10.5–16.7)	0.27 (0.22–0.37)	251 (198–320)	11.4 (7.5–18.6)
Repeat blood measurements, 2003 (n = 472)					
Mean ± SD in 1995	79 ± 43	12.3 ± 4.0	0.28 ± 0.15	274 ± 101	16.4 ± 12.7
Mean ± SD in 2003	70 ± 44	16.5 ± 6.2	0.39 ± 0.39	274 ± 139	12.8 ± 11.42
P values for t tests for blood concentrations in 1995 compared with 2003	0.62	<0.001	<0.001	0.52	<0.001
Geometric mean in 2003	60	15.5	0.31	257	10.0
Intraclass correlation coefficient (1995 compared with 2003)	0.53	0.27	0.53	0.26	0.20

[1] tHcy, total homocysteine; MMA, methylmalonic acid.
[2] Among the participants, 11 with vitamin B-12 concentrations > 1000 pmol/L were excluded, as were others with missing data for some biochemical variables.

Cross-sectional analyses of cognitive function with vitamin status

The cross-sectional associations of cognitive function in 1993 and in 2003 with age, sex, level of education, history of vascular disease, presence of any *APOE* ε4 allele, and tertiles of vitamin status in 1995 are shown in **Table 3**. In addition to the significant associations of cognitive function with established risk factors, such as age, sex, level of education, and prior history of vascular disease, cognitive function at baseline and at 10 y was also associated with holoTC, MMA, and tHcy concentrations.

Longitudinal analyses of cognitive decline with vitamin status

The longitudinal associations between cognitive decline and markers of vitamin status are shown in **Table 4** and Table S1 (*See* Table S1 under "Supplemental Data" in the online issue.). On average, the mean MMSE score (maximum score: 30) declined by ≈1.3 points during 10 y. The rate of cognitive decline was more rapid in women than in men (P = 0.01), in participants with higher systolic blood pressure (P = 0.01), and in participants with the *APOE* ε4 allele (P = 0.02), but it did not appear to vary by level of education, smoking, or history of vascular disease at enrollment. After adjustment for sex, education, smoking, history of vascular disease, systolic blood pressure, and *APOE* ε4, the effects of differences in individual markers of vitamin status when examined separately on the rates of cognitive decline in linear mixed models are shown in Table 4 (single-vitamin model) and **Figure 3**. In these multivariate analyses, a doubling in holoTC concentrations (eg, from 50 to 100 pmol/L) was associated with 30% slower rate of cognitive decline (−0.137 to −0.083), whereas a doubling in tHcy (eg, from 10 to 20 μmol/L) or MMA (eg, from 0.25 to 0.50 μmol/L) concentrations was associated with >50% more rapid cognitive decline (−0.090 to −0.169) and (−0.104 to −0.169), respectively. In contrast, cognitive decline was unrelated to either total vitamin B-12 or folate status (both of which provide a less precise measurement of vitamin status). These analyses were repeated with 3-factor interaction terms for folate and holoTC by age to assess any interaction in the rates of cognitive decline associated with low vitamin B-12 status and high folate status, and the interaction terms were not statistically significant (data not shown).

The effect on the rate of cognitive decline when all markers of vitamin status were examined simultaneously in the linear mixed models, which also included sex, education, smoking, systolic blood pressure, vascular disease, and *APOE*, is shown in Table 4 (multiple-vitamin model). These analyses showed significant independent associations of cognitive decline with low holoTC concentrations and with high MMA concentrations, but the association of cognitive decline with tHcy was attenuated and was no longer statistically significant after including the other markers of vitamin status (Table 4, multiple-vitamin model).

DISCUSSION

This longitudinal cohort study showed that low serum concentrations of holoTC (the biologically active fraction of vitamin B-12) and high MMA (a metabolic indicator of vitamin B-12 function) both reflecting low vitamin B-12 status were each independently and significantly associated with a more rapid cognitive decline during a 10-y period. In contrast, cognitive decline was not significantly associated with serum concentrations of folate or tHcy, after adjustment for the other markers of vitamin status. Assuming that these associations are causal, a doubling in the serum concentration of holoTC, achievable by oral vitamin B-12 supplementation (31), was associated with a slowing in the rate of age-associated cognitive decline by about one third. Moreover, if the associations with low vitamin B-12 status are fully reversible by therapy, then strategies to double the concentration of holoTC could delay the rate of cognitive decline by one third. In contrast with previous reports (19, 20), the present study found no interaction in the rate of cognitive decline between low vitamin B-12 and high folate status, providing no support for the hypothesis that an increased folate status was associated with a more rapid rate of cognitive decline among those with reduced vitamin B-12 status.

The American Journal of Clinical Nutrition

TABLE 3
Cognitive function values in participants at enrollment and at 10 y according to values for risk factors and vitamin status[1]

Known risk factors and vitamin status at baseline	At enrollment			At 10 y		
	Participants	MMSE score[2]	P[3]	Participants	MMSE score[2]	P[3]
	n			*n*		
Age						
<70 y	627	27.0 ± 3.4[4]	<0.0001	278	27.2 ± 3.6	<0.0001
70–74 y	700	26.4 ± 3.6		227	26.5 ± 3.3	
75–79 y	572	24.8 ± 4.8		112	24.6 ± 5.1	
80–84 y	486	23.3 ± 6.3		55	22.5 ± 7.6	
≥85 y	354	20.0 ± 8.2		20	20.6 ± 6.2	
Sex						
Male	1054	25.6 ± 4.6	<0.0001	273	26.6 ± 3.9	0.002
Female	1685	24.3 ± 6.2		419	25.6 ± 5.0	
Education						
<10 y	1370	23.5 ± 6.5	<0.0001	292	25.3 ± 4.5	0.0003
≥10 y	1275	26.4 ± 3.8		395	26.6 ± 4.4	
Vascular disease						
No	1746	25.4 ± 4.6	0.05	499	26.4 ± 3.9	0.0002
Yes	927	25.1 ± 4.7		192	25.0 ± 5.6	
APOE (ε4)						
No	1224	26.3 ± 3.5	NS	487	26.1 ± 4.4	NS
Yes	390	25.9 ± 4.4		124	26.2 ± 4.4	
holoTC						
<52 pmol/L	512	25.8 ± 3.6	0.001	146	25.4 ± 5.4	0.01
52–78 pmol/L	457	26.3 ± 3.6		195	26.1 ± 3.5	
≥79 pmol/L	502	26.6 ± 3.4		233	26.7 ± 3.9	
tHcy						
<11.3 μmol/L	507	26.8 ± 3.1	<0.0001	265	26.4 ± 4.1	0.004
11.3–15.2 μmol/L	512	26.5 ± 3.4		203	26.5 ± 3.6	
≥15.3 μmol/L	513	25.5 ± 4.0		130	25.1 ± 5.0	
MMA						
<0.24 μmol/L	510	26.9 ± 3.2	0.0001	263	26.5 ± 4.2	0.02
0.24–0.32 μmol/L	511	26.5 ± 3.3		196	26.4 ± 3.9	
≥0.33 μmol/L	510	25.4 ± 4.0		138	25.3 ± 4.7	

[1] Risk factor data were available for 2741 participants, and 1648 participants provided a blood sample. Data from some participants were missing for some characteristics. *APOE,* apolipoprotein E gene; holoTC, holotranscobalamin; tHcy, total homocysteine; MMA, methylmalonic acid; MMSE, Mini-Mental State Examination.
[2] Maximum score was 30.
[3] Determined with ANOVA.
[4] $\bar{x}$ ± SD (all such values).

The longitudinal design of this study suggests that differences in vitamin B-12 status preceded the onset of cognitive decline. The reliability of these associations is supported by the high level of agreement between replicate measurements of holoTC and MMA within participants during an 8-y period. Although participants were younger and had a higher level of cognitive function than did nonparticipants in the study, the effect of any nonresponse bias is to underestimate any associations of cognitive decline with vitamin status.

The strength of the associations of cognitive decline with vitamin B-12 status (Table 4) when assessed longitudinally (within-participant) was more modest than that observed in cross-sectional associations (between-participants) (Table 3). Moreover, the longitudinal associations of cognitive decline with tHcy concentrations in this study and in other longitudinal studies (16–18) were less extreme than those reported in cross-sectional or in retrospective studies (1, 4–17). In contrast with previous reports (1, 7, 11, 13), the present study suggests that the variability in rates of cognitive decline are primarily explained by reduced vitamin B-12 status. The Nurses Health Study of 635 women in the United States also had a longitudinal study design and a 10-y follow-up (1990 to about 2000), but it failed to identify any association of cognitive decline with plasma folate and vitamin B-12 concentrations (32). The introduction of mandatory folic acid fortification in 1996 in the United States may have obscured any associations with folate status in that study (32). In contrast, the present study conducted in a population without mandatory folic acid fortification used holoTC and MMA, which are more sensitive assays of vitamin B-12 status than are serum total vitamin B-12 concentrations alone (33). The results of the present longitudinal study are consistent with the findings of previous cross-sectional studies that reported associations of holoTC or MMA with cognitive impairment (34, 35) and with Alzheimer disease (36). Polymorphisms for the transcobalamin gene that influence vitamin B-12 status were not examined (37); hence, the present study was unable to address the relevance of these genetic variants for cognitive decline. Although the rate of cognitive decline was more rapid among

TABLE 4
Effect on cognitive decline during 10 y associated with differences in serum concentrations of vitamin status when examined separately and in combination[1]

	Single-vitamin model: separate effects of each marker in the multivariate regression model[2]			Multiple-vitamin model: joint effects of each marker in the multivariate regression model[2]		
	Coefficient (SE) for a doubling of vitamin concentration	Example of effect		Coefficient (SE) for a doubling of vitamin concentration	Example of effect	
		Change in vitamin status	Change in rate of cognitive decline		Change in vitamin status	Change in rate of cognitive decline
			MMSE/y			*MMSE/y*
holoTC (pmol/L)	0.053 (0.015)	from 50 to 100	−0.137 to −0.083	0.047 (0.019)	from 50 to 100	−0.136 to −0.089
tHcy (μmol/L)	−0.079 (0.023)	from 10 to 20	−0.090 to −0.169	−0.033 (0.029)	from 10 to 20	−0.105 to −0.138
MMA (μmol/L)	−0.065 (0.017)	from 0.25 to 0.50	−0.104 to −0.169	−0.044 (0.023)	from 0.25 to 0.50	−0.113 to −0.157
Vitamin B-12 (pmol/L)	0.022 (0.023)	from 150 to 300	−0.136 to −0.114	−0.044 (0.010)	from 150 to 300	−0.085 to −0.130
Folate (nmol/L)	0.015 (0.012)	from 10 to 20	−0.126 to −0.111	0.004 (0.013)	from 10 to 20	−0.119 to −0.115

[1] Values are for a change in cognitive function with age after adjustment for sex, education, smoking, history of vascular disease, systolic blood pressure, and apolipoprotein E (APOE), genotypes with each marker of vitamin status added separately (single-vitamin model) and with all markers of vitamin status included simultaneously (multiple-vitamin model). holoTC, holotranscobalamin; tHcy, total homocysteine; MMA, methylmalonic acid; MMSE, Mini-Mental State Examination.

[2] The absolute values are provided for men, aged 65 y with 10 y of education without *APOE* ε4 genotype and with median values of systolic blood pressure and median values of all other markers of vitamin status.

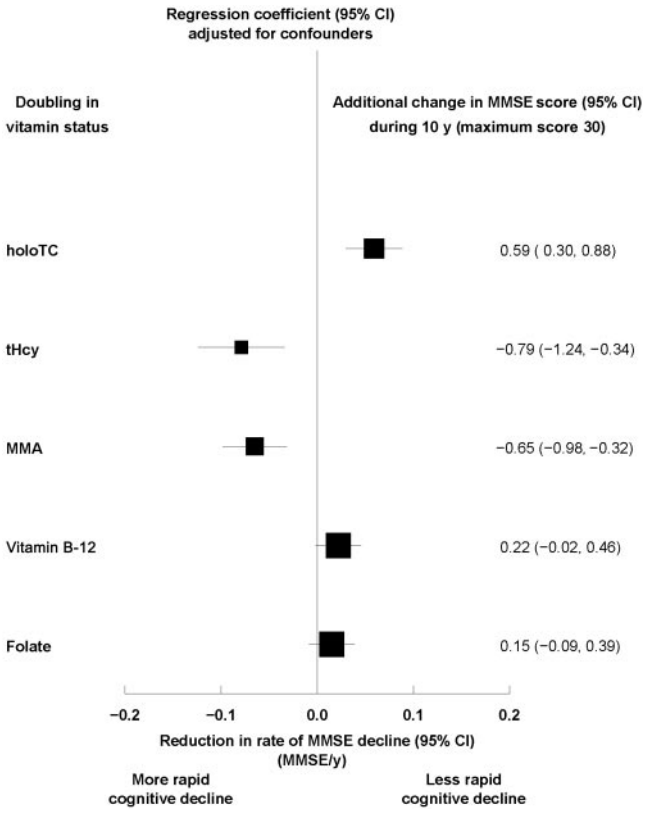

FIGURE 3. Associations of cognitive decline during 10 y (1993–2003) with a doubling in serum concentrations of markers of vitamin status. The values shown are regression coefficients for a doubling in serum concentrations of vitamin status after adjustment for sex, smoking, vascular disease, systolic blood pressure, education, apolipoprotein E gene ε4 alleles, and where each marker of vitamin status is examined separately. The point estimates are the regression coefficients for a reduction in the rate of cognitive decline, and the horizontal lines are the 95% CIs. holoTC, holotranscobalamin; tHcy, total homocysteine; MMA, methylmalonic acid.

participants with higher systolic blood pressure, no interactions of systolic blood pressure were observed with any of the markers of vitamin B-12 status.

The associations between low vitamin B-12 status and cognitive decline observed in the present study may well be relevant to older adults with cognitive impairment. Correction of vitamin B-12 deficiency may be appropriate among those with relevant symptoms. However, it is still not known whether correction of vitamin B-12 deficiency could attenuate the rate of cognitive decline because previous trials that have addressed this question have been too small and involved an inadequate duration of treatment to address this question reliably (38, 39). Randomized trials for the effects of B vitamins on cognitive function should be available from ongoing trials of B vitamins for prevention of cardiovascular disease (2, 40). Further trials in older adults are required to assess the effects of vitamin B-12 supplementation for the maintenance of cognitive function.

We thank all the participants in the Oxford Healthy Aging Project (OHAP), Olive Goddard and Pippa Whitbread for performing the data collection; and Kathleen Emmens for laboratory support. David Rubinsztein measured the Apo E polymorphisms and Paul Sherliker produced the figures. The MRC CFAS provided administrative support for the main interview data.

The author's responsibilities were as follows—RC, JB and JGE: conception and design of the study and acquisition of data; EN, PMU, J Schneede, J Scott, and AM: analyzed the laboratory data and critically reviewed the manuscript; JB: analyzed and interpreted the data; RC: wrote the draft of the manuscript; all authors critically reviewed the manuscript; RC and JGE: obtained funding and provided administrative, technical, and material support. None of the authors had a personal or financial conflict of interest.

REFERENCES

1. Clarke R, Smith AD, Jobst KA, Refsum H, Sutton L, Ueland PM. Folate, vitamin B12, and serum total homocysteine levels in confirmed Alzheimer disease. Arch Neurol 1998;55:1449–55.
2. Clarke R. Vitamin B12, folic acid, and the prevention of dementia. N Engl J Med 2006;354:2817–9.
3. Homocysteine Lower Trialists' Collaboration. Dose-dependent effects

of folic acid on blood concentrations of homocysteine: a meta-analysis of the randomized trials. Am J Clin Nutr 2005;82:806–12.
4. Joosten E, Lesaffre E, Riezler R, et al. Is metabolic evidence for vitamin B-12 and folate deficiency more frequent in elderly patients with Alzheimer's disease? J Gerontol A Biol Sci Med Sci 1997;52:76–9.
5. McCaddon A, Davies G, Hudson P, Tandy S, Cattell H. Total serum homocysteine in senile dementia of Alzheimer type. Int J Geriatr Psychiatry 1998;13:235–9.
6. Riggs KM, Spiro A III, Tucker K, Rush D. Relations of vitamin B-12, vitamin B-6, folate, and homocysteine to cognitive performance in the Normative Aging Study. Am J Clin Nutr 1996;63:306–14.
7. McCaddon A, Hudson P, Davies G, Hughes A, Williams JH, Wilkinson C. Homocysteine and cognitive decline in healthy elderly. Dement Geriatr Cogn Disord 2001;12:309–13.
8. Morris MS, Jacques PF, Rosenberg IH, Selhub J; National Health and Nutrition Examination Survey. Hyperhomocysteinemia associated with poor recall in the third National Health and Nutrition Examination Survey. Am J Clin Nutr 2001;73:927–33.
9. Duthie SJ, Whalley LJ, Collins AR, Leaper S, Berger K, Deary IJ. Homocysteine, B vitamin status, and cognitive function in the elderly. Am J Clin Nutr 2002;75:908–13.
10. Budge M, Johnston C, Hogervorst E, et al. Plasma total homocysteine and cognitive performance in a volunteer elderly population. Ann N Y Acad Sci 2000;903:407–10.
11. Tucker KL, Qiao N, Scott T, Rosenberg I, Spiro A III. High homocysteine and low B vitamins predict cognitive decline in aging men: the Veterans Affairs Normative Aging Study. Am J Clin Nutr 2005;82:627–35.
12. Kado DM, Bucur A, Selhub J, Rowe JW, Seeman T. Homocysteine levels and decline in physical function: MacArthur Studies of Successful Aging. Am J Med 2002;113:537–42.
13. Mooijaart SP, Gussekloo J, Frolich M, et al. Homocysteine, vitamin B-12, and folic acid and the risk of cognitive decline in old age: the Leiden 85-Plus study. Am J Clin Nutr 2005;4:866–71.
14. Nurk E, Refsum H, Tell GS, et al. Plasma total homocysteine and memory in the elderly: the Hordaland Homocysteine Study. Ann Neurol 2005;58:847–57.
15. Prins ND, Den Heijer T, van Dijk EJ, et al. Homocysteine and cognitive function in the elderly: the Rotterdam study. Neurology 2002;59:1375–80.
16. 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;346:476–83.
17. Ravaglia G, Forti P, Maioli F, et al. Homocysteine and folate as risk factors for dementia and Alzheimer's disease. Am J Clin Nutr 2005;82:636–43.
18. Luchsinger JA, Tang MX, Shea S, Miller J, Green R, Mayeux R. Plasma homocysteine levels and risk of Alzheimer disease. Neurology 2004;62:1972–6.
19. Clarke R, Refsum H, Birks J, et al. Screening for vitamin B12 and folate deficiency in older people. Am J Clin Nutr 2003;77:1241–7.
20. Morris MC, Evans DA, Bienias JL, et al. Dietary folate and vitamin B12 intake and cognitive decline among community-dwelling older persons. Arch Neurol 2005;62:641–5.
21. Morris MS, Jacques PF, Rosenberg IW, 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.
22. Lindenbaum J, Healton EB, Savage DG, et al. Neuropsychiatric disorders caused by cobalamin deficiency in the absence of anemia or macrocytosis. N Engl J Med 1988;318:1720–8.
23. Cognitive function and dementia in six areas of England and Wales: the distribution of MMSE and prevalence of GMS organicity level in the MRC CFA Study. The Medical Research Council Cognitive Function and Ageing Study (MRC CFAS). Psychol Med 1998;28:319–35.
24. Matthews F, Brayne C. The incidence of dementia in England and Wales: findings from the five identical sites of the MRC CFA Study. PLoS Med 2005;8:e193.
25. Rose G, Blackburn H, Gillum RF, Prineas RJ. Cardiovascular survey. 2nd ed. Geneva, Switzerland: World Health Organization, 1982.
26. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state." A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975;3:189–98.
27. Nexo E, Christensen AL, Hvas AM, Petersen TE, Fedosov SN. Quantification of holo-transcobalamin, a marker of vitamin B12 deficiency. Clin Chem 2002;48:561–2.
28. Molloy AM, Scott JM. Microbiological assay for serum, serum and red-cell folate using cryopreserved, microliter plate method. Meth Enzymol 1997;281:43–53.
29. Husek P. Chloroformates in gas chromatography as general purpose derivatizating agents. J Chromatogr B Biomed Sci Appl 1998;717:57–91.
30. Rubin DB. Inference and missing data. Biometrika 1976;63:581–92.
31. Eussen SJPM, deGroot CPGM, Clark R, et al. Oral vitamin B12 supplementation in elderly people with vitamin B12 deficiency: a dose finding trial. Arch Intern Med 2005;165:1167–72.
32. Kang JH, Irizarry MC, Grodstein F. Prospective study of plasma folate, vitamin B12, and cognitive function and decline. Epidemiology 2006;17:650–7.
33. Clarke R, Sherliker P, Hin H, et al. Detection of vitamin B12 deficiency in older people by measuring vitamin B12 or the active fraction of vitamin B12, holotranscobalamin. Clin Chem 2007;53:963–70.
34. McCracken C, Hudson P, Ellis R, McCaddon A; Medical Research Council Cognitive Function and Ageing Study. Methylmalonic acid and cognitive function in the Medical Research Council Cognitive Function and Ageing Study. Am J Clin Nut 2006;84:1406–11.
35. Lewis MS, Miller LS, Johnson MA, Dolce EB, Allen RH, Stabler SP. Elevated methylmalonic acid is related to cognitive impairment in older adults enrolled in an elderly nutrition program. J Nutr Elder 2005;24:47–65.
36. Refsum H, Smith AD. Low vitamin B-12 status in confirmed Alzheimer's disease as revealed by serum holotranscobalamin. J Neurol Neurosurg Psychiatry 2003;74:959–61.
37. Miller JW, Ramos MI, Garrod MG, Flynn MA, Green R. Transcobalamin II 775G>C polymorphism and indices of vitamin B12 status in healthy older adults. Blood 2002;100:718–20.
38. Eussen SJ, deGroot LC, Joosten LW, et al. Effect of oral vitamin B-12 with or without folic acid on cognitive function in older people with mild vitamin B-12 deficiency: a randomized, placebo-controlled trial. Am J Clin Nutr 2006;84:361–70.
39. McMahon JA, Green TJ, Skeaff CM, Knight RG, Mann JI, Williams SM. A controlled trial of homocysteine-lowering on cognitive performance in older people. N Engl J Med 2006;354:2764–72.
40. Clarke R, Armitage JM, Lewington S, Sherliker P, Collins R; B-Vitamin Treatment Trialists' Collaboration. Homocysteine-lowering trials for prevention of cardiovascular events: a review of the design and power of the large randomized trials. Am Heart J 2006;151:282–7.

The American Journal of Clinical Nutrition

Table S1: Associations of cognitive decline measured using MMSE over 10 years (1993-2003) associated with established risk factors and with markers of vitamin status at baseline. The coefficients for age and additional annual decline in MMSE were obtained from linear mixed models regressing serial MMSE scores by age after adjustment for sex, smoking, education, vascular disease, SBP and *APOE* gene variants. The models are presented separately for each marker of vitamin status, where the coefficients (age*lnX) for each marker of vitamin status (X) are shown in bold.

Fixed effects	**Identity of X**	**HoloTC (pmol/L)**	**Homocysteine (:mol/L)**	**MMA (:mol/L)**	**Vitamin B12 (pmol/L)**	**Folate (nmol/L)**
intercept	28.10 (0.243)	28.04 (0.251)	28.08 (0.246)	28.00 (0.244)	28.02 0.245)	28.05 (0.246)
Age, yr	-0.130 (0.023)	-0.117 (0.024)	-0.121 (0.023)	-0.111 (0.023)	-0.119 (0.023)	-0.123 (0.023)
Education, yr	0.191 (0.043)	0.164 (0.046)	0.164 (0.045)	0.160 (0.045)	0.158 (0.045)	0.153 (0.045)
SBP, mmHg	0.011 (0.006)	0.014 (0.006)	0.013 (0.006)	0.014 (0.006)	0.013 (0.006)	0.014 (0.006)
Female	0.436 (0.257)	0.424 (0.267)	0.443 (0.260)	0.374 (0.258)	0.453 (0.263)	0.404 (0.259)
Smoker	-0.830 (0.321)	-0.519 (0.332)	-0.503 (0.327)	-0.438 (0.325)	-0.497 (0.327)	-0.491 (0.328)
Vascular disease	-0.145 (0.273)	-0.064 (0.284)	-0.134 (0.277)	-0.103 (0.276)	-0.061 (0.277)	-0.073 (0.277)
APOE, ,4	-0.012 (0.293)	0.133 (0.301)	0.085 (0.294)	0.102 (0.292)	0.098 (0.294)	0.083 (0.294)
Log(X)	-	-0.430 (0.236)	1.004 (0.388)	0.451(0.296)	-0.406 (0.364)	-0.121 (0.177)
Age*education,	0.007 (0.004)	0.007 (0.004)	0.007 (0.004)	0.006 (0.004)	0.008 (0.004)	0.009 (0.004)
Age*SBP	-0.001 (0.0005)	-0.001 (0.0005)	-0.001 (0.0005)	-0.001 (0.0005)	-0.001 (0.0005)	-0.001 (0.0005)
age*female	-0.066 (0.024)	-0.068 (0.025)	-0.067 (0.024)	-0.060 (0.024)	-0.067 (0.025)	-0.063 (0.024)

age*smoker	0.030 (0.033)	-0.001 (0.035)	-0.002 (0.034)	-0.012 (0.034)	-0.002 (0.034)	-0.001 (0.034)
age* vascular disease	-0.029 (0.025)	-0.038 (0.026)	-0.027 (0.025)	-0.027 (0.025)	-0.036 (0.025)	-0.035 (0.026)
age*APOE	-0.051 (0.028)	-0.067 (0.029)	-0.064 (0.028)	-0.065 (0.028)	-0.064 (0.029)	-0.063 (0.028)
age*log(X)	**-**	**0.077 (0.022)**	**-0.114 (0.033)**	**-0.094 (0.025)**	**0.032 (0.034)**	**0.022 (0.017)**
Random effects						
Variance of intercepts	2.982 (0.677)	2.549 (0.683)	2.575 (0.666)	2.460 (0.657)	2.572 (0.668)	2.578 (0.672)
Variance of gradients	0.052 (0.007)	0.049 (0.007)	0.048 (0.007)	0.046 (0.007)	0.049 (0.007)	0.049 (0.007)
Number of people	1343	1206	1256	1256	1256	1254
Number of observations	4235	3831	3978	3978	3978	3973
Median value of X	-	65	13.2	0.27	251	11.4
Ln(median value of X)	-	4.174	2.580	-1.309	5.525	2.434