British Journal of Nutrition (2014), **111**, 1085–1095
doi:10.1017/S000711451300336X

Interactions between plasma concentrations of folate and markers of vitamin B_{12} status with cognitive performance in elderly people not exposed to folic acid fortification: the Hordaland Health Study

Esmée L. Doets[1]*, Per M. Ueland[2,3], Grethe S. Tell[4], Stein Emil Vollset[4], Ottar K. Nygård[5,6], Pieter van't Veer[1], Lisette C. P. G. M. de Groot[1], Eha Nurk[7,8], Helga Refsum[7,9], A. David Smith[9] and Simone J. P. M. Eussen[2,4]

[1]*Division of Human Nutrition, Wageningen University, PO Box 8129, 6700 EV, Wageningen, The Netherlands*
[2]*Section for Pharmacology, Department of Clinical Science, University of Bergen, Bergen, Norway*
[3]*Laboratory of Clinical Biochemistry, Haukeland University Hospital, Bergen, Norway*
[4]*Department of Global Public Health and Primary Care, University of Bergen, Bergen, Norway*
[5]*Section for Cardiology, Department of Clinical Science, University of Bergen, Bergen, Norway*
[6]*Department of Heart Disease, Haukeland University Hospital, Bergen, Norway*
[7]*Department of Nutrition, Faculty of Medicine, Institute of Basic Medical Sciences, University of Oslo, Oslo, Norway*
[8]*Department of Surveillance and Evaluation, National Institute for Health Development, Tallinn, Estonia*
[9]*Department of Pharmacology, Oxford Project to Investigate Memory and Ageing (OPTIMA), University of Oxford, Oxford, UK*

(Submitted 26 November 2012 – Final revision received 27 August 2013 – Accepted 27 August 2013 – First published online 11 November 2013)

Abstract

A combination of high folate with low vitamin B_{12} plasma status has been associated with cognitive impairment in a population exposed to mandatory folic acid fortification. The objective of the present study was to examine the interactions between plasma concentrations of folate and vitamin B_{12} markers in relation to cognitive performance in Norwegian elderly who were unexposed to mandatory or voluntary folic acid fortification. Cognitive performance was assessed by six cognitive tests in 2203 individuals aged 72–74 years. A combined score was calculated using principal component analysis. The associations of folate concentrations, vitamin B_{12} markers (total vitamin B_{12}, holotranscobalamin (holoTC) and methylmalonic acid (MMA)) and their interactions in relation to cognitive performance were evaluated by quantile regression and least-squares regression, adjusted for sex, education, apo-ε4 genotype, history of CVD/hypertension and creatinine. Cross-sectional analyses revealed an interaction ($P=0{\cdot}009$) between plasma concentrations of folate and vitamin B_{12} in relation to cognitive performance. Plasma vitamin B_{12} concentrations in the lowest quartile (<274 pmol/l) combined with plasma folate concentrations in the highest quartile (>18·5 nmol/l) were associated with a reduced risk of cognitive impairment compared with plasma concentrations in the middle quartiles of both vitamins (OR 0·22, 95% CI 0·05, 0·92). The interaction between folate and holoTC or MMA in relation to cognitive performance was not significant. In conclusion, this large study population unexposed to mandatory folic acid fortification showed that plasma folate, but not plasma vitamin B_{12}, was associated with cognitive performance. Among the elderly participants with vitamin B_{12} concentrations in the lower range, the association between plasma folate and cognitive performance was strongest.

Key words: **Vitamin B_{12}: Folate: Cognition: Elderly**

Folate and vitamin B_{12} status have been directly associated with cognitive performance in cross-sectional and prospective studies[1–4]. Some earlier case reports have observed accelerated neurological deterioration in patients with pernicious anaemia and severe vitamin B_{12} deficiency after treatment with folic acid[5,6]. These observations in combination with the known metabolic interrelation of folate and vitamin B_{12} suggest that the effects of one of these B-vitamins on cognitive

Abbreviations: HADS-D, Hospital Anxiety and Depression Scale depression subscale; holoTC, holotranscobalamin; MMA, methylmalonic acid; m-MMSE, modified version of the Mini-Mental State Examination; NHANES, National Health and Nutrition Examination Survey; pABG, p-aminobenzoyl-glutamate; PCA, principal component analysis; tHcy, total homocysteine.

* **Corresponding author:** E. L. Doets, fax +31 317 483342, email doets@pallashrc.com

performance might be modified by blood concentrations of the other B-vitamin. In line with this, cross-sectional analyses within the National Health and Nutrition Examination Survey (NHANES) study revealed that high serum folate status was generally associated with protection against cognitive impairment; however, individuals with low vitamin B_{12} status combined with high folate status had an increased risk of cognitive impairment compared with those with a normal status of both vitamins[7].

It has been hypothesised that unmetabolised folic acid, which is likely to be present in individuals living in areas with mandatory folic acid fortification of food items[8,9], may mask or exacerbate metabolic and clinical consequences of vitamin B_{12} deficiency[10]. The findings from the NHANES study that high folate concentrations were associated with an increased risk of cognitive impairment in individuals with vitamin B_{12} deficiency are in line with the results from the Framingham Heart Study including individuals unexposed to folic acid fortification[11]. In other large study populations unexposed[12,13] or exposed[14] to mandatory folic acid fortification, the NHANES findings could not be confirmed. These previous studies differed from the NHANES study in several aspects. First, they used a lower cut-off value for high folate concentrations; second, they used only one or two cognitive performance tests; third, they may have lacked power; and fourth, they measured a single marker of vitamin B_{12} status, either total vitamin B_{12} or holotranscobalamin (holoTC) II. HoloTC II refers to the fraction of circulating vitamin B_{12} bound to the transporter protein transcobalamin which delivers the vitamin into the cells, and it has been suggested to be a more sensitive and specific marker of vitamin B_{12} status than total concentrations of vitamin B_{12} in plasma[15].

It remains unclear whether the combination of high folate and low vitamin B_{12} status worsens cognitive performance when compared with that of normal folate and normal vitamin B_{12} status. We therefore investigated the interaction between various markers of vitamin B_{12} and folate status in relation to cognitive performance based on six cognitive tests in a large population-based study not exposed to mandatory or voluntary food fortification with folic acid.

Methods

Study population

The study population consisted of apparently healthy residents of Bergen (Norway) born between 1925 and 1927, who participated both in the Hordaland Homocysteine Study in 1992–3 and in the Hordaland Health Study in 1997–9 (http://www.husk.b.uib.no). A total of 2841 community-dwelling elderly individuals were invited to participate in a substudy on cognitive tests in 1997–9, of whom 2203 (77·5 %) agreed. Details of this study have been described elsewhere[16–18]. The study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Regional Committee for Medical Research Ethics of Western Norway. Written informed consent was obtained from all the subjects.

Assessment of cognitive performance

Cognitive performance was assessed at the study location by trained nurses and included six tests[19]: a modified version of the Mini-Mental State Examination (m-MMSE; global cognition, maximum score 12)[20,21]; a modified version of the Digit Symbol Test (perceptual speed, score reflects the number of digits recalled)[22]; a short form of the Block Design (visuospatial skills, maximum score 16)[22]; the Kendrick Object Learning Test (episodic memory, maximum score 70)[23]; an abridged version of the Controlled Oral Word Association Test (access to semantic memory, score reflects the number of words recalled)[24]; the Trail Making Test Part A (executive function, score reflects the number of seconds needed to complete the task)[25]. For all tests, a higher score indicates better performance, except for the Trail Making Test Part A where a shorter time used indicates better performance.

Other covariates

Both in 1992–3 and 1997–9, participants underwent a brief health examination including measurements of height and weight. In addition, information on cardiovascular risk factors and lifestyle factors including smoking status (current smokers, ex-smokers or never smokers), consumption of coffee (0–1, 1–4 or more than five cups/d), alcohol use (number of glasses/week) and use of multivitamin or B-vitamin supplements was collected via self-administered questionnaires, as described previously[17,26]. Users of folic acid supplements were included in the study. Dietary intakes of folate and vitamin B_{12} were measured in 1997–9 by a quantitative FFQ[27].

History of CVD was based on self-reported information on the history of myocardial infarction, angina pectoris and stroke as recorded both in 1992–3 and 1997–9, and on the history of thrombosis and phlebitis as recorded in 1992–3. Of the self-reported CVD cases, 79 % were validated with hospitalisation records used in an earlier study[28], whereas the remaining 21 % were presumably less severe and did not require hospitalisation or occurred before 1992. A history of hypertension was defined as current or previous use of antihypertensive drugs, and was based on self-reported data collected in 1997–9. Diabetes was based on self-reported information collected in 1997–9. Depression score was assessed in 1997–9 by a seven-item subscale for depression from the Hospital Anxiety and Depression Scale depression subscale (HADS-D)[29]. Educational level was classified as no primary school, primary school ($\leq$9 years), vocational secondary school (10–12 years), theoretical secondary school (10–12 years), college or university ($<$4 years), and college or university ($\geq$4 years).

Plasma measurements

Non-fasting EDTA blood samples were collected for the analyses of plasma markers of folate and vitamin B_{12} status. The EDTA samples were kept at 4°C until centrifugation. Samples collected in 1992–3 were stored at −20°C for up to 10 years, whereas samples collected in 1997–9 were

stored at −80°C for up to 12 months before analyses. Plasma concentrations of folate and vitamin B_{12} were determined by microbiological assays[30,31]. A recent study has shown that folate concentrations in plasma are not stable during storage[32]. However, folate determined as p-aminobenzoylglutamate (pABG) equivalents only decreases slowly during storage. We therefore measured pABG equivalents using liquid chromatography–MS/MS in 200 randomly selected samples collected in 1992–3 and 1997–9[32]. Based on the results of these analyses, we corrected for folate degradation during storage by using separate correction factors for the samples collected at baseline (corrected folate concentration 1992–3 = 5·3373 + 1·4045 × folate concentration measured in 1992–3) and those collected at follow-up (corrected folate concentration 1997–9 = 8·051 + 1·101 × folate concentration measured in 1997–9).

Plasma concentrations of methylmalonic acid (MMA), an inverse marker for vitamin B_{12} status[33,34], were determined by a modified GC–MS method based on ethylchloroformate derivatisation[35], and plasma concentrations of holoTC II were analysed by microbiological assays[36]. These indicators of vitamin B_{12} status were only measured in the samples collected in 1997–9.

Plasma total homocysteine (tHcy) concentration was determined using an automated HPLC assay[37,38].

Within-day CV for plasma measurements were < 5 % for concentrations of folate, vitamin B_{12}, MMA, holoTC II and tHcy.

Serum creatinine levels were analysed in the samples collected in 1997–9 by a modification of a liquid chromatography–MS/MS procedure[39]. ApoE-ε4 genotypes (0, 1 or 2 apoE-ε4 alleles) were determined using a one-stage PCR method[40], and methylenetetrahydrofolate reductase genotyping (677C → T) was performed by a real-time PCR[41].

Statistical analyses

Descriptive analyses. Plasma concentrations of folate and vitamin B_{12} measured in 1992–3 and 1997–9 were compared with a paired sample *t* test. Relationships between the different markers of vitamin B_{12} status measured in 1997–9 were evaluated with Spearman's correlation tests.

Principal component analysis (PCA) was used to create a summary score for cognitive performance that accounted for the correlations between the different cognitive performance tests and, thereby, maximised the explained variance. The number of components to be retained was determined according to two criteria: (1) eigenvalues > 1 and (2) Cattell's scree plot which shows the total variance related to each component. For comparison of cognitive performance on the individual tests across the quartiles of the cognitive performance component created with PCA, univariate ANOVA was used.

Quantile regression analyses. In order to investigate the interactions between folate and markers of vitamin B_{12} status in relation to cognitive performance, multivariate quantile regression and ordinary least-squares regression were used including the cognitive performance component extracted with PCA as the dependent variable. Interactions between folate and markers of vitamin B_{12} status in relation to cognitive performance were assessed with models including folate, a marker for vitamin B_{12} status, and their product term reflecting interaction, as continuous, independent variables. The quantile regression technique was used to provide distribution-free tests of whether the associations between folate, vitamin B_{12} markers and their interactions vary along the cognitive performance distribution. Plasma concentrations of folate and markers of vitamin B_{12} status were expressed as standardised *z*-scores to provide comparable associations per SD increase.

Logistic regression analyses. We further studied the interaction by estimating OR for cognitive impairment according to categories of combined folate and vitamin B_{12} status using logistic regression analyses. Cognitive impairment was defined as the lowest 10th percentile of the combined cognitive performance component as derived from the PCA. We determined quartiles for the markers of folate and vitamin B_{12} status, and defined the first quartile as 'low', the fourth quartile as 'high' and the two middle quartiles as 'normal'. Categories were created in which we combined 'low', 'normal' or 'high' folate status with 'low', 'normal' or 'high' vitamin B_{12} status. The combination of normal folate and normal vitamin B_{12} status was used as the reference group. All analyses were adjusted for sex, education level, history of CVD/hypertension, apoE-ε4 genotype and creatinine. These covariates were strong predictors for cognitive performance or associated with both B-vitamin levels and cognitive performance, as demonstrated with ANOVA or Pearson's correlation coefficients. BMI, smoking status, consumption of coffee, alcohol use, methylenetetrahydrofolate reductase 677 C → T genotype, diabetes and depression score were associated with either plasma folate or the markers of vitamin B_{12} or with cognitive performance, but adjusting for these biological and lifestyle factors did not markedly change the results of the analysis, and are therefore not included in the final model.

Descriptive analyses and PCA were performed using SAS version 9.2 (SAS Institute Inc.). Quantile regression analyses were performed in R version 2.13.1 (R Foundation for Statistical Computing) using the package 'quantreg'. Multiple imputation of missing values was carried out by chained equations with fully conditional specifications using the package 'mice'. *P* values < 0·05 were considered as statistically significant.

Results

Characteristics of the study population

The characteristics of the study population in 1997–9 are presented in Table 1. The mean age of the participants was 72·5 years and 44·9 % were men. Of the participants, about one-half (51 %) reported a history of CVD, 17 % suffered from depressive symptoms as indicated by a HADS-D score ≥ 8 and 14 % were current smokers. The median plasma folate concentration measured in 1992–3 was 12·5 (5th–95th percentile 8·7–20·9) nmol/l after correction for folate degradation during storage, which was lower than the concentration measured in 1997–9 (median 15·8 (5th–95th percentile 12·0–34·0) nmol/l) (*P* for difference < 0·0001). The median

British Journal of Nutrition

Table 1. Characteristics of the study population in 1997–1999

(Number of subjects and percentages; mean values and 95 % confidence intervals; median values and 5th and 95th percentiles)

Characteristics	*n**	Subjects (*n*)	%
Age (years)	2203		
Mean		72·5	
95 % CI		71·5, 73·6	
Male sex	2203	990	44·9
Education	2024		
No primary school		149	7·4
Primary school (≤9 years)		648	32·0
Vocational secondary school (10–12 years)		607	30·0
Theoretical secondary school (10–12 years)		238	11·8
College or university <4 years		215	10·6
College or university ≥4 years		167	8·3
History of CVD or hypertension†	2073	1050	50·7
Diabetes‡	2170	145	6·7
ApoE genotype	2192		
0 ApoE-ε4 alleles		1490	68·0
1 ApoE-ε4 allele§		633	28·9
2 ApoE-ε4 alleles		69	3·1
MTHFR C677T status	2202		
CC		1104	50·1
CT		915	41·6
TT		183	8·3
Depression	1999		
HADS-D score			
Mean		4·6	
95 % CI		4·4, 4·7	
Smoking status	2203		
Smokers		310	14·1
Ex-smokers		943	42·8
Never smokers		950	43·1
Daily coffee consumption	2146		
< 1 cup		156	7·3
1–4 cups		1656	77·2
≥ 5 cups		334	15·6
Alcohol consumption (glasses/week)	1848		
Mean		2	
95 % CI		1·8, 2·1	
Users of supplements containing B-vitamins	2032	193	9·5
Vitamin B_{12} intake including supplements (μg/d)	2031		
Mean		6·7	
95 % CI		6·5, 6·9	
Vitamin B_{12} intake without supplements (μg/d)	2031		
Mean		6·7	
95 % CI		6·5, 6·9	
Folate intake including supplements (μg/d)	2031		
Mean		290	
95 % CI		284, 295	
Folate intake without supplements (μg/d)	2031		
Mean		275	
95 % CI		270, 280	
Plasma vitamin B_{12} (pmol/l)	2194		
Median		339	
95 % CI		192, 651	
Plasma folate (nmol/l)‖	2186		
Median		15·8	
5th–95th percentile		12·0, 34·0	
Plasma MMA (μmol/l)	2192		
Median		0·19	
95 % CI		0·12, 0·36	
Plasma holoTC II (pmol/l)	2041		
Median		90	
95 % CI		43, 192	
Serum creatinine (mmol/l)	2202		
Mean		93	
95 % CI		92, 94	
m-MMSE	2181		
Median		12	
95 % CI		10, 12	

Table 1. *Continued*

Characteristics	*n**	Subjects (*n*)	%
m-DST	2188		
Median		9	
95 % CI		5, 18	
m-BD	2186		
Median		16	
95 % CI		10, 16	
KOLT	2197		
Median		35	
95 % CI		23, 48	
COWAT	2193		
Median		15	
95 % CI		7, 25	
TMT-A	2193		
Median		44	
95 % CI		28, 124	

MTHFR, methylenetetrahydrofolate reductase; HADS-D, Hospital Anxiety and Depression Scale depression subscale; MMA, methylmalonic acid; holoTC II, holotranscobalamin; m-MMSE, modified version of the Mini-Mental State Examination; m-DST, modified version of the Digit Symbol Test; m-BD, short form of the Block Design; KOLT, Kendrick Object Learning Test; COWAT, abridged version of the Controlled Oral Word Association Test; TMT-A, Trail Making Test Part A.

* Sample numbers may vary across the different variables due to different numbers of missing data.

† Based on self-reported CVD (myocardial infarction, angina pectoris, stroke, thrombosis and phlebitis) or hypertension at baseline or follow-up.

‡ Based on self-reported diabetes.

§ E2/E4 and E3/E4 genotypes.

‖ Folate concentrations are corrected for degradation during storage[32] as explained in the Methods section.

plasma vitamin B_{12} concentration in 1992–3 was 338 (5th–95th percentile 196–595) pmol/l and comparable with the concentration measured in 1997–9 (median 339 (5th–95th percentile 192–651) pmol/l) (P for difference = 0·12). In 1997–9, 4·9 % of the participants had vitamin B_{12} deficiency, defined as plasma concentrations of vitamin B_{12} < 150 pmol/l or MMA > 0·37 μmol/l. Plasma folate concentrations > 30, > 45 and > 59 nmol/l were observed in 6·6, 1·6 and < 1 % of the participants, respectively. Among the participants with vitamin B_{12} deficiency, only three had a folate concentration > 30 nmol/l.

Spearman's correlations (r) between plasma concentrations in 1992–3 and 1997–9 were 0·41 (P < 0·0001) for folate and 0·63 (P < 0·0001) for vitamin B_{12}. Plasma vitamin B_{12} (in 1997–9) correlated significantly with holoTC II (r 0·66, P < 0·0001) and MMA (r −0·20, P < 0·0001), and holoTC II also correlated significantly with MMA (r −0·24, P < 0·0001). Furthermore, in individuals with vitamin B_{12} concentrations < 274 pmol/l, holoTC II concentrations were decreased and MMA and tHcy concentrations were elevated compared with individuals with vitamin B_{12} concentrations > 274 pmol/l (Table S1, available online). tHcy concentrations were not elevated in participants with high folate concentrations (> 18·5 nmol/l) compared with those with folate concentrations ≤ 18·5 nmol/l.

Cognitive performance factors

Median (5th–95th percentile) scores for the six cognitive performance tests are presented in Table 1. Based on m-MMSE scores ≤ 10[19], 36 % of the study population suffered from mild cognitive impairment. Eigenvalues and Cattell's scree plot revealed that one component derived by the PCA should be retained, explaining 40·5 % of the total variance, whereas factors 2–4 explained less than 14·6 % each. The factor loading matrix is presented in Table S2 (available online). The first component strongly correlated with all six cognitive tests performed and is further referred to as 'overall cognitive performance'. Overall cognitive performance scores ranged from −4·80 to 2·49 with a mean of 0 and a SD of 1. Across the increasing quartiles of overall cognitive performance, the subjects performed better on each individual cognitive test (for all tests, P for difference < 0·0001; data not shown).

Cross-sectional associations of plasma concentrations of folate and vitamin B_{12} markers with overall cognitive performance assessed in 1997–9

Fig. 1 shows that plasma vitamin B_{12} is directly associated with cognitive performance at the lower quantiles of the cognitive performance distribution and inversely associated at the upper quantiles of the distribution. However, overall, this association is not significantly different from zero. Furthermore, Fig. 1 indicates a significant direct association of plasma folate, and a significant inverse interaction between plasma concentrations of folate and vitamin B_{12} in relation to cognitive performance. The associations of sex (male *v.* female), education (direct), history of CVD/hypertension (inverse) and apoE-ε4 status (inverse) with overall cognitive performance were as expected[42]. Notably, the associations of folate, education and apoE-ε4 with overall cognitive performance were asymmetric with the strongest effects in the lowest ranges of the overall cognitive performance scores.

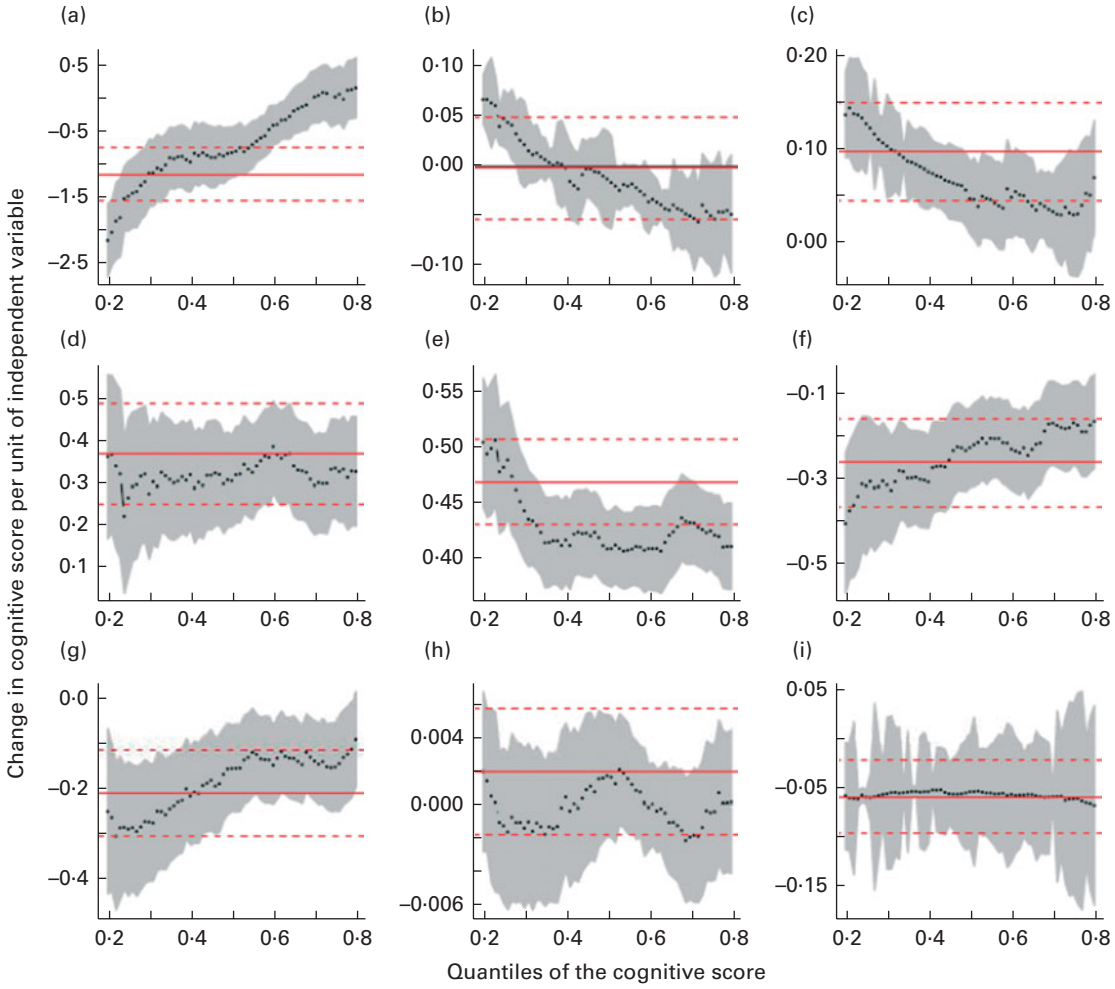

Fig. 1. Changes in cognitive score according to plasma vitamin B_{12}, folate and other determinants by quantile regression. The *y*-axis represents the associations between independent variables and the summary score for cognitive performance (based on six cognitive performance tests) extracted with the principal component analysis. The *x*-axis represents the quantiles of the cognitive performance distribution as observed within the present study population. The direction (positive and negative) and strength of the association are given by the number on the *y*-axis, whereas the 'slope' of the graph demonstrates the asymmetry or tail effects. The black points represent quantile regression fits, the dark-shaded grey zones represent 95 % CI for the estimates. The black horizontal lines at $y = 0$ indicate no (zero) changes in cognition. An upward or downward slope indicates the highest or lowest response at the upper or lower tail, respectively, of the distribution of the cognitive scores, whereas a horizontal graph below or above zero indicates similar effects through the whole distribution. The red horizontal solid lines represent the ordinary least-squares estimates of the conditional mean effects, and the red horizontal dotted lines represent the conventional 95 % CI for the least-squares estimates. (a) Intercept. (b) Plasma vitamin B_{12} and (c) folate were given as *z*-scores; (d) sex categorised as (1) men and (2) women; (e) education as (0) no primary school, (1) primary school (≤9 years), (2) vocational secondary school (10–12 years), (3) theoretical secondary school (10–12 years), (4) college or university (<4 years) and (5) college or university (≥4 years); (f) history of CVD/hypertension as (1) yes or (0) no; (g) apoE-ε4 genotype as 0, 1 or 2 apoE-ε4 alleles; (h) creatinine was given as μmol/l and (i) interaction (vitamin B_{12} × folate).

When including holoTC II or MMA instead of plasma vitamin B_{12} in the model, the quantile regression plots showed non-significant associations between holoTC II and MMA, a non-significant interaction between plasma folate and vitamin B_{12} markers, but similar associations of the other covariates (data not shown).

Table 2 presents the multivariate-adjusted ordinary least-squares regression estimates for the associations of plasma folate concentrations and the markers of vitamin B_{12}, as well as the interaction between plasma folate concentrations and the markers of vitamin B_{12} in relation to the overall cognitive performance score. In agreement with Fig. 1, plasma folate was directly associated and plasma vitamin B_{12} was not associated with the overall cognitive performance score. The negative interaction between plasma concentrations of folate and vitamin B_{12} indicates that the linear association between plasma folate and the overall cognitive performance score changes at different plasma concentrations of vitamin B_{12} and vice versa. The observed association for the interaction term suggests that, among the elderly with vitamin B_{12} concentrations in the lower range of the population, for example 2 SD below the mean, the association between plasma folate and the overall cognitive performance changes from $\beta = 0{\cdot}097$ to $\beta = 0{\cdot}097 - 2 \times (-0{\cdot}058) = 0{\cdot}213$, indicating that the positive effect of plasma folate concentrations on cognition becomes stronger at lower vitamin B_{12} concentrations. In contrast, among the elderly with vitamin B_{12} concentrations, 2 SD above the mean, the significant positive association between plasma folate and the overall cognitive performance changes from $\beta = 0{\cdot}097$ to $\beta = 0{\cdot}097 + 2\times$

British Journal of Nutrition

Table 2. Cross-sectional associations between plasma concentrations of folate, markers of vitamin B_{12} status and their interactions in relation to overall cognitive performance measured in 1997–9*

(β-Coefficients and standard errors)

	Marker of vitamin B_{12} status								
	Vitamin B_{12} (pmol/l)			HoloTC II (pmol/l)			MMA (μmol/l)		
	β	SE	P	β	SE	P	β	SE	P
n		1848			1721			1845	
Intercept	−1·10	0·24	<0·001	−1·09	0·24	<0·001	−0·86,	0·23	<0·001
Vitamin B_{12} marker†	0·00	0·03	0·997	0·03	0·03	0·320	−0·01	0·02	0·401
Folate† (nmol/l)	0·10	0·03	0·002	0·09	0·03	0·004	0·04	0·01	0·007
Vitamin B_{12} marker × folate‡	−0·06	0·02	0·009	−0·04	0·03	0·131	−0·01	0·03	0·880
Sex (women)	0·36	0·07	<0·001	0·36	0·07	<0·001	0·33	0·07	<0·001
Education level§	0·48	0·02	<0·001	0·48	0·02	<0·001	0·41	0·02	<0·001
History of CVD/hypertension	−0·26	0·06	<0·001	−0·26	0·06	<0·001	−0·23	0·06	<0·001
ApoE-ε4 genotype (0, 1 or 2 of alleles)	−0·22	0·06	<0·001	−0·22	0·06	<0·001	−0·15	0·06	0·015
Creatinine (mmol/l)	0·00	0·00	0·566	0·00	0·00	0·580	0·00	0·00	0·375

HoloTC, holotranscobalamin; MMA, methylmalonic acid.

* Ordinary least-squares regression coefficients adjusted for sex, education, apoE-ε4 status, history of CVD/hypertension and creatinine.

† Standardised B-vitamin concentrations (*z*-scores).

‡ The coefficient for the product term indicates how the association between folate status and the cognitive performance score changes when the concentration of the marker for vitamin B_{12} status increases.

§ Education level is defined as follows: no primary school; primary school (≤9 years); vocational secondary school (10–12 years); theoretical secondary school (10–12 years); college or university (<4 years); college or university (≥4 years).

(−0·058) = −0·019, indicating that the positive effect of plasma folate concentrations on cognition is slightly reduced. The vitamin B_{12} markers MMA and holoTC II did not show significant associations with the overall cognitive performance factor, nor were any of the interactions with folate significant (Table 2).

We further evaluated the observed interaction between plasma concentrations of folate and vitamin B_{12} using logistic regression analysis (Table 3). Plasma vitamin B_{12} concentrations in the lowest quartile of its distribution (<274 pmol/l) in combination with plasma folate concentrations in the highest quartile (>18·5 nmol/l) (*n* 102) when compared with normal plasma concentrations in the second and third quartiles of both vitamins (*n* 549) were associated with a reduced risk of cognitive impairment (OR 0·22, 95 % CI 0·05, 0·92).

Prospective associations of plasma concentrations of folate and total vitamin B_{12} in 1992–3 with cognitive performance assessed in 1997–9

Multivariate quantile regression and ordinary least-squares regression revealed no significant associations of plasma folate (β = 0·019, SE = 0·031, P=0·540) or plasma vitamin B_{12} (β = 0·020, SE = 0·033, P=0·541) measured in 1992–3 along the distribution of overall cognitive performance. In addition, the interaction between plasma folate and vitamin B_{12} sampled 6 years before the measurement of overall cognitive performance was not significant (β = 0·024, SE = 0·028, P=0·394; data not shown).

Discussion

The present population-based study investigated the hypothesis that high folate status in combination with low vitamin B_{12} status increased the risk for cognitive impairment, as was observed within the NHANES study[7], and was conducted in a population that was not exposed to mandatory or voluntary fortification of food items with folic acid. The study, which included 2203 elderly people aged 71–74 years, revealed that plasma folate, but not plasma vitamin B_{12}, was associated with better cognitive performance. Although the subgroup was rather small, a combination of plasma folate >18·5 nmol/l with vitamin B_{12} <274 pmol/l was associated with a reduced risk of cognitive impairment when compared with having normal concentrations of both vitamins.

Methodological considerations

It has been suggested that folate and vitamin B_{12} are related to different cognitive outcomes, and that there may even also be a difference in outcomes related to indicators of functional vitamin B_{12} status (MMA and holoTC II) *v.* total concentrations of vitamin B_{12} in plasma[43]. A major strength of the present study is the use of an extensive cognitive test battery covering global cognition, perceptual speed, visuospatial skills, episodic memory, access to semantic memory and executive function. We derived a cognitive performance component by the PCA, which is a robust measure for cognitive performance that reduces the possibility of measurement error and chance findings[44,45].

There is currently no 'gold standard' to define vitamin B_{12} deficiency with respect to potential markers and cut-off points to be used[46,47], although it has been recommended to include holoTC II and MMA as additional markers of vitamin B_{12} status[48]. Fedosov[49] presented a mathematic model combining the concentrations of vitamin B_{12}, MMA, holoTC II and tHcy to classify vitamin B_{12} status without the use of pre-defined cut-off levels. In addition, single markers were examined for their potential to predict vitamin B_{12} deficiency. They showed concentrations of holoTC II and

Table 3. Risk of cognitive impairment according to vitamin B_{12} and folate status by logistic regression*

(Number of subjects, odds ratios and 95 % confidence intervals)

B-vitamin status				
Vitamin B_{12}†	Folate‡	Subjects (*n*)	OR	95 % CI
Normal	Normal	549	1·0	
Normal	High	273	0·77	0·43, 1·40
Normal	Low	270	0·98	0·58, 1·66
Low	Normal	280	0·94	0·54, 1·63
Low	High	102	0·22	0·05, 0·92
Low	Low	166	1·01	0·51, 2·01
High	Normal	263	0·84	0·47, 1·49
High	High	170	0·68	0·32, 1·44
High	Low	113	0·82	0·38, 1·79

* Adjusted for sex, education, apoE-ε4 status, history of CVD/hypertension and creatinine.

† The categories 'low', 'normal' and 'high' plasma vitamin B_{12} were based on quartiles (Q) and defined as follows: <274 pmol/l (Q1), 274–432 pmol/l (Q2 and Q3) and >432 pmol/l (Q4), respectively.

‡ The categories 'low', 'normal' and 'high' plasma vitamin folate were based on quartiles (Q) and defined as follows: <14·1 nmol/l (Q1), 14·1–18·5 nmol/l (Q2 and Q3) and >18·5 nmol/l (Q4), respectively.

MMA to be more reliable than vitamin B_{12} or tHcy concentrations for predicting vitamin B_{12} deficiency. Vogiatzoglou *et al.*[50] observed that vitamin B_{12} concentrations below 400 pmol/l were associated with elevated concentrations of tHcy and MMA in the Hordaland Health cohort. In line with this, it has been suggested that desirable blood vitamin B_{12} concentrations may be higher than the current clinical recommendations since many studies have observed associations between 'low–normal' vitamin B_{12} and several negative health outcomes[51]. To avoid the use of cut-off levels for low vitamin B_{12} status, elevated folate status and cognitive impairment, as for the latter two, a consensus definition is lacking as well, we performed the present analyses by quantile regression, in which we plotted the associations between B-vitamins and the overall cognitive score through the distribution of the overall cognitive score. To facilitate comparison with the literature, we additionally created population-based quartile categories to define high and low concentrations of B-vitamins.

Our study group previously demonstrated that folate is degraded in stored samples to compounds that are almost completely recoverable as pAGB[32]. In the present study, we used the pABG assay[52] in a subsample and subsequently corrected for folate degradation in the full cohort. Nevertheless, the concentrations measured in 1992–3 were still lower than those measured in 1997–9. This may indicate either improved folate status over time or insufficient correction for folate degradation. Although, the pABG assay has been demonstrated to be the best available method to determine folate status in samples stored for a longer time, about 1 % of folate measured as pABG is lost per year[32]. Therefore, actual folate concentrations of the participants at the time of blood withdrawal may have been underestimated, and differences between folate concentrations in 1992–3 and 1997–9 may have been smaller than observed. However, it seems unlikely that this would have changed the present results.

Comparison with the literature

The present main observation that a combination of plasma folate >18·5 nmol/l with plasma vitamin B_{12} <274 pmol/l was associated with better cognitive performance contrasts the findings from the NHANES[7] and the Framingham Heart Study[11]. The NHANES showed worse cognitive performance in individuals with a combination of high plasma folate (>59 nmol/l) with low vitamin B_{12} (plasma vitamin B_{12} <148 pmol/l or MMA >0·21 μmol/l)[7]. Several aspects related to study design might explain this discrepancy. First, the NHANES was conducted in an area of mandatory folic acid fortification, and the use of supplements containing folic acid was common, which led to increased concentrations of serum folate[53] and unmetabolised folic acid[54]. In contrast, supplement use was uncommon among Norwegian elderly, and mean folate intake including folate from supplements did not differ from folate intake from foods (Table 1). As a result, very high folate concentrations (>59 nmol/l) were present in 20·7 % of the NHANES study population (*n* 1459), compared with less than 1 % in the present study.

Second, the Norwegian elderly population had better vitamin B_{12} status, with only 1·5 % suffering from vitamin B_{12} deficiency (≤148 pmol/l), which was used as a cut-off by the NHANES. The unique combination of very high folate with very low vitamin B_{12} status was present in a relatively small subgroup of forty-two individuals in the NHANES study, and even lower in the present study population (*n* 3). The better vitamin B_{12} status of the present study population may be explained by an adequate dietary intake of vitamin B_{12} (total mean intake 6·73 μg/d), probably related to the high intake of milk and fish in this population as shown previously[55].

The present findings also differed from the results of the Framingham Heart Study, unexposed to mandatory folic acid fortification, that showed a faster rate of decline in MMSE scores associated with having low vitamin B_{12} plasma concentrations (<257 pmol/l) at high folate levels (>20·2 nmol/l) ($P_{interaction}$ <0·001)[11]. Cut-off levels used to define 'low' vitamin B_{12} and 'high' folate status were similar to the present study; however, the Framingham Heart Study population showed more variation in plasma concentrations of both vitamin B_{12} (18·6–695 pmol/l) and folate (0·54–149 nmol/l), which may explain the discrepant findings.

Overall, the contrasting findings between the NHANES study[7] and the present study, supported by the findings of the Framingham Heart Study[11], suggest that only supraphysiologic folate status, resulting from fortification or supplemental folic acid intake, may exacerbate adverse cognitive consequences of low plasma vitamin B_{12} status. This hypothesis is supported by recent data published by Morris[43].

Metabolic effects of a combination of high folate and low vitamin B_{12} status

Some[7,14,56,57], but not all[13] studies have shown that elderly individuals with high folate and low vitamin B_{12} status have a higher prevalence of anaemia, and higher concentrations of tHcy and MMA. It has been proposed that high folate

concentrations may exacerbate the negative consequences of vitamin B_{12} deficiency. It is possible that subjects with a combination of low vitamin B_{12} and high folate status in these studies[7,13,14,56,57] suffered from severe vitamin B_{12} deficiency due to disorders that affected vitamin B_{12} absorption, such as pernicious anaemia[58,59]. A recent study in healthy young adults without any medical conditions that could induce anaemia or affect folate or vitamin B_{12} absorption did not observe any adverse effects of high folate concentrations on biochemical markers related to vitamin B_{12} deficiency[60]. In our population, MMA concentrations were highest in participants with plasma vitamin B_{12} in the lowest quartile and folate status in the highest quartile, which is in line with previous findings[14,57]. In contrast, tHcy concentration was substantially lower in subjects with folate concentration in the highest quartile (>18·5 nmol/l) when compared with the other quartiles, independent of vitamin B_{12} status.

The view prevails that low vitamin B_{12} status is associated with cognitive impairment[3]. For instance, six decades ago, it was proposed that high folate status in vitamin B_{12}-deficient subjects may deteriorate cognitive performance[5,6]. The adverse effect of high folate status may be confined to subjects with severe vitamin B_{12} deficiency, leading to the methylfolate trap[61]. The Hordaland Health population was replete with vitamin B_{12}, and only 4·9 % had plasma concentrations of vitamin B_{12} <150 pmol/l or MMA >0·37 μmol/l. Under these conditions, high folate levels may accelerate methionine synthesis, thereby increasing biological methylation including compounds involved in neurotransmission[62]. This is in line with the present observation that folate is associated with better cognitive performance, which remains significant even at vitamin B_{12} concentrations <274 pmol/l. We did not observe an interaction of folate with holoTC II and MMA. The utility of the vitamin B_{12} markers MMA and holoTC II in relation to cognitive performance has been evaluated in some observational studies[3,63]. The discrepancy between total vitamin B_{12} and the other markers in the present study could perhaps be related to the fact that total vitamin B_{12} may be a less sensitive indicator of vitamin B_{12} status at concentrations above the traditional clinical cut-off level of 150 pmol/l due to buffering capacities of vitamin B_{12} body stores[48]. Furthermore, similar to the present study, previous studies have observed poor correlations between the different indicators of vitamin B_{12} status[64–67]. The optimal combinations of markers, cut-off levels and assays for vitamin B_{12} status assessment have been the topic of debate since many years. Mathematic models combining the results of different markers may provide the best alternative for assessing vitamin B_{12} status in the future[49].

Conclusion

In conclusion, the present large study population unexposed to mandatory or voluntary folic acid fortification showed that plasma folate, but not plasma vitamin B_{12}, was associated with cognitive performance. Among the elderly participants with vitamin B_{12} concentrations in the lower range, the association between plasma folate and cognitive performance was strongest. No interaction between folate and vitamin B_{12} status was observed when considering more sensitive markers of vitamin B_{12} status, MMA and holoTC II. Therefore, the clinical relevance of these observations is uncertain.

Supplementary material

To view supplementary material for this article, please visit http://dx.doi.org/10.1017/S000711451300336X

Acknowledgements

The present study was supported by the Norman Collisson Foundation, UK. The laboratory measurements were supported by the Foundation to Promote Research into Functional Vitamin B_{12} Deficiency, Norway. Both the Norman Collisson Foundation and the Foundation to Promote Research into Functional Vitamin B_{12} Deficiency had no role in the design, analysis or writing of this article.

We thank Lisette de Groot for her financial contribution to perform additional analysis on the folate samples. Lisette de Groot contributed to the preparation of the manuscript.

The authors' contributions were as follows: E. L. D. and S. J. P. M. E. designed the research; E. L. D. conducted the research; G. S. T., A. D. S. and H. R. provided the essential materials; E. L. D., S. J. P. M. E. and P. M. U. analysed the data and performed the statistical analysis; E. L. D. and S. J. P. M. E. wrote the paper; P. M. U., G. S. T., S. E. V., O. K. N., P. v. V., L. C. P. G. M. d. G., E. N., H. R. and S. J. P. M. E. provided a critical review of the manuscript; E. L. D. had primary responsibility for the final content. All authors read and approved the final manuscript.

None of the authors has any conflict of interest.

References

1. Raman G, Tatsioni A, Chung M, *et al.* (2007) Heterogeneity and lack of good quality studies limit association between folate, vitamins B-6 and B-12, and cognitive function. *J Nutr* **137**, 1789–1794.
2. Smith AD (2008) The worldwide challenge of the dementias: a role for B vitamins and homocysteine? *Food Nutr Bull* **29**, S143–SS72.
3. Smith AD & Refsum H (2009) Vitamin B-12 and cognition in the elderly. *Am J Clin Nutr* **89**, 707S–711S.
4. Vogel T, Dali-Youcef N, Kaltenbach G, *et al.* (2009) Homocysteine, vitamin B_{12}, folate and cognitive functions: a systematic and critical review of the literature. *Int J Clin Pract* **63**, 1061–1067.
5. Chodos RB & Ross JF (1951) The effects of combined folic acid and liver extract therapy. *Blood* **6**, 1213–1234.
6. Israels MCG & Wilkinson JF (1949) Risk of neurological complications in pernicious anaemia treated with folic acid. *Br Med J* **2**, 1072–1075.
7. Morris MS, Jacques PF, Rosenberg IH, *et al.* (2007) 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* **85**, 193–200.
8. Bailey RL, Mills JL, Yetley EA, *et al.* (2010) Unmetabolized serum folic acid and its relation to folic acid intake from diet and supplements in a nationally representative sample

of adults aged >or =60 y in the United States. *Am J Clin Nutr* **92**, 383–389.
9. Kalmbach RD, Choumenkovitch SF, Troen AM, *et al.* (2008) Circulating folic acid in plasma: relation to folic acid fortification. *Am J Clin Nutr* **88**, 763–768.
10. Morris MS, Jacques PF, Rosenberg IH, *et al.* (2010) Circulating unmetabolized folic acid and 5-methyltetrahydrofolate in relation to anemia, macrocytosis, and cognitive test performance in American seniors. *Am J Clin Nutr* **91**, 1733–1744.
11. Morris MS, Selhub J & Jacques PF (2012) Vitamin B-12 and folate status in relation to decline in scores on the mini-mental state examination in the Framingham heart study. *J Am Geriatr Soc* **60**, 1457–1464.
12. Clarke R, Birks J, Nexo E, *et al.* (2007) Low vitamin B-12 status and risk of cognitive decline in older adults. *Am J Clin Nutr* **86**, 1384–1391.
13. Clarke R, Sherliker P, Hin H, *et al.* (2008) Folate and vitamin B-12 status in relation to cognitive impairment and anaemia in the setting of voluntary fortification in the UK. *Br J Nutr* **100**, 1054–1059.
14. Miller JW, Garrod MG, Allen LH, *et al.* (2009) Metabolic evidence of vitamin B-12 deficiency, including high homocysteine and methylmalonic acid and low holotranscobalamin, is more pronounced in older adults with elevated plasma folate. *Am J Clin Nutr* **90**, 1586–1592.
15. Obeid R & Herrmann W (2007) Holotranscobalamin in laboratory diagnosis of cobalamin deficiency compared to total cobalamin and methylmalonic acid. *Clin Chem Lab Med* **45**, 1746–1750.
16. HUSK. Recruitment into the cognitive sub-study of the Hordaland Homocysteine Study. http://www.uib.no/isf/husk/Vedlegg_dokumenter/Cognitive_Sub_study.pdf (accessed 12 August 2013).
17. Refsum H, Nurk E, Smith AD, *et al.* (2006) The hordaland homocysteine study: a community-based study of homocysteine, its determinants, and associations with disease. *J Nutr* **136**, 1731S–1740S.
18. Ueland PM, Nygard O, Vollset SE, *et al.* (2001) The Hordaland homocysteine studies. *Lipids* **36**, S33–S39.
19. Nurk E, Drevon CA, Refsum H, *et al.* (2007) Cognitive performance among the elderly and dietary fish intake: the Hordaland Health Study. *Am J Clin Nutr* **86**, 1470–1478.
20. Braekhus A, Laake K & Engedal K (1992) The Mini-Mental State Examination: identifying the most efficient variables for detecting cognitive impairment in the elderly. *J Am Geriatr Soc* **40**, 1139–1145.
21. Folstein MF, Folstein SE & McHugh PR (1975) Mini-Mental State – practical method for grading cognitive state of patients for clinician. *J Psychiatr Res* **12**, 189–198.
22. Wechsler D (1981) *Wechsler Adult Intelligence Scale-Revised*. New York, NY: Psychological Corporation.
23. Kendrick D (1985) *Kendrick Cognitive Tests for the Elderly*. Windsor: NFER-NELSON Publishing Company Ltd.
24. Benton A & Hamsher K (1989) *Multilingual Aphasia Examination*. Iowa city, IA: AJA Associates.
25. Reitan R (1958) Validity of the trail making test as an indicator of organic brain damage. *Percept Mot Skills* **8**, 271–276.
26. Nygard O, Vollset SE, Refsum H, *et al.* (1995) Total plasma homocysteine and cardiovascular risk profile. The Hordaland Homocysteine Study. *JAMA* **274**, 1526–1533.
27. Nes M, Andersen LF, Solvoll K, *et al.* (1992) Accuracy of a quantitative food frequency questionnaire applied in elderly Norwegian women. *Eur J Clin Nutr* **46**, 809–821.
28. Nurk E, Tell GS, Vollset SE, *et al.* (2002) Plasma total homocysteine and hospitalizations for cardiovascular disease – the Hordaland Homocysteine Study. *Arch Intern Med* **162**, 1374–1381.
29. Zigmond AS & Snaith RP (1983) The hospital anxiety and depression scale. *Acta Psychiatr Scand* **67**, 361–370.
30. Kelleher B & Broin S (1991) Microbiological assay for vitamin B_{12} performed in 96-well microtitre plates. *J Clin Pathol* **44**, 592–595.
31. O'Broin S & Kelleher B (1992) Microbiological assay on microtitre plates of folate in serum and red cells. *J Clin Pathol* **45**, 344–347.
32. Hannisdal R, Gislefoss RE, Grimsrud TK, *et al.* (2010) Analytical recovery of folate and its degradation products in human serum stored at −25 degrees C for up to 29 years. *J Nutr* **140**, 522–526.
33. Lindenbaum J, Savage DG, Stabler SP, *et al.* (1990) Diagnosis of cobalamin deficiency. 2. Relative sensitivities of serum cobalamin, methylmalonic acid, and total homocysteine concentrations. *Am J Hematol* **34**, 99–107.
34. Moelby L, Rasmussen K, Jensen MK, *et al.* (1990) The relationship between clinically confirmed cobalamin deficiency and serum methylmalonic acid. *J Intern Med* **228**, 373–378.
35. Husek P (1995) Simultaneous profile analysis of plasma amino and organic-acids by capillary gas-chromatography. *J Chromatogr B Biomed Appl* **669**, 352–357.
36. Refsum H, Johnston C, Guttormsen AB, *et al.* (2006) Holotranscobalamin and total transcobalamin in human plasma: determination, determinants, and reference values in healthy adults. *Clin Chem* **52**, 129–137.
37. Fiskerstrand T, Refsum H, Kvalheim G, *et al.* (1993) Homocysteine and other thiols in plasma and urine: automated determination and sample stability. *Clin Chem* **39**, 263–271.
38. Refsum H, Ueland PM & Svardal AM (1989) Fully automated fluorescence assay for determining total homocysteine in plasma. *Clin Chem* **35**, 1921–1927.
39. Holm PI, Ueland PM, Kvalheim G, *et al.* (2003) Determination of choline, betaine, and dimethylglycine in plasma by a high-throughput method based on normal-phase chromatography-tandem mass spectrometry. *Clin Chem* **49**, 286–294.
40. Wenham PR, Price WH & Blundell G (1991) Apolipoprotein-E genotyping by one-stage PCR. *Lancet* **337**, 1158–1159.
41. Ulvik A & Ueland PM (2001) Single nucleotide polymorphism (SNP) genotyping in unprocessed whole blood and serum by real-time PCR: application to SNPs affecting homocysteine and folate metabolism. *Clin Chem* **47**, 2050–2053.
42. Anstey K & Christensen H (2000) Education, activity, health, blood pressure and apolipoprotein E as predictors of cognitive change in old age: a review. *Gerontology* **46**, 163–177.
43. Morris MS (2012) The role of B vitamins in preventing and treating cognitive impairment and decline. *Adv Nutr* **3**, 801–812.
44. Barnes LL, Wilson RS, Schneider JA, *et al.* (2003) Gender, cognitive decline, and risk of AD in older persons. *Neurology* **60**, 1777–1781.
45. Visser PJ (2006) Role of cognitive testing in disease modifying AD trials. *J Nutr Health Aging* **10**, 131–132.
46. Clarke R, Sherliker P, Hin H, *et al.* (2007) Detection of vitamin B-12 deficiency in older people by measuring vitamin B-12 or the active fraction of vitamin B-12, holotranscobalamin. *Clin Chem* **53**, 963–970.
47. Hoey L, Strain JJ & McNulty H (2009) Studies of biomarker responses to intervention with vitamin B-12: a systematic review of randomized controlled trials. *Am J Clin Nutr* **89**, 1981S–1996S.
48. Carmel R (2011) Biomarkers of cobalamin (vitamin B-12) status in the epidemiologic setting: a critical overview of

context, applications, and performance characteristics of cobalamin, methylmalonic acid, and holotranscobalamin II. *Am J Clin Nutr* **94**, 348S–358S.
49. Fedosov SN (2010) Metabolic signs of vitamin B(12) deficiency in humans: computational model and its implications for diagnostics. *Metabolism* **59**, 1124–1138.
50. Vogiatzoglou A, Oulhaj A, Smith AD, *et al.* (2009) Determinants of plasma methylmalonic acid in a large population: implications for assessment of vitamin B-12 status. *Clin Chem* **55**, 2198–2206.
51. Smith AD & Refsum H (2012) Do we need to reconsider the desirable blood level of vitamin B_{12}? *J Intern Med* **271**, 179–182.
52. Hannisdal R, Ueland PM, Eussen SJ, *et al.* (2009) Analytical recovery of folate degradation products formed in human serum and plasma at room temperature. *J Nutr* **139**, 1415–1418.
53. Choumenkovitch SF, Jacques PF, Nadeau MR, *et al.* (2001) Folic acid fortification increases red blood cell folate concentrations in the Framingham Study. *J Nutr* **131**, 3277–3280.
54. Yang Q, Cogswell ME & Hamner HC (2010) Folic acid source, usual intake, and folate and vitamin B-12 status in US adults: National Health and Nutrition Examination Survey (NHANES) 2003–2006. *Am J Clin Nutr* **91**, 64–72.
55. Vogiatzoglou A, Smith AD, Nurk E, *et al.* (2009) Dietary sources of vitamin B-12 and their association with plasma vitamin B-12 concentrations in the general population: the Hordaland Homocysteine Study. *Am J Clin Nutr* **89**, 1078–1087.
56. Selhub J, Morris MS, Jacques PF, *et al.* (2009) Folate–vitamin B-12 interaction in relation to cognitive impairment, anemia, and biochemical indicators of vitamin B-12 deficiency. *Am J Clin Nutr* **89**, 702S–706S.
57. Selhub J, Morris MS, Jacques PF, *et al.* (2007) In vitamin B_{12} deficiency, higher serum folate is associated with increased total homocysteine and methylmalonic acid concentrations. *Proc Natl Acad Sci U S A* **104**, 19995–20000.
58. Berry RJ, Carter HK, Yang Q, *et al.* (2007) Cognitive impairment in older Americans in the age of folic acid fortification. *Am J Clin Nutr* **86**, 265–267 (author reply 7–9).
59. Carmel R (2009) Does high folic acid intake affect unrecognized cobalamin deficiency, and how will we know it if we see it? *Am J Clin Nutr* **90**, 1449–1450.
60. Mills JL, Carter TC, Scott JM, *et al.* (2011) Do high blood folate concentrations exacerbate metabolic abnormalities in people with low vitamin B-12 status? *Am J Clin Nutr* **94**, 495–500.
61. Scott J & Weir D (1994) Folate/vitamin B_{12} inter-relationships. *Essays Biochem* **28**, 63–72.
62. Weir DG & Scott JM (1999) Brain function in the elderly: role of vitamin B-12 and folate. *Br Med Bull* **55**, 669–682.
63. Doets EL, van Wijngaarden JP, Szczecinska A, *et al.* (2012) Vitamin B_{12} intake and status and cognitive function in elderly people. *Epidemiol Rev* **35**, 2–21.
64. Carmel R, Green R, Rosenblatt DS, *et al.* (2003) Update on cobalamin, folate, and homocysteine. *Hematology* **2003**, 62–81.
65. Chen X, Remacha AF, Sarda MP, *et al.* (2005) Influence of cobalamin deficiency compared with that of cobalamin absorption on serum holo-transcobalamin II. *Am J Clin Nutr* **81**, 110–114.
66. Herrmann W, Obeid R, Schorr H, *et al.* (2005) The usefulness of holotranscobalamin in predicting vitamin B_{12} status in different clinical settings. *Curr Drug Metab* **6**, 47–53.
67. Miller JW, Garrod MG, Rockwood AL, *et al.* (2006) Measurement of total vitamin B_{12} and holotranscobalamin, singly and in combination, in screening for metabolic vitamin B_{12} deficiency. *Clin Chem* **52**, 278–285.

British Journal of Nutrition