RESEARCH ARTICLE

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Cognitive and clinical outcomes of homocysteine-lowering B-vitamin treatment in mild cognitive impairment: a randomized controlled trial

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Background: Homocysteine is a risk factor for Alzheimer's disease. In the first report on the VITACOG trial, we showed that homocysteine-lowering treatment with B vitamins slows the rate of brain atrophy in mild cognitive impairment (MCI). Here we report the effect of B vitamins on cognitive and clinical decline (secondary outcomes) in the same study.

Methods: This was a double-blind, single-centre study, which included participants with MCI, aged ≥70 y, randomly assigned to receive a daily dose of 0.8 mg folic acid, 0.5 mg vitamin B_{12} and 20 mg vitamin B_6 (133 participants) or placebo (133 participants) for 2 y. Changes in cognitive or clinical function were analysed by generalized linear models or mixed-effects models.

Results: The mean plasma total homocysteine was 30% lower in those treated with B vitamins relative to placebo. B vitamins stabilized executive function (CLOX) relative to placebo ($P=0.015$). There was significant benefit of B-vitamin treatment among participants with baseline homocysteine above the median (11.3 μmol/L) in global cognition (Mini Mental State Examination, $P<0.001$), episodic memory (Hopkins Verbal Learning Test–delayed recall, $P=0.001$) and semantic memory (category fluency, $P=0.037$). Clinical benefit occurred in the B-vitamin group for those in the upper quartile of homocysteine at baseline in global clinical dementia rating score ($P=0.02$) and IQCODE score ($P=0.01$).

Conclusion: In this small intervention trial, B vitamins appear to slow cognitive and clinical decline in people with MCI, in particular in those with elevated homocysteine. Further trials are needed to see if this treatment will slow or prevent conversion from MCI to dementia. Copyright © 2011 John Wiley & Sons, Ltd.

Supporting information may be found in the online version of this article.

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Introduction

Mild cognitive impairment (MCI) is a syndrome defined as 'cognitive decline greater than that expected for an individual's age and education level but that does not interfere notably with activities of daily life' (Gauthier *et al.*, 2006). The prevalence of MCI is about 16% in those over 70 years old (Graham *et al.*, 1997; Petersen *et al.*, 2009), which means that there are about 5 million in the USA and 14 million in Europe with this condition. Because about half of those with MCI will develop dementia within 5 years of diagnosis

(Gauthier *et al.*, 2006), there is an urgent need to identify ways of slowing cognitive decline in this sector of the population.

Low levels of B vitamins are associated with cognitive impairment (Selhub *et al.*, 2000; McCaddon 2006; Smith, 2008). The biological pathways involved are probably related to formation of the amino acid homocysteine. If B-vitamin levels are lower than normal, total homocysteine (tHcy) levels in plasma increase. Plasma tHcy levels are known to increase with age (Refsum *et al.*, 2004), and community-dwelling older people with higher-than-average levels of tHcy perform less well on cognitive tests than those with lower levels (Smith, 2008). Patients with Alzheimer's disease (AD) have higher plasma tHcy than normal older people (Clarke *et al.*, 1998; McCaddon *et al.*, 1998; Seshadri *et al.*, 2002; Smith, 2008), and tHcy influences the rate of cognitive decline (Oulhaj *et al.*, 2010). Thus, tHcy is recognized as a risk factor for cognitive impairment and AD.

We have reported the primary outcome of a clinical trial (VITACOG) designed to determine if lowering of tHcy levels with B-vitamin supplements (folic acid, vitamins B_6 and B_{12}) over 2 years would slow the rate of brain atrophy in older participants with MCI (Smith *et al.*, 2010). B-vitamin treatment reduced average brain atrophy rate by 30% compared with placebo, and the effect of the treatment was greater with higher baseline concentration of tHcy; atrophy rate was slowed by 53% in participants with tHcy in the upper quartile. The final cognitive test scores were inversely related to the rate of atrophy. In this paper, we ask the question: Are the secondary cognitive and clinical outcomes also influenced by B-vitamin treatment, especially in those participants with high baseline tHcy concentrations?

Methods

The study was carried out under the principles of the Declaration of Helsinki and was approved by National Health Service Oxfordshire Research Ethics Committee A (04/Q1604/100). All participants gave written informed consent. The trial was registered under ISRCTN94410159.

Study protocol

The study protocol for this 2-year, placebo-controlled, randomized clinical trial has been described (Smith *et al.*, 2010). Respondents to recruitment advertising ($n=646$) from the Oxford area were screened for entry criteria by telephone and for MCI using a questionnaire, the Telephone Interview for Cognitive Status-modified (TICS-M) (Brandt *et al.*, 1993) (≥17 and ≤29 out of 39) and a category fluency test (animals) (Morris *et al.*, 1989). For borderline cases, if TICS-M was >29 but category fluency <19 or TICS-M word recall ≤10/20, then participants were eligible. Likewise, if TICS-M was <17 but category fluency was ≥19 or word recall was ≥10/20, participants were also eligible. Those with MCI who were ≥70 y, had a study partner and had no exclusion criteria (Smith *et al.*, 2010) were invited to the study.

Other measures to confirm the MCI diagnosis (Petersen, 2004) were collected, including the Mini Mental State Examination (MMSE) (Folstein *et al.*, 1975) (≥24/30), a subjective memory complaint with corroboration from a study partner using questions from the Cambridge examination for mental disorders of the elderly (CAMDEX) (Roth *et al.*, 1986) and normal activities of daily living using five questions from the Cambridge Behavioural Inventory (Wedderburn *et al.*, 2008). The clinical dementia rating scale (CDR) (Morris, 1993) was assessed, but was not used for MCI classification. Amnestic MCI was not differentiated from non-amnestic MCI in the inclusion criteria.

At the first clinic visit (baseline), 271 participants gave written consent and were randomized to treatment or placebo. Centralized telephone randomization was used with full allocation concealment and minimization for age, gender, TICS-M score and MRI consent. Five participants never started taking the tablets (failed on screening criteria or withdrew consent), leaving 266 participants that entered the trial. The flow of participants is shown in Figure S1. Participants, study partners and those assessing outcomes were blind to the assignment of interventions.

The B-vitamin group received TrioBe Plus® (Meda AB/Recip AB, Box 906, Pipers väg 2A, SE-170 09 Solna, Sweden), containing 0.8 mg folic acid, 0.5 mg cyanocobalamin and 20 mg pyridoxine HCl. The placebo group received vitamin-free tablets of similar appearance. Both at baseline and at the second visit, blood samples were obtained for routine biochemical tests and assessment of plasma tHcy, folate, vitamin B_{12}, holotranscobalamin and apolipoprotein E (*APOE*) genotype, as previously described (Smith *et al.*, 2010).

Primary and secondary outcome measures

The trial was powered for the primary outcome, a change in the rate of brain atrophy over 2 y, and the

results have been reported previously (Smith *et al.*, 2010). In that paper, we also reported some secondary outcomes, including compliance by tablet count and biological compliance (change in plasma vitamin status), and evaluation of safety and adverse effects (Smith *et al.*, 2010).

Changes in cognitive and clinical status were secondary outcome measures and have not been reported previously. At baseline and follow-up, a neuropsychological test battery, described in the trial protocol (Smith *et al.*, 2010), was conducted by trained research nurses and psychologists blind to CDR and informant information. Tests reported here are representative of particular cognitive domains important in MCI: global cognition (MMSE) (Folstein *et al.*, 1975); episodic memory (Hopkins Verbal Learning Test-revised with delayed recall, HVLT-R) (Brandt, 1991); semantic memory (category fluency, CERAD) (Morris *et al.*, 1989); executive function (CLOX) (Royall *et al.*, 1998). The HVLT-R was administered using the six different versions consecutively throughout the trial, at baseline and 3, 6, 9, 15, 18 and 24 months, to reduce practice effects. Other tests reported here were only administered at baseline and 24 months. The clinical outcome measures were the global CDR (Morris, 1993) and the short form of the Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE) scores (Jorm, 2004). These tests are described more fully in the Supplementary Appendix.

Statistical analyses

Efficacy analyses were performed on the intention-to-treat population, defined as participants who were randomly assigned to treatment and received at least one dose of study medication.

The HVLT-delayed recall (DR) score was analysed by a longitudinal method (using data from five time points) by logistic regression with Generalized Linear Mixed Model (GLMM; binomial errors, logit link). The HVLT-DR score at 3 months was used as a starting point to reduce the practice effects up to 3 months.

Other neuropsychological tests, for which only baseline and last follow-up measures were collected, were analysed cross-sectionally. These include cognitive tests, namely MMSE, Category fluency and CLOX and clinical measures, namely the CDR and IQCODE. There were fewer clinical measures at follow-up ($n=191$) compared with cognitive measures ($n=223$) due to some study partners being unavailable to complete the CDR and IQCODE.

For each test, rather than analysing the change from baseline to last follow-up, we assessed the effect of treatment by modelling the score at last follow-up (24 months), controlling for its baseline value as well as for potential confounders such as age, gender, education and *APOE* status. For the CLOX test, which has two components, the effect of treatment was assessed by modelling CLOX1 (executive function) at 24 months, controlling for CLOX1 at baseline and for CLOX2 (praxis element) at 24 months, in addition to potential confounders described above, as explained in the Supplementary Information (McGuinness *et al.*, 2010). Generalized linear models (GLM) were fitted using different distributions depending on the nature of the outcomes. For the CLOX1 (ranging from 0 to 15) and MMSE (ranging from 0 to 30 points), we used the binomial distribution with the logit link. The Category fluency score is the number of correct words given in a timed interval, and therefore was analysed using the Poisson distribution and log link. For the IQCODE, we used the Gaussian distribution (which corresponds to linear regression). The CDR overall score is an ordered categorical outcome and was recorded as a binary outcome, 0 or ≥0.5, and analysed via logistic regression.

The models were initially fitted without interaction terms to determine the overall effect of treatment on cognition, controlling for covariates including age, *APOE* ε4 allele (present or absent), sex and education. Thereafter, pre-specified subgroup analyses were carried out with baseline tHcy included in the interaction term as a binary variable, wherein study participants were classified as 'low tHcy group' if their baseline tHcy level was below the median (11.3 μmol/L) or 'high tHcy group' for the remainder. For all outcomes, the analysis started with a saturated model, which was then reduced hierarchically using the likelihood ratio test and the Aikake information criterion. There was no multiple testing. Statistical analysis was carried out using the R statistical program (www.R-project.org). Reported *P* values were two-sided, and *P* values <0.05 were considered significant.

Results

Participants

Of the 266 participants starting the intervention, 223 participants (83.8%) completed the second visit 2 years later. Reasons for withdrawal have been previously described (Smith *et al.*, 2010) and are summarized in Figure S1. There was no difference between baseline demographics for the 43 participants who failed to complete the trial compared with the 223

participants who completed the trial (Table S1). Baseline demographics for the B-vitamin-treated and placebo groups for the participants who completed the trial are presented in Table 1. There were no significant differences between the groups, except for the depression score.

Biochemical response to treatment

The baseline and follow-up data for plasma concentrations of tHcy and B vitamins are shown in Table 2. Plasma tHcy increased modestly but significantly in the placebo group, but decreased in the B-vitamin group, so that at the end of the trial, there was a nearly 30% difference between the two groups ($P<0.001$).

Treatment effects on cognitive decline

Overall effect. Cognitive data at baseline and at the end of trial for all participants by treatment and by tHcy group are shown in Table 3 as descriptive statistics. The fitted models show no significant overall effect of treatment for MMSE ($P=0.57$), HVLT-DR ($P=0.23$) or category fluency ($P=0.92$). On the other hand, the CLOX test of executive function showed an overall significant difference according to treatment. The odds of a correctly drawn item from CLOX1 at follow-up (24 months), controlling for CLOX2 at follow-up and CLOX1 at baseline, as well as for age, education, *APOE* ε4 status and sex, was 30% higher in B-vitamin-treated participants ($P=0.015$) relative to placebo.

Table 1 Baseline characteristics of the study participants

Characteristic	Placebo group ($n=113$)	B-vitamin group ($n=110$)	*P* value
Age at baseline (years)	76.7±4.8	76.8±5.1	0.81
Female sex	73 (64.6)	70 (63.6)	0.88
Total education (years)	14.9±3.5	14.2±3.5	0.16
APOE ε4 carrier	35 (31.0)	34 (30.9)	0.99
Smoker—ever	57 (50.4)	47 (42.7)	0.27
GDS (0–30)[a]	7.5±5.0	5.7±4.3	0.005
Haemoglobin (g/dL)	13.8±1.2	13.8±1.2	0.68
MCV (fL)	92.9±4.1	92.5±4.4	0.44
Diabetes—ever	11 (9.7)	5 (4.5)	0.11
Previous stroke, TIA, MRI infarct	23 (20.3)	17 (15.6)	0.36
Previous MI	9 (8.0)	8 (7.3)	0.85
Systolic BP (mmHg)	146±20	147±23	0.77
Diastolic BP (mmHg	80±11	80±11	0.92
BMI (kg/m^2)	26.1±4.0	25.7±3.6	0.40
Alcohol (units/week)	7.1±8.5	9.1±9.2	0.11
Creatinine (μmol/L)	98±16	96±17	0.45
Treatment period (years)	2.1±0.1	2.1±0.1	0.74
TICS-M (0–39)	24.9±2.8	24.9±2.8	0.98
CDR ≥0.5	82 (72.6)[b]	80 (72.7)[c]	0.98

Data are for the participants who completed the trial; shown as mean± SD or *n* (%). Student's *t*-test for comparison of continuous variables and χ^2 for comparison of categorical variables. *APOE*, gene for apolipoprotein E; CDR, global clinical dementia rating; GDS, Geriatric Depression Scale; MCV, mean red cell volume; MI, myocardial infarct; TIA, transient ischemic attack; TICS-M, telephone interview of cognitive status, modified.

[a]GDS: 0–10, mild; 11–20, moderate; 21–30, severe depressive symptoms.

[b]Ninety-five participants completed the trial with CDR.

[c]Ninety-six participants completed the trial with CDR.

Subgroup analysis: interaction with homocysteine at baseline. The effect of B-vitamin treatment on cognitive outcomes using subgroup analysis defined by the tHcy concentration at baseline gave consistent and significant results.

The final model for HVLT-DR revealed that participants taking placebo in the high tHcy group (≥median, i.e. ≥11.3 μmol/L) showed significant decline, whereas participants receiving B vitamins in the high tHcy group showed no significant decline. The average HVLT-DR scores in the low tHcy group did not decline over time for either treatment category (Table 4). The odds of correctly remembering a word from the list of 12 in the HVLT for a person in the high tHcy group at the end of the trial was 69% greater if they were taking B vitamins than if they were taking placebo (odds ratio $=1.69$, $P=0.001$) (Table 4). Figure 1 shows plots of the raw data for the mean HVLT-DR scores for all time points between 3 and 24 months. The plot justifies why we added a quadratic term for time when modelling the log odds ratio by GLMM. It also shows that B-vitamin treatment resulted in maintained performance in those with elevated tHcy, whereas placebo treatment was associated with decline in performance over time.

Table 5 summarizes the cross-sectional results for all other tests, assessed at only two time points. For the MMSE, those in the high tHcy group who were treated with B vitamins were 1.58 times more likely to give a correct answer than those receiving placebo ($P<0.001$). In the low tHcy group, no significant difference was found in the odds ratio comparing treated and placebo. For category fluency, in the high tHcy group, the average number of words at follow-up was 9.4% greater in those on treatment compared with those on placebo ($P=0.037$). In the low tHcy group, no significant difference was found when comparing treated with placebo. For CLOX1, no interaction with tHcy was found.

Table 2 Folate and cobalamin markers in plasma before and after 2y of intervention

Variable		Placebo group (n=113)		B-vitamin group (n=110)[a]		
		Geometric mean	95% CI	Geometric mean	95% CI	P value[b]
tHcy (μmol/L)	Before	11.6	10.9–12.9	11.3	10.7–11.9	0.53
	After	12.4	11.8–13.1	8.7	8.2–9.1	<0.001
P value[c]		<0.001		<0.001		
Folate (nmol/L)	Before	23.0	20.4–26.0	22.6	20.0–25.5	0.83
	After	24.7	21.9–27.9	83.8	74.3–94.6	<0.001
P value[c]		0.35		<0.001		
Vitamin B_{12} (pmol/L)	Before	324	303–347	332	310–356	0.62
	After	348	326–373	690	644–740	<0.001
P value[c]		0.021		<0.001		
HoloTC (pmol/L)	Before	66	59–73	63	57–70	0.62
	After	68	62–75	188	171–208	<0.001
P value[c]		0.34		<0.001		

Data are for the participants who completed the trial. Variables were log transformed prior to analysis. Geometric means and the 95% CI of the means are shown. Active treatment group received daily supplements of folic acid (0.8mg), vitamin B_{12} (0.5mg) and vitamin B_6 (20mg) for 24 months. Abbreviations: tHcy, plasma total homocysteine; HoloTC, holotranscobalamin.
[a]The values after treatment refer to 109 participants because one declined to give a blood sample.
[b]Student's t-test for unpaired samples.
[c]Student's t-test for paired samples.

Table 3 Cognitive test scores at baseline and after 2y of intervention by median split of homocysteine

Cognitive test	Placebo group (n=113)		B-vitamin group (n=110)	
	Baseline	Follow-up	Baseline	Follow-up
MMSE				
Low tHcy	28.1±1.6	28.1±1.9	28.3±1.8	27.8±2.4
High tHcy	28.4±1.2	27.2±2.5	28.2±1.8	27.9±2.1
HVLT DR				
Low tHcy	7.4±2.5[a]	7.9±3.2	7.0±2.7[a]	7.6±3.7
High tHcy	6.9±2.8[a]	5.9±3.6	6.8±2.6[a]	7.2±3.3
Cat. fluency				
Low tHcy	21.0±4.7	21.1±5.5	21.5±5.0	20.7±5.0
High tHcy	18.6±5.1	17.6±5.6	19.5±4.5	19.6±4.7
CLOX1				
Low tHcy	12.4±1.9	13.0±1.7	12.8±1.6	13.5±1.7
High tHcy	12.1±2.5	12.3±2.4	12.3±2.2	12.6±1.9
CLOX2				
Low tHcy	14.1±1.0	14.5±0.7	14.6±0.6	14.3±1.1
High tHcy	14.0±1.1	14.1±1.1	14.3±0.7	14.1±1.3
IQCODE				
Low tHcy	3.2±0.44	3.1±0.37	3.2±0.28	2.9±0.76
High tHcy	3.2±0.48	3.1±0.68	3.3±0.30	3.1±0.56
CDR sob				
Low tHcy	0.82±0.7	0.75±0.7	0.84±0.79	0.65±0.94
High tHcy	0.89±0.7	1.4±1.6	1.1±0.87	1.0±1.3

Data are for the participants who completed the trial; shown as means±SD. P values were not derived from these data. 'High tHcy' is defined as at or above the median (11.3μmol/L) for this cohort, and 'Low tHcy' is below the median. CDR sob, CDR sum of boxes.
[a]HVLT baseline scores (0month) were excluded in preference of 3month test scores to avoid the learning effect.

Treatment effects on clinical outcomes

Overall effect. In the whole intention-to-treat cohort, there was no significant effect of B vitamins on CDR (P=0.23) or IQCODE (P=0.26).

Subgroup analysis: interaction with homocysteine at baseline. There was no significant interaction effect when tHcy was categorized by median split for IQCODE and CDR. However, when we compared the upper quartile of tHcy (≥13.1μmol/L) to the lower quartiles,

Table 4 Homocysteine subgroup analysis for the efficacy of B-vitamin treatment on longitudinal HVLT-DR shown by the Generalized Linear Mixed-effects Model (GLMM)

Cognitive test	Parameter	Estimate	SE	*Z*-value	*P* value
HVLT-DR (Distribution=Binomial)	Intercept	0.74	0.085	10.11	<0.001
	Linear term	0.73	0.11	6.434	<0.001
	Quadratic term	−0.36	0.06	−5.63	<0.001
	Interaction term	−0.17	0.05	−3.25	0.001

The column 'Parameter' gives the names of the beta coefficients associated with the linear and quadratic terms for time in the log odds ratio of correctly answering an item from HVLT-DR. This odds ratio compares B-vitamins to placebo. The model estimates the trend over time of the odds ratio in both the Low tHcy and High tHcy groups (tHcy cut-off 11.3μmol/L).

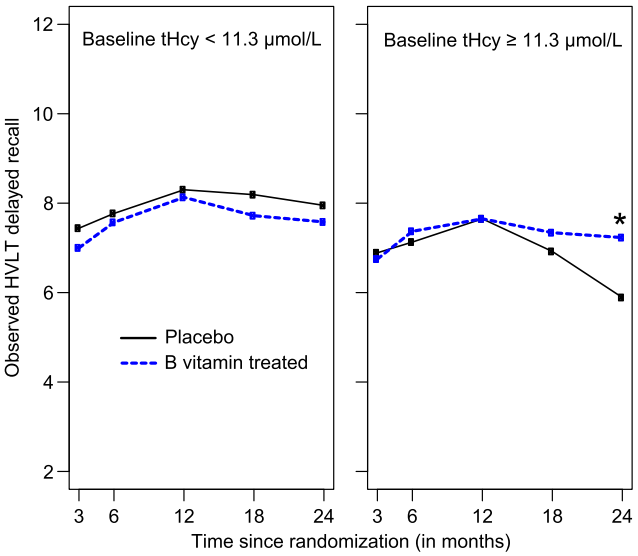

Figure 1 Effect of B-vitamin treatment on a test of episodic memory. The graphs illustrate the longitudinal effect of treatment on unadjusted mean HVLT-DR scores and its interaction with baseline tHcy level. 'Low' and 'High' baseline tHcy refer to values below and above the median (11.3μmol/L), respectively. *P=0.001 compared with placebo by logistic regression using GLMM (Table 4).

both IQCODE and CDR showed a significant interaction of baseline tHcy levels with treatment (Table 5). For the IQCODE, in the high tHcy group, treated participants had better average IQCODE scores at follow-up compared with placebo (regression coefficient=0.23; P=0.011. In the low tHcy group, no significant difference between treated and placebo was found.

The distribution of global CDR scores at baseline was almost the same for the placebo (CDR=0: 29.8%; CDR=0.5: 70.2%; CDR=1: 0%) and B-vitamin groups (28.9%, 70%, 1.1%). At the end of the trial, the corresponding distribution was 42.1%, 54.7%, 3.2% for the placebo group, and 50%, 47.9%, 2.1% for the B-vitamin group. When stratified by tHcy quartiles at baseline, there was a significant effect of treatment on global CDR scores, but only in those in the highest quartile (≥13.1μmol/L), with a shift to a larger proportion having a CDR score of zero (Table S2 and Figure 2). A CDR score of zero was found in 25.0% of the B-vitamin-treated group who were in the top quartile of tHcy at baseline and in 58.3% at follow-up (P=0.039, Fisher's exact test). In contrast, those in the high tHcy group taking placebo showed no change in the proportion with CDR of zero from baseline (24%) to follow-up (28%, P=1.0). The results were not changed when we controlled for covariates including age, sex, ApoE4 and education using logistic regression. In the upper-quartile tHcy group, the odds of having CDR=0 at follow-up is five times greater in the active-treatment group compared with placebo (P=0.02; Table 5).

As a secondary analysis, we fitted the GLMM to confirm the GLM results obtained for outcomes at two time points (except for CLOX). The results are shown in Supplementary Figures S2–S4. The beneficial effect of active treatment was significant in each test. Adjusting for other potential confounders, in addition to those above, such as depression score, smoking, diabetes and systolic blood pressure, did not materially alter the results for all cognitive and clinical outcomes in the GLMM and GLM models (data not shown).

Discussion

In the intention-to-treat cohort of participants with MCI who completed the trial, B-vitamin treatment did not improve performance in tests of global cognitive function (MMSE), episodic memory (HVLT-DR) or semantic memory (category fluency) or on measures of clinical status (CDR and IQCODE). However, B-vitamin treatment did stabilize performance on the CLOX test of executive and planning function, and this effect was independent of baseline tHcy, perhaps indicating a direct effect of one or more of the B vitamins.

Table 5 Homocysteine subgroup analysis for the efficacy of B-vitamin treatment on cross-sectional test performance shown by Generalized Linear Model (GLM)

Cognitive test	Parameter	Estimate	SE	*Z*-value	*P* value
MMSE at last visit (Distribution=Binomial)	Intercept	1.61	0.20	8.0	<0.001
	Treatment category	0.46	0.13	3.4	<0.001
	Baseline tHcy	0.49	0.14	3.6	<0.001
	Treatment_x_tHcy	−0.69	0.19	−3.6	<0.001
Category fluency at last visit (Distribution=Poisson)	Intercept	3.07	0.08	40.3	<0.001
	Treatment category	0.09	0.04	2.1	0.037
	Baseline tHcy	0.10	0.04	2.3	0.023
	Treatment_x_tHcy	−0.13	0.06	−2.2	0.030
Clinical dementia rating (CDR) at last visit (Distribution=Binomial)	Intercept	0.84	0.58	1.46	0.14
	Treatment category	1.62	0.70	2.32	0.02
	Baseline tHcy	0.66	0.57	1.17	0.240
	Treatment_x_tHcy	−1.62	0.79	−2.06	0.039
IQCODE at last visit (Distribution=Gaussian)	Intercept	1.50	0.23	6.40	<0.001
	Treatment category	−0.22	0.08	−2.57	0.011
	Baseline tHcy	−0.10	0.07	−1.45	0.150
	Treatment_x_tHcy	0.23	0.10	2.34	0.020
CLOX1 at follow-up (Distribution=Binomial)	Intercept	−5.47	0.62	−8.8	<0.001
	Treatment category	0.26	0.11	2.4	0.015
	Clox1_baseline	0.13	0.02	5.7	<0.001
	Clox2_24 months	0.37	0.04	8.8	<0.001

The table shows those cognitive outcomes where there was only one follow-up observation (24months). Treatment category: 0, placebo; 1, B vitamins. Baseline tHcy: 0, high; 1, low. High tHcy is defined as ≥median (11.3 μmol/L) for the cognitive tests and ≥13.1 μmol/L for CDR and IQCODE. Adjusted for age, sex, *APOE* ε4 status and education. For more details, see Supplementary Information.

On the other hand, when analysis was done according to predefined subgroups based on the baseline tHcy concentration, there were clear beneficial effects of B vitamins on tests of episodic memory, semantic memory and global cognition in participants with baseline tHcy ≥11.3 μmol/L. In participants with high baseline tHcy treated with placebo, significant cognitive decline occurred, but decline was prevented in those on treatment with B vitamins.

A similar result was found for the clinical outcomes (CDR and IQCODE), where B-vitamin treatment actually improved the clinical outcome, but only in participants with baseline tHcy ≥13.1 μmol/L. Particularly striking was the effect of B-vitamin treatment on the proportion of participants with a CDR score of zero, which doubled after 2 y of treatment.

Although the sizes of the effects of B-vitamin treatment were relatively modest, the fact that they were highly significant and were found in several cognitive domains and also in clinical assessments is consistent with an effect of the intervention on disease progression.

Our results contrast with several negative trials on homocysteine-lowering treatment and cognition or dementia (reviewed by Wald *et al.*, 2010). The reason may be related to several differences between our trial and previous studies. We included participants with MCI, who were followed for 2 y, during which the placebo group underwent significant cognitive decline.

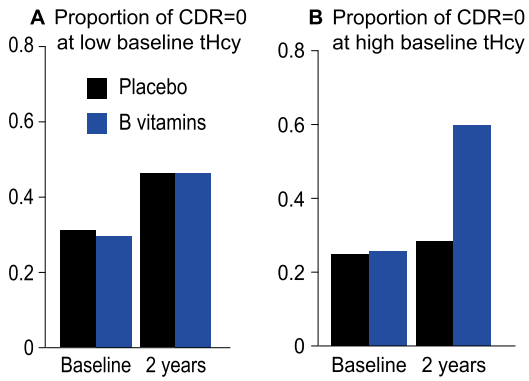

Figure 2 Effect of B-vitamin treatment on the proportion of participants with a CDR score of zero. 'Low' and 'High' baseline tHcy refer to values below and above the upper quartile (13.1 μmol/L), respectively.

Several of the trials without treatment effect may have been too short in duration, excluded vitamin B_{12} or B_6 supplements (Wald *et al.*, 2010) or had participants who were either healthy and therefore did not decline (Stott *et al.*, 2005; McMahon *et al.*, 2006) or included patients who already have dementia too advanced for an improvement to be readily detected (Aisen *et al.*, 2008). It is noteworthy that in the VITAL trial, participants with mild AD, but not those with moderately severe AD, showed some improvement upon B-vitamin treatment (Aisen *et al.*, 2008).

Our study confirms the positive findings in the FACIT trial (Durga *et al.*, 2007) in which high tHcy (>13 μmol/L) was an inclusion criterion and where there was improvement in several of the test scores, in particular episodic memory, in non-cognitively impaired participants receiving folic acid. The observations in FACIT and in our trial that improvement was mainly apparent in participants with higher baseline tHcy concentrations may explain the lack of treatment effect in B-vitamin trials with low tHcy at baseline. Among our tests, the effect of B-vitamin treatment was most striking for episodic memory, where intervention for 2 y in participants with high levels of tHcy (>11.3 μmol/L) gave a 69% higher likelihood of correct word recall compared with placebo. Furthermore, there was a significant difference in the rate of decline between the treatment and placebo groups. The same pattern was also observed for the MMSE (global) and category fluency (semantic memory) tests. Thus, although the study was not originally powered for effects on cognitive performance, we have observed significant effects of B-vitamin treatment in MCI-relevant cognitive domains in the pre-specified analyses according to baseline tHcy.

The clinical improvement in the CDR suggests a possible reversal of early cognitive impairment in some of those with MCI on B-vitamin treatment. Similar to most of the cognitive tests, the effect was only significant for those with raised tHcy levels, in this case in the upper quartile (≥13.1 μmol/L). It is striking that a similar threshold was observed for the effect of treatment on the IQCODE, another indicator of clinical status.

A limitation of the trial is the small sample size. As a result, we could not investigate other subgroups (e.g. *APOE* ε4 status, presence of disease at baseline, drug use, etc.), which will require larger studies. The compliance was relatively good (Smith *et al.*, 2010), but nevertheless, a per-protocol analysis using biological compliance based on plasma vitamin response may have given even more significant results.

Is homocysteine a causative factor in cognitive decline or just a marker (Obeid and Herrmann, 2006; Smith, 2008)? In this trial population, B-vitamin treatment markedly slowed the rate of brain atrophy relative to placebo (Smith *et al.*, 2010). The effect of treatment was highly dependent on plasma tHcy: In those with tHcy in the lowest quartile (i.e. in those with low tHcy), B vitamins had no effect on atrophy rate. In contrast, in those in the top quartile (≥13.1 μmol/L), B vitamins had a dramatic effect by halving the rate of atrophy. Furthermore, the rate of atrophy was a major determinant of cognitive function at the end of the trial (Smith *et al.*, 2010). Thus, one interpretation is that lowering tHcy concentrations by administering B vitamins slows brain atrophy, which in turn slows both cognitive and clinical decline. Such an interpretation is consistent with several studies showing that whole brain atrophy rate is strongly correlated with decline in various cognitive measures, including the CDR, in participants with MCI (Jack *et al.*, 2008). The findings of the VITACOG trial give strong support to the idea of using measures of brain atrophy as end points in clinical trials of disease-modifying treatments (Jack *et al.*, 2008).

In conclusion, our data indicate that B vitamins may slow cognitive and clinical decline in participants with MCI, in particular in those who have high tHcy concentrations.

Role of the funding sources

The sponsor (University of Oxford), the funders of the study and the company providing the tablets had no role in the study design, data collection, data analysis, data interpretation or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Conflicts of interest

A. D. Smith is named as an inventor on three patents held by the University of Oxford on the use of folic acid to treat AD or MCI (US6008221; US6127370; PCT/GB2010/051557); under the University's rules, he could benefit financially if the patent is exploited. Drs Refsum and Smith report having in the past received speaking honoraria from Recip AB, the company that donated the vitamin tablets, and from Axis-Shield, who make the equipment used to assay homocysteine. None of the other authors have any financial disclosures.

Key points

- B-vitamin treatment over 2 years slows the decline in cognitive test performance in MCI.
- B-vitamin treatment over 2 years leads to apparent improvement in clinical status.
- These effects are mainly found only in those with a high baseline level of plasma total homocysteine (>11 μmol/L for cognitive tests and >13 μmol/L for clinical outcomes).

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