



Plasma concentrations of vitamin B₁₂ and folate and global cognitive function in an older population: cross-sectional findings from The Irish Longitudinal Study on Ageing (TILDA)

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Abstract

The uncertainty surrounding high intakes of folic acid and associations with cognitive decline in older adults with low vitamin B₁₂ status has been an obstacle to mandatory folic acid fortification for many years. We estimated the prevalence of combinations of low/normal/high vitamin B₁₂ and folate status and compared associations with global cognitive function using two approaches, of individuals in a population-based study of those aged ≥50 years in the Republic of Ireland. Cross-sectional data from 3781 men and women from Wave 1 of The Irish Longitudinal Study on Ageing were analysed. Global cognitive function was assessed by the Mini Mental State Examination (MMSE) and Montreal Cognitive Assessment (MoCA). Prevalence estimates for combinations of vitamin B₁₂ (plasma vitamin B₁₂ < or ≥258 pmol/l) and folate (plasma folate ≤ or >45.3 nmol/l) concentrations were generated. Negative binomial regression models were used to investigate the associations of vitamin B₁₂ and folate status with global cognitive function. Of the participants, 1.5% (*n* 51) had low vitamin B₁₂ (<258 pmol/l) and high folate (>45.3 nmol/l) status. Global cognitive performance was not significantly reduced in these individuals when compared with those with normal status for both B-vitamins (*n* 2433). Those with normal vitamin B₁₂/high folate status (7.6%) had better cognitive performance (MMSE: incidence rate ratio (IRR) 0.82, 95% CI 0.68, 0.99; *P* = 0.043, MoCA: IRR 0.89, 95% CI 0.80, 0.99; *P* = 0.025). We demonstrated that high folate status was not associated with lower cognitive scores in older adults with low vitamin B₁₂ status. These findings provide important safety information that could guide fortification policy recommendations in Europe.

Key words: Vitamin B₁₂: Folate: Ageing: Older people: Cognitive function: Epidemiology

Cognitive impairment has been associated with low vitamin B₁₂⁽¹⁾ and low folate status⁽²⁾. In addition, some previous observational data have suggested that older adults with low vitamin B₁₂ and high folate status had higher risks of anaemia and cognitive impairment or decline^(3–5); however, the causal relevance of these associations remains uncertain.

Vitamin B₁₂ and folate participate in one carbon metabolism, critical for the formation of DNA and RNA, neurotransmitters, membrane lipids and proteins. Because of these functions, there has been growing interest in the possibility that age-related conditions, in particular loss of cognitive function, may partly be explained by inadequate status of these vitamins^(6–9). Vitamin B₁₂ deficiency (plasma vitamin B₁₂ < 148 pmol/l) is associated with severe neurological consequences, including sensory and

motor neuropathy, altered mental status and cognitive decline⁽¹⁾. Vitamin B₁₂ deficiency also reduces the availability of folate for DNA synthesis⁽¹⁾.

Since the introduction of mandatory fortification of staple foods with folic acid to reduce neural tube defects (NTD), high folate status (>45.3 nmol/l) is frequently observed in countries such as the USA and Canada. Recent research has raised concerns regarding the adverse implications of simultaneous low vitamin B₁₂ and high folate status given that high-dose folic acid treatment was shown to temporarily mask clinical symptoms in persons with pernicious anaemia⁽¹⁰⁾.

While some studies have reported that older adults with low vitamin B₁₂ and high folate status had higher risks of anaemia, cognitive impairment and decline^(3–5), other large studies

Abbreviations: MMSE, Mini Mental State Examination; MoCA, Montreal Cognitive Assessment; TILDA, The Irish Longitudinal Study on Ageing.

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conducted in diverse populations have not replicated such adverse associations⁽¹¹⁻¹³⁾. There is considerable heterogeneity among the published studies, including differences in vitamin status cut-offs, biochemical methodologies and the instruments used to assess cognitive function⁽¹⁴⁾. Likewise, large-scale randomised controlled trials have failed to demonstrate any beneficial effects of supplementation with vitamin B₁₂ or folic acid on global or domain-specific cognitive function⁽¹⁵⁾.

Ireland has one of the highest rates of neural tube defects in Europe⁽¹⁶⁾, and supplementation initiatives have been shown to be ineffective⁽¹⁷⁾, yet one major obstacle impeding population-based food fortification is the concern that high folate status in the presence of low vitamin B₁₂ status could have a detrimental effect on cognitive function in older people. In our population study, The Irish Longitudinal Study on Ageing (TILDA), the prevalence of older people with deficient or low vitamin B₁₂ status (<185 pmol/l) was 12%⁽¹⁸⁾, while high folate status (>45.3 nmol/l) was observed in 9% of the population⁽¹⁸⁾. TILDA affords a unique opportunity to investigate whether there is an association between cognitive performance and variations of vitamin B₁₂ and folate in a large, nationally representative population sample of older adults. We hypothesise that a low vitamin B₁₂ status coupled with high blood concentrations of folate does not confer greater risk to global cognitive performance in this older population group.

Methods

Study population

Cross-sectional data from Wave 1 of TILDA were analysed. TILDA is a nationally representative prospective cohort study on ageing

of community-dwelling adults aged 50 years and older, living in the Republic of Ireland. Details of the study design have been reported previously⁽¹⁹⁻²¹⁾. Briefly, Wave 1 took place between October 2009 and February 2011. Eight thousand hundred and seventy-four adults aged ≥50 years from 6279 households completed a computer-aided personal interview, representing a response rate of 62%. Of the total sample, 84.6% completed the self-completion questionnaire (*n* 7196) and 72.1% (*n* 5895) participated in a health assessment which included blood measurements. Plasma vitamin B₁₂ measurements were available for 88.5% (*n* 5219) and plasma folate for 90.7% (*n* 5350) of participants. Missing data were due to insufficient sample and/or assay failures. Individuals who did not complete both standardised assessments of cognitive function, the Mini Mental State Examination (MMSE)⁽²²⁾ and Montreal Cognitive Assessment (MoCA)⁽²³⁾, were not included in this analysis, giving a final analytical sample of 3871 (Fig. 1). Ethical approval was obtained from the Faculty of Health Sciences Research Ethics Committee of Trinity College Dublin. All participants were fully informed of the study and gave written consent.

Assessment of cognitive function

Global cognitive function was assessed using the MMSE and MoCA. The MMSE is a brief 30-point test that is used to screen for cognitive impairment, commonly used in clinical practice to screen for dementia and assesses orientation, recall, attention, calculation, language abilities and visuospatial ability. The MoCA, also used the screen for dementia, is particularly sensitive to mild cognitive deficits^(23,24), with additional assessments of executive function, abstraction and visuospatial ability. Both tools have scores ranging from 0 to 30.

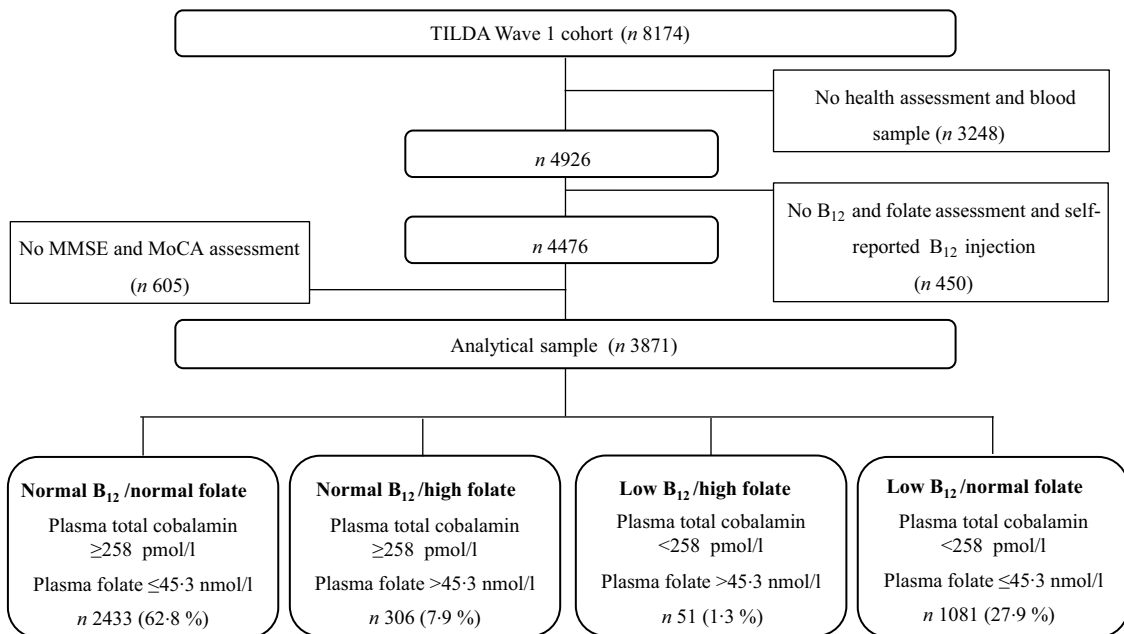


Fig. 1. Flow diagram – analytical sample design and selection. TILDA, The Irish Longitudinal Study on Ageing; MMSE, Mini Mental State Examination; MoCA, Montreal Cognitive Assessment; B₁₂, vitamin B₁₂.

Analysis of B-vitamins

A non-fasting blood sample was collected by venepuncture into one 10 ml K₂EDTA tube (BD, Becton, Dickinson Limited) for immediate analysis and two 10 ml EDTA (BD, Becton, Dickinson Limited) tubes for long-term storage (Sarstedt). Samples were kept chilled and were centrifuged (3000 rpm for 15 min). Aliquots of plasma were labelled and stored at -80°C within 48 h of blood collection and stored until required for analysis. The TILDA protocol for blood sample collection, processing, and storage is detailed elsewhere⁽²¹⁾. Plasma vitamin B₁₂ and folate concentrations were determined by microbiological assay; *Lactobacillus leichmannii* (*L. leichmannii* (*delbrueckii*) (NCIB 12519, ATCC 43787)), limit of detection 3.7 pmol/l⁽²⁵⁾ and *Lactobacillus casei* (*L. rhamnosis* (NCIB 10463; ATCC 27773)), limit of detection 0.6 nmol/l⁽²⁶⁾, respectively. Inter-assay CV for both methods were <10.9%.

Other covariates

Sociodemographic characteristics examined included age, sex, educational attainment, mean asset wealth and habitation status. Lifestyle characteristics comprised self-reported physical activity (International Physical Activity Questionnaire), smoking status, alcohol consumption, depressive symptoms (Center for Epidemiologic Studies Depression Scale)⁽²⁷⁾ and history of stroke. Use of medications (up to twenty prescription and non-prescription medications, including food supplements) was classified by World Health Organization, Anatomical Therapeutic Chemical classification codes.

Anthropometric measurements included height, weight, BMI and handgrip strength (average two recordings from dominant hand using a dynamometer). Hypertension was defined as systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg and/or antihypertensive medication use. Diabetes was defined as diabetes or prediabetes by measurement of glycated Hb (HbA_{1c})⁽²⁸⁾, self-reported diabetes diagnosis and/or use of anti-glycaemic medications.

Exclusion criteria

Individuals without a health assessment and blood sample were excluded (n 3248). Those for whom vitamin B₁₂ and/or folate concentrations could not be measured, or whose measurement values exceeded the 99th percentiles and participants in receipt of vitamin B₁₂ injections, were omitted from the analysis (n 450). In order to rule out participants with possible mild cognitive impairment, additional exclusionary criteria were applied to both MMSE and MoCA results. Those with MMSE scores of <24⁽²⁹⁾ and MoCA below the age and education normative cut-offs of Rossetti *et al.*⁽³⁰⁾ were also omitted (n 605).

Statistical methods

While there is no international consensus regarding the cut-offs for both vitamin B₁₂ and folate status, we adopted the widely used cut-offs identified in the literature. For plasma vitamin B₁₂ (pmol/l), these included <148 (deficient); 148–<185 (low status); 185–<258 (low/normal status); >258–>601 (normal

and >601 (high)^(31,32). For plasma folate (nmol/l), the cut-offs included 10–23.0 (low/normal) and >23.0–45.3 (normal) >45.3 (high)^(12,33). To compare with previous studies, a four-level categorical variable was created on the basis of vitamin B₁₂ (< or ≥ 258 pmol/l)^(31,32) and folate concentrations (\leq or >45.3 nmol/l)⁽³⁴⁾ and was defined as: (i) normal vitamin B₁₂/normal folate (≥ 258 pmol/l, ≤ 45.3 nmol/l), (ii) normal vitamin B₁₂/high folate (≥ 258 pmol/l, >45.3 nmol/l), (iii) low vitamin B₁₂/high folate (<258 pmol/l, >45.3 nmol/l) and (iv) low vitamin B₁₂/normal folate (<258 pmol/l, ≤ 45.3 nmol/l).

Non-normally distributed continuous variables were described as medians and interquartile ranges for each group, stratified by sample characteristics, biomarkers and cognitive tests. These were compared using Kruskal–Wallis and Mann–Whitney U tests for pairwise comparisons. Categorical variables were compared using χ^2 tests or Fisher's exact test, as appropriate. A false discovery rate correction for multiple comparisons was applied ($P < 0.013$, achieving false discovery rate $q < 0.05$) to adjust for the number of individual tests carried out (i.e. q indicates that the average alpha level across the family of tests does not exceed 0.05, following correction).

Associations of vitamin B₁₂ and folate with cognitive function and other covariates were explored in a series of regression analyses. The relationship between cognitive function and groups of vitamin B₁₂ and folate status was analysed with models appropriate for the distribution of the outcome variables, MMSE (continuous) and MoCA (continuous). Visualisation of the data and detailed inspection of model residuals demonstrated that neither outcome was normally distributed after appropriate removal of outliers. Therefore, MMSE and MoCA were analysed using negative binomial regression models (due to overdispersion) with the outcome representing the count of errors made during the test (30 minus the raw score) due to their left-skewed distributions, consistent with previous reports using TILDA data⁽³⁵⁾.

The results of these analyses are presented as the incidence rate ratio for each model, relative to the reference category, normal vitamin B₁₂/normal folate, as previously performed in this cohort⁽³⁶⁾. For both cognitive tests, three models were investigated: unadjusted (model 0); adjusted for sociodemographic covariates – age, sex, educational attainment, mean asset wealth and habitation status (model 1) and adjusted for sociodemographic, lifestyle, health indicators and medications – physical activity, smoking, alcohol consumption, BMI, mean grip strength, hypertension, diabetes, depressive symptoms, folic acid supplement use, proton pump inhibitor, statin and thyroid medication use and history of previous stroke (model 2).

These analyses were then repeated in the sample aged ≥ 60 years (n 2070), for more direct comparison with other studies in this field. Further, a sensitivity analysis was performed including the participants with lower cognitive scores, aged ≥ 50 and ≥ 60 years, omitted from the main analysis.

Additionally, quintiles of vitamin B₁₂ concentrations were created based on plasma vitamin B₁₂ concentrations in those aged ≥ 60 years. An interaction term was included to examine whether the association between plasma folate (as a continuous variable) differed between quintiles of vitamin B₁₂, using the highest quintile of vitamin B₁₂/folate (Q5) as the reference



category (the reverse instantiation was also analysed, that is, continuous vitamin B₁₂ and quintiles of folate).

Population weights were calculated for the regression analyses by comparing the TILDA population with the 2011 Irish Census with respect to age, sex, educational attainment, marital status and urban *v.* rural residence (weights reflected the reciprocal of the selection probability). Prevalence estimates, descriptive statistics and model coefficients were adjusted using modified base weights that accounted for survey non-response, non-attendance at the health assessment component of the study and whether a blood sample was provided. The staged, stratified and clustered sample design of TILDA was considered when estimating confidence intervals and standard errors. Analyses were carried out using STATA 15 (StataCorp).

Results

Baseline characteristics of the study sample

The study design and exclusion criteria are detailed in Fig. 1. The mean age was 61.8 years (sd 8.8) (range 50–98 years); 50.7% were female (*n* 3871). Median (interquartile ranges) MMSE and MoCA scores were 29 (28–30) and 26 (24–28), respectively. Vitamin B₁₂ concentrations ranged from 45.4 to 719 pmol/l, with 4.8% (95% CI 4.1, 5.7) classified as deficient (<148 pmol/l) and 25.5% (95% CI 24.1, 27.0) with low vitamin B₁₂ status (148–258 pmol/l). Folate concentrations were 0.9–82 nmol/l, 5.7% (95% CI 4.9, 6.7) had high folate status (45.3–60 nmol/l), with an additional 3.3% (95% CI 2.8, 4.0) classified as very high folate status (>60 nmol/l), similar to rates observed in our full population⁽¹⁸⁾. The prevalence of normal vitamin B₁₂/normal folate status was 62% (95% CI 60.2, 63.8), normal vitamin B₁₂/high folate was 7.6% (95% CI 6.6, 8.8), low vitamin B₁₂/high folate was 1.5% (95% CI 1.1, 2.0) and low vitamin B₁₂/normal folate was 28.9% (95% CI 27.3, 30.6).

Sample characteristics, by vitamin B₁₂ and folate groups

Sample characteristics, stratified by vitamin B₁₂ and folate groups, are detailed in Table 1, with additional sample characteristics displayed in online Supplementary Table S1. All groups were older than the reference category, normal vitamin B₁₂/normal folate, and were more likely to be hypertensive. When compared with the reference category, subjects with normal vitamin B₁₂/high folate were more likely to be female, have above average household wealth, lower mean grip strength and BMI and were less likely to be categorised as obese. This group was also more likely to report taking a folic acid supplement and had better global cognitive scores than the reference group. Those with low vitamin B₁₂/high folate status were more likely to be obese, have above average household wealth and be hypertensive and diabetic relative to the reference category. Subjects with low vitamin B₁₂/normal folate status had lower levels of educational attainment, higher overall BMI and were more likely to smoke and to be obese, hypertensive, diabetic and thus to use metformin (online Supplementary Table S1). These groups were further examined by age group (online Supplementary Table S2), though no additional relevant significant findings were observed.

Associations with combinations of vitamin B₁₂ and folate status

Fig. 2 and online Supplementary Table S3 display the associations between the groups of vitamin B₁₂ and folate status in those aged ≥50 and ≥60 years for MMSE. Relative to normal vitamin B₁₂/normal folate, having low vitamin B₁₂/high folate was not associated with a significant increase in MMSE error rates for models 0, 1 or 2. Further, for all three models, there were statistically significant decreases in MMSE error rates for those in the normal vitamin B₁₂/high folate group in both the ≥50 and ≥60 years groups (model 2: 0.84 (95% CI 0.71, 1.00); *P*=0.047 and 0.82 (95% CI 0.68, 0.99); *P*=0.043, respectively). Fig. 3 and online Supplementary Table S3 show the associations for MoCA. Similarly, low vitamin B₁₂/high folate status was not associated with increases in error rates in the three models tested. However, having normal vitamin B₁₂/high folate was associated with significant decreases in MoCA error rates for models 0, 1 and 2, respectively, in those aged ≥50 years (0.91 (95% CI 0.83, 0.99); *P*=0.031, 0.92 (95% CI 0.85, 1.00); *P*=0.040 and 0.91 (95% CI 0.85, 1.00); *P*=0.042) and ≥60 years (0.87 (95% CI 0.78, 0.97); *P*=0.014, 0.91 (95% CI 0.83, 1.00); *P*=0.044 and 0.89 (95% CI 0.80, 1.00); *P*=0.025). Results of the full multivariate analyses for models 1 and 2 are presented in online Supplementary Tables S4 and S5. Sensitivity analysis indicated that those in the low vitamin B₁₂/high folate groups for each model and in the subgroup aged ≥60 years were not more likely to make errors with the MMSE or MoCA compared with those within the reference category (online Supplementary Table S6).

Associations with quintiles of vitamin B₁₂ and continuous folate concentrations

Additional models, in subjects aged ≥60 years, examined folate concentrations on a continuum, interacted with quintiles (Q1–Q5) of vitamin B₁₂ (Q1 vitamin B₁₂, 45.4–225 pmol/l (*n* 409); Q2 225–289 pmol/l (*n* 427); Q3 289–350 pmol/l (*n* 408); Q4 350–428 pmol/l (*n* 415); Q5 428–719 pmol/l (*n* 412)). These demonstrated there was no evidence of interactions with respect to MMSE and MoCA error rates (Fig. 4). For MoCA, model 2 revealed a small yet statistically significant decrease in the rate of errors for those with the highest vitamin B₁₂ concentrations (online Supplementary Table S7). The reverse instantiation was also tested (i.e. vitamin B₁₂ as the continuous variable, interacted with quintiles of folate). This analysis showed no association of error rates with decreasing concentrations of vitamin B₁₂ (online Supplementary Table S8).

Discussion

The present study estimated the prevalence of groups of vitamin B₁₂ and folate status in a nationally representative sample of older adults in Ireland and investigated the hypothesis that low vitamin B₁₂ status in conjunction with high folate status is associated with increased risk of cognitive decline in older people^(3–5). We demonstrated that the 1.5% of the population with low vitamin B₁₂/high folate status did not have any adverse associations with cognitive performance compared with those with normal status for both B-vitamins. In contrast, the present study

Table 1. Basic sample characteristics, grouped by concentrations of vitamin B₁₂ (B₁₂) and folate† (Numbers and percentages; medians and interquartile ranges (IQR))

	n	Vitamin B ₁₂ ≥ 258 pmol/l				Vitamin B ₁₂ < 258 pmol/l				P for comparison across all groups
		Folate ≤ 45.3 nmol/l		Folate > 45.3 nmol/l		Folate > 45.3 nmol/l		Folate ≤ 45.3 nmol/l		
		Normal B ₁₂ /normal folate		Normal B ₁₂ /high folate		Low B ₁₂ /high folate		Low B ₁₂ /normal folate		
		Median	IQR	Median	IQR	Median	IQR	Median	IQR	
Subjects (n)	3871	2433		306		51		1081		
Age (years)	3871	60	55, 67	62	56, 68	66	57, 73	61	55, 68	0.0001
P		Reference		0.0050*		0.0015*		0.0105*		
Over 80 years	95/3871									0.017
n		52		5		4		34		
%		2.1		1.6		7.8		3.2		
P		Reference		0.5204		0.1347		0.0967		
Females	2045/3871									0.0000
n		1292		198		23		531		
%		53.1		64.7		45.1		49.1		
P		Reference		0.0001*		0.2602		0.0294		
Obese	1274/3866									0.0000
n		777		72		24		401		
%		31.9		23.5		47.1		37.1		
P		Reference		0.0013*		0.0338*		0.0032*		
Vitamin B ₁₂ (pmol/l)	3871	361.3	308.7, 435.2	421.0	344.7, 514.0	214.2	160.1, 241.3	205.9	170.3, 235.4	0.0001
P		Reference		0.0000*		0.0000*		0.0000*		
Folate (nmol/l)	3871	18.8	13.1, 26.9	56.4	50.0, 65.5	55.4	49.1, 61.5	14.7	10.7, 22.0	0.0001
P		Reference		0.0000*		0.0000*		0.0000*		
Folic acid supplement use	123/3871									0.0000
n		68		22		7		26		
%		2.8		7.2		13.7		2.4		
P		Reference		0.0038*		0.0251		0.4969		
MMSE (global cognition)	3871	29	28.0, 30.0	29	29.0, 30.0	29	28.0, 30.0	29	28.0, 30.0	0.0170
P		Reference		0.0003*		0.9448		0.7175		
MoCA (global cognition)	3871	26	24.0, 28.0	26	25.0, 28.0	26	24.0, 27.0	26	24.0, 28.0	0.0054
P		Reference		0.0243*		0.5625		0.0338		

MMSE, Mini Mental State Examination; MoCA, Montreal Cognitive Assessment.

* Statistically significant after adjustment for multiple comparisons ($P < 0.013$).

† Non-normally distributed continuous variables are described as medians and IQR (all such values) and compared using the Kruskal–Wallis test as appropriate, and the Mann–Whitney U test for pairwise comparisons with the reference category. Categorical variables were compared using χ^2 tests of Fisher's exact test, as appropriate.

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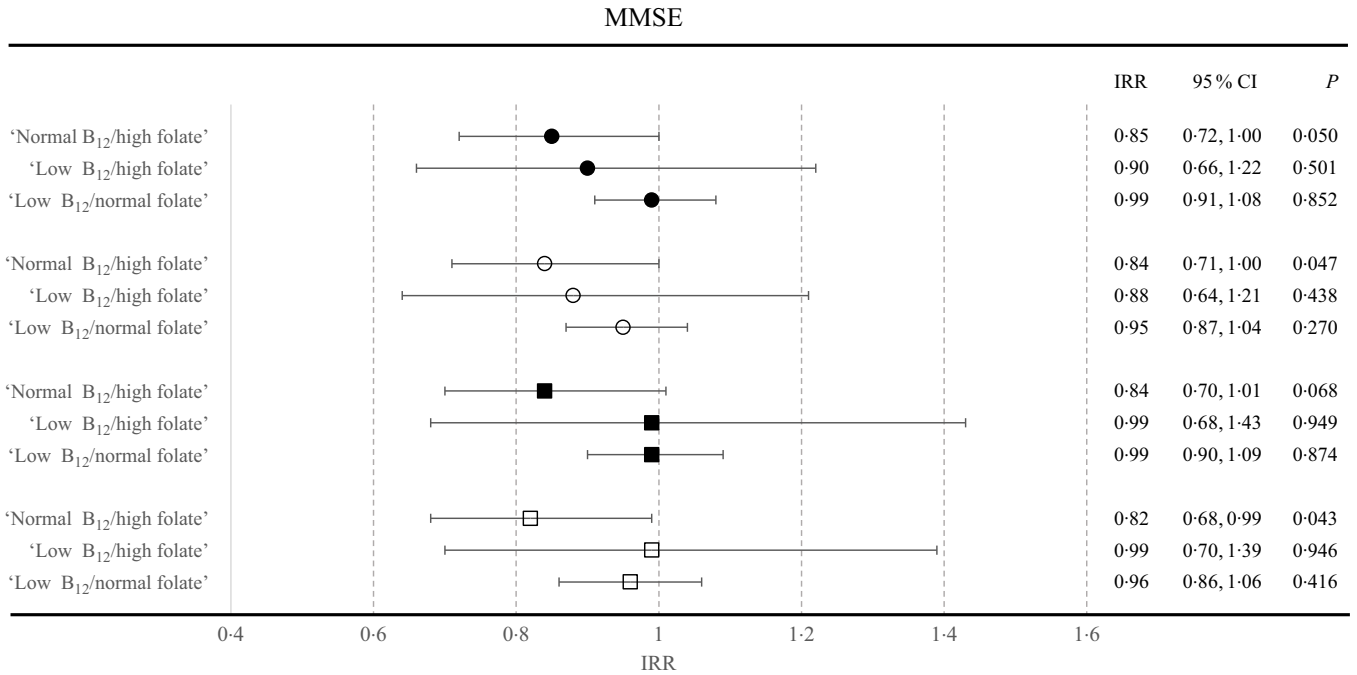


Fig. 2. Associations of vitamin B₁₂ (B₁₂) and folate status and global cognition (Mini Mental State Examination (MMSE)) – aged ≥ 50 and ≥ 60 years (incidence rate ratios (IRR) and 95 % confidence intervals). (●), Aged ≥ 50 years, model 1 (Sociodemographic) (MMSE error rate and vitamin B₁₂ and folate groups with controls for age, sex, educational attainment, mean asset wealth and habitation status). (○), Aged ≥ 50 years, model 2 (Sociodemographic, Lifestyle, Health Indicators and Medications) (model 1 + controls for physical activity, smoking status and alcohol consumption, BMI, grip strength, hypertension status, diabetes status and depressive symptoms, folic acid supplement use, proton pump inhibitor, statin and thyroid medication use and history of stroke). (■), Aged ≥ 60 years, model 1 (Sociodemographic) (MMSE error rate and B₁₂ and folate groups controls for age, sex, educational attainment, mean asset wealth and habitation status). (□), Aged ≥ 60 years, model 2 (Sociodemographic, Lifestyle, Health Indicators and Medications) (model 1 + controls for physical activity, smoking status and alcohol consumption, BMI, grip strength, hypertension status, diabetes status and depressive symptoms, folic acid supplement use, proton pump inhibitor, statin and thyroid medication use and history of stroke).

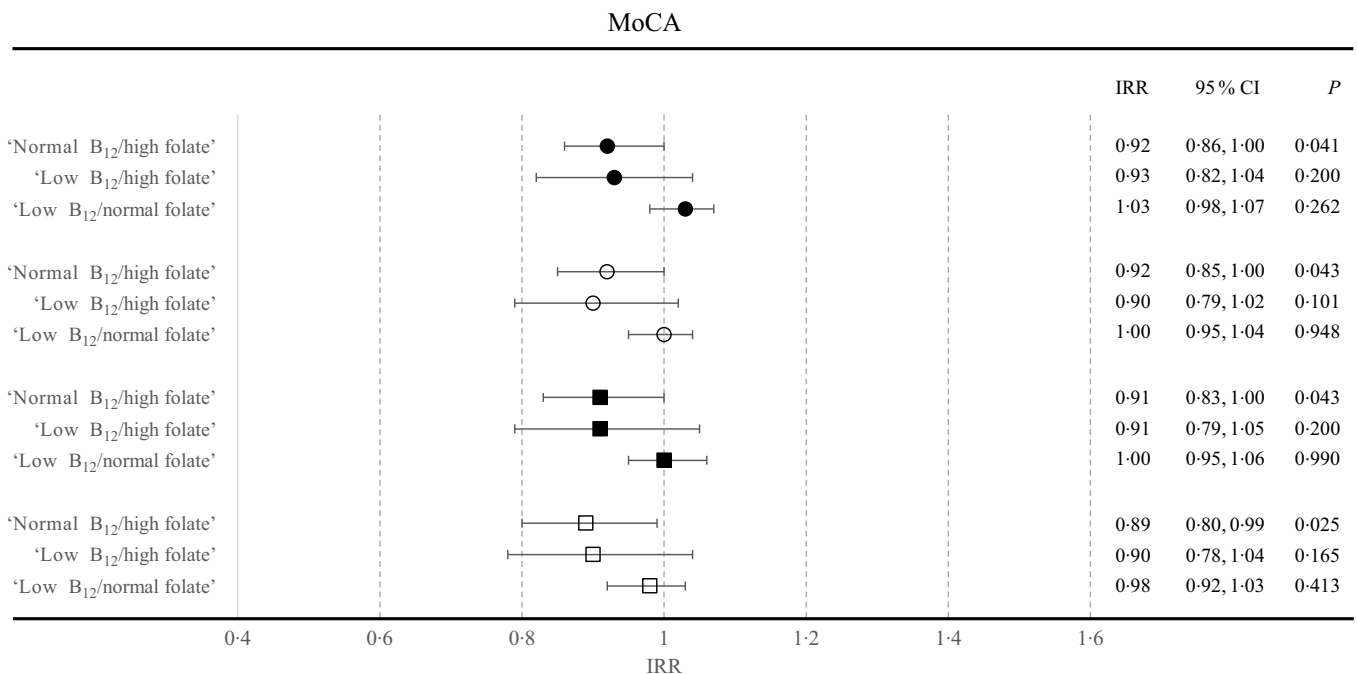


Fig. 3. Associations of vitamin B₁₂ (B₁₂) and folate status and global cognition (Montreal Cognitive Assessment (MoCA)) – aged ≥ 50 and ≥ 60 years (incidence rate ratios (IRR) and 95 % confidence intervals). (●), Aged ≥ 50 years, model 1 (Sociodemographic) (MoCA error rate and vitamin B₁₂ and folate groups with controls for age, sex, educational attainment, mean asset wealth and habitation status). (○), Aged ≥ 50 years, model 2 (Sociodemographic, Lifestyle, Health Indicators and Medications) (model 1 + controls for physical activity, smoking status and alcohol consumption, BMI, grip strength, hypertension status, diabetes status and depressive symptoms, folic acid supplement use, proton pump inhibitor, statin and thyroid medication use and history of stroke). (■), Aged ≥ 60 years, model 1 (Sociodemographic) (MoCA error rate and vitamin B₁₂ and folate groups with controls for age, sex, educational attainment, mean asset wealth and habitation status). (□), Aged ≥ 60 years, model 2 (Sociodemographic, Lifestyle, Health Indicators and Medications) (model 1 + controls for physical activity, smoking status and alcohol consumption, BMI, grip strength, hypertension status, diabetes status and depressive symptoms, folic acid supplement use, proton pump inhibitor, statin and thyroid medication use and history of stroke).

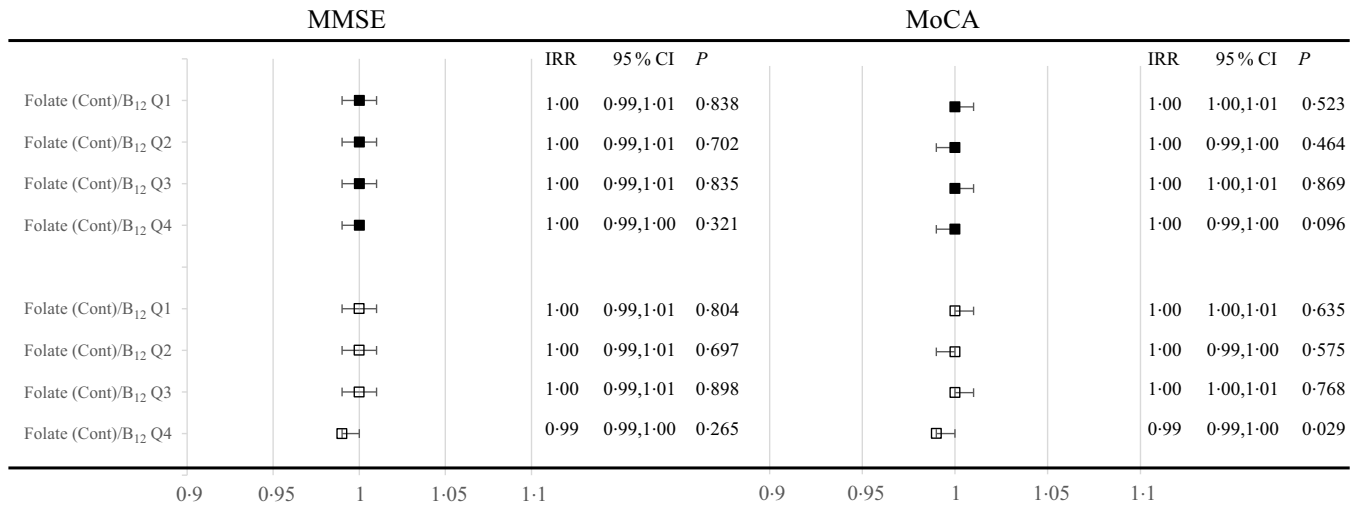


Fig. 4. Associations between concentrations of folate (continuous (Cont)) and quintiles (Q) of vitamin B₁₂ and global cognition – aged ≥60 years (incidence rate ratios (IRR) and 95 % confidence intervals). (■), Aged ≥60 years, model 1 (Sociodemographic) (Global cognition measure error and vitamin B₁₂ and folate concentrations with controls for age, sex, educational attainment, mean asset wealth and habitation status). (□), Aged ≥60 years, model 2 (Sociodemographic, Lifestyle, Health Indicators and Medications) (model 1 + controls for physical activity, smoking status and alcohol consumption, BMI, grip strength, hypertension status, diabetes status and depressive symptoms, folic acid supplement use, proton pump inhibitor, statin and thyroid medication use and history of stroke). MMSE, Mini Mental State Examination; MoCA, Montreal Cognitive Assessment.

demonstrated that higher concentrations of folate were associated with small, but statistically significant higher scores for MMSE and MoCA performance. In contrast to previous research using single measures of global cognition in older cohorts, we employed two clinically validated methods of global cognitive function measurement simultaneously.

There are a number of possible explanations as to why our findings diverge with those of the National Health and Nutrition Examination Survey (NHANES), the Framingham Heart Study and three combined cohorts of Moore and colleagues (2014)⁽³⁻⁵⁾. In NHANES (*n* 1457), concentrations of high plasma folate (>59 nmol/l) with low vitamin B₁₂ (plasma B₁₂ < 148 pmol/l or methylmalonic acid > 0.21 mmol/l) (*n* 42) were associated with poorer cognitive performance cross-sectionally. The latter study was conducted in the USA, a region implementing mandatory folic acid fortification and where the use of supplements containing folic acid was common, leading to extremely high concentrations of plasma folate and the presence of circulating unmetabolised folic acid^(3,37,38). Thus, very high concentrations (plasma folate >59 nmol/l) were observed in 20.7 % of the NHANES cohort, compared with just 3.3 % (plasma folate ≥ 60 nmol/l) in the present study. The use of folic acid supplements was uncommon amongst older adults in Ireland, ranging between 7.2 and 13.7 % amongst the two groups with the highest status. In addition, the Digit Symbol-Substitution subtest of the Wechsler Adult Intelligence Scale III was used in NHANES to examine cognitive performance in attention and processing speed, while the present study assessed global cognitive performance using both MMSE and MoCA. From the Framingham Heart Study⁽⁴⁾, the data of which pertained to pre-mandatory fortification in the USA (1986–1990), Morris *et al.*⁽⁴⁾ reported faster rates of decline in MMSE were associated with vitamin B₁₂ concentrations of <257 pmol/l and plasma folate of >20.2 nmol/l – after 8-year follow-up (*n* 24). While the vitamin B₁₂ cut-off used was similar to the present study, we applied a

higher threshold to define high folate status and used a cognitively intact sample. Furthermore, there were twice as many participants included in the present analysis.

Moore *et al.*⁽⁵⁾ also suggested that high folate or folic acid supplements may be detrimental to cognition in older people with low vitamin B₁₂ concentrations (*n* 1354)⁽⁵⁾. Compared with the present study, a similar vitamin B₁₂ cut-off (<250 pmol/l) was used, and red cell folate of >1594 nmol/l, which approximate to those employed in the Framingham Heart Study investigation and had thirty-nine participants in the low vitamin B₁₂/high folate group. However, the sample was recruited from three separate clinical cohorts in Australia, a region also implementing mandatory folic acid fortification. In addition, 480 subjects had established Alzheimer's disease and 187 had confirmed mild cognitive impairment. This is in contrast with our study, all of whom were free living and cognitively robust (*n* 3871). In addition, Moore *et al.*⁽⁵⁾ used the binary outcome of MMSE of <24 to define cognitive impairment, whereas our study used MMSE and MoCA as a continuous outcome to investigate the range of risk to cognitive performance.

The findings of the present study are consistent with other studies using European population data; those of Clarke *et al.*⁽¹¹⁾ and Doets *et al.*⁽¹³⁾ showing no association of low vitamin B₁₂/high folate status with an increased risk to cognitive function. In addition, the Sacramento Area Latino Study on Aging in America⁽¹²⁾ did not demonstrate a negative association with cognitive function (*n* 1535) using a vitamin B₁₂ deficiency cut-off of <148 pmol/l and high folate of >45.3 nmol/l, although biochemical anomalies were observed.

To our knowledge, this is the largest such study to use a nationally representative sample of community-dwelling older adults that were cognitively unimpaired, employing rigorous exclusion criteria for cognitive performance to ensure accurate investigation of early cognitive changes and to mitigate potential confounding. Another strength of the present study is the use of

two tests of global cognitive performance in parallel. Most other studies in this field have investigated the MMSE or variations of it to determine cognitive performance or impairment. We also measured global cognitive performance using the MoCA, an arguably better screening tool for subtle cognitive decline and mild cognitive deficits^(23,24). In addition, we are the first study to explore interactions of vitamin B₁₂ and folate concentrations with respect to MoCA and we controlled for a large extent of covariates to account for factors that may affect B-vitamin status, particularly medications and food supplement use.

Consistent with previous studies, the present study was limited by the low numbers of participants in the 'at-risk' group. This determined the cut-off for vitamin B₁₂ (<258 pmol/l) utilised in the present study, as there were too few individuals with vitamin B₁₂ concentrations in the deficient range. The present study had only fifty-one participants in the low vitamin B₁₂/high folate group, albeit this number exceeded previous studies addressing this hypothesis. The low number in the group of interest may reflect the large proportion of the older population of Ireland captured by 'sub-optimal' but not deficient status, and the inherent selection bias in analysis of the most robust participants in a study population. However, the vitamin B₁₂ cut-off used in the present study is comparable with those used by the Framingham and Moore *et al.* studies^(4,5). Further, the small number of such individuals included in the present study reflected the application of strict exclusion criteria, and the authors are satisfied that this is representative of the proportion of those aged ≥50 years in this interest group. Similarly, our investigation using continuous concentrations of plasma folate was conducted to compensate for the low proportions of participants with vitamin B₁₂ concentrations below 148 pmol/l.

Another limitation was the cross-sectional nature of the present study, and therefore we cannot predict decline in the at-risk group. Moreover, the present study was limited to the use of plasma vitamin B₁₂ and folate as indicators of status, acknowledging the advantages and disadvantages associated with these biochemical measures (i.e. total vitamin B₁₂ in blood has relatively low sensitivity for estimating vitamin B₁₂ deficiency⁽³⁹⁾ when compared with other biomarkers, whereas plasma folate can be subject to variations from recent dietary intakes). In addition, dietary intake data at Wave 1 were not ascertained, therefore we could not estimate dietary exposure of both vitamin B₁₂ and folate. It is also important to note that because of the recruitment sampling criteria and rigorous exclusion criteria in the present study, these findings may not be generalisable to non-Caucasians, older adults in institutional care or those with cognitive impairment. Finally, we acknowledge the potential reverse causality and bidirectional relationship between global cognitive performance and vitamin B₁₂ and folate status and that the possible association between B-vitamin status and cognitive impairment in older adults might reflect effects of such vitamins on Alzheimer's disease or cerebrovascular disease, which are the primary pathogenic processes underlying cognitive impairment in older adults.

In summary, we undertook the present study to assess the possible hazards for cognitive function in older people associated with voluntary folic acid fortification in Ireland and found no evidence that high blood folate concentrations affected the

associations of low vitamin B₁₂ status with cognitive impairment. Our inability to detect differences in cognitive performance using these instruments implies that elevated folate status should not cause an increased risk to the present study population. In contrast, our data suggest that higher folate status may be protective of global cognitive function irrespective of vitamin B₁₂ status, in a representative sample of community-dwelling population of older people. This is particularly relevant, as our group has previously determined that in addition to 12% of older adults in the Republic of Ireland having low or deficient vitamin B₁₂ status, up to 15% also had deficient or low folate status⁽¹⁸⁾ – demonstrating that the policy of voluntary fortification with folic acid is ineffective for older people in the community.

These findings have significant implications for public policy on folic acid fortification in Ireland and elsewhere in Europe and provide reassurance to both healthcare providers and policy makers that high folate status is not associated with increased risk of cognitive impairment in older people.

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The authors declare that there are no conflicts of interest.

Supplementary material

For supplementary material referred to in this article, please visit <https://doi.org/10.1017/S0007114520001427>

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