Low vitamin B_{12} but not folate is associated with incident depressive symptoms in community-dwelling older adults: a 4 year longitudinal study

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Abstract

The objective was to examine the prospective relationship between folate and vitamin B_{12} (B_{12}) status and incident depressive symptoms in a representative cohort of community-dwelling older people. This was a longitudinal study utilising the Irish Longitudinal Study on Aging (n =3,849 aged ≥50 years) and investigated the relationship between blood plasma folate and B_{12} levels at baseline (wave 1) and incident depressive symptoms at 2 and 4 years (waves 2 and 3). Participants with depression at wave 1 were excluded. A score ≥9 on the Center for Epidemiologic Studies Depression Scale-8 at wave 2 or 3 was indicative of incident depressive symptoms. Plasma B_{12} and folate concentrations were determined by microbiological assay. B12 status profiles (pmol/l) were defined as: <185, deficient-low; 185 - <258, low normal; >258 - 601, normal and >601 high. Folate status profiles (nmol/l) were defined as: ≤10.0, deficient-low; >10 - 23.0, low normal; >23.0 - 45.0, normal; >45.0, high. Logistic regression models reporting odds ratios were used to analyse the longitudinal association of B-vitamin categories with incident depression. Both B_{12} and folate plasma concentrations were lower in the group with incident depressive symptoms vs. non depressed (folate: 21.4 vs. 25.1 nmol/L; P=0.0003); (B_{12}: 315.7 vs. 335.9 pmol/L; P=0.0148). Regression models demonstrated that participants with deficient-low B_{12} status at baseline had a significantly higher likelihood of incident depression four years later (odds ratio 1.51, 95% CI 1.01-2.27, P=0.043). This finding remained robust after controlling for relevant covariates including physical activity, chronic disease burden, vitamin D status. cardiovascular disease and antidepressant use. No associations of folate status with incident depression were observed. Older adults with deficient-low B_{12} status had a 51% increased likelihood of developing depressive symptoms over 4 years. Given the high rates of B_{12} deficiency, these findings are important and highlight the need to further explore the low cost benefits of optimising vitamin B_{12} status for depression in older adults.

Introduction

Deficiency and low status of the B-vitamins such as folate and vitamin B_{12} are highly prevalent in older populations. Estimates of vitamin B_{12} (B_{12}) deficiency in those aged ≥50 yrs range from 5 to 40 % depending on the marker of measurement and the deficiency cut-off selected.[1] The consequences of low B_{12} status can include megaloblastic anaemia, irreversible demyelinating neurological disease/paresthesia, and the potential for impaired cognitive function.[2] Folate status is highly dependent on whether the country of residence has a mandatory folic acid (FA) food fortification policy. For instance, in the US, mandatory food fortification with FA has resulted in folate deficiency/low status rates of just 1.2%[3] in those aged ≥60 yrs. This is in direct contrast to countries such as the UK which have no such policy (deficiency rates range from 5 to 31 %).[4] Recent data from the older Irish population has shown that one in seven people aged >50 years has low folate status.[5] Similarly, one in eight older adults are reported to have low B_{12} status[5] whilst low dietary intakes and low blood status have been reported throughout all age groups in the Irish population.[6] This is not surprising given that Ireland, like the UK, has no policy of fortification with FA but allows voluntary food fortification. The foods that are most commonly fortified with FA are breakfast cereals[7] while there is varying but inconsistent FA enrichment of other food items.[8] In terms of B_{12}, foods that are fortified or rich in this micronutrient are not always regularly consumed by the older population and recent data has shown that dairy foods (which are rich in B-vitamins and B_{12}), are only consumed in the recommended amounts by 4 % of older Irish adults.[9]

These high deficiency rates and poor access to micronutrient rich foods are of concern given the reported linkage of folate and B_{12} with depression. Low folate and B_{12} concentrations have been correlated with depressive disorders[10-15] whilst evidence has suggested that both of these vitamins may enhance the effectiveness of anti-depressants.[16] More recently, a large Irish cohort study of older Irish adults (n 5,186) reported that the lowest quintile of red blood cell folate compared with the highest was associated with an increased risk of depression.[17] These associations are plausible given these B-vitamins are required for the synthesis of methionine which subsequently forms S-adenosylmethionine (SAM)[18] which is the main methyl donor for the formation of monoamine neurotransmitters, phospholipids, and nucleotides[19]. However the majority of studies examining the associations of these B-vitamins with depression are cross-sectional and have not controlled for important covariates, such as disease status, medication use or vitamin D status, which has been shown to an

important predictor of depression.[20] Understanding the link between folate/B_{12} status and depression in later life is important as depression is a risk factor for functional decline[21], admission to residential care[22] and early mortality.[23] Thus the identification of risk or protective factors for this condition is of the upmost importance. Moreover there is a growing momentum for the introduction of mandatory food fortification of these B-vitamins in Europe and the UK and there is a need to understand links between these micronutrients with chronic conditions and health before fortification can be implemented.

Thus, the aim of this study is to examine the longitudinal relationship between baseline folate and B_{12} status and incident depressive symptoms up to four years later in a representative sample of the community-living population of Ireland aged 50 years and over.

Methods

Study Design and participants

This study utilizes data from the Irish Longitudinal Study on Ageing (TILDA), a nationally population-based representative sample of community dwelling older Irish adults aged ≥ 50 years. As described in detail elsewhere[24] the first wave of data collection (Wave 1, 2009-2011) was conducted using a stratified clustered procedure to randomly sample postal addresses from the Irish Geo-Directory (a listing of all residential addresses in the Republic of Ireland). Wave 2 was conducted between 2011-2012 and Wave 3 between 2014-2015. This sub-study within TILDA investigates the association of folate and vitamin B_{12} (Wave 1) with incident depression at later recruitment Waves. Therefore, participants were included in this study if they were aged ≥50 years and underwent assessment at Wave 1 including measurement of plasma folate and plasma total B_{12} and screening for depression. Participants were excluded if they were missing blood data or had depression at Wave 1 or did not complete 4-year follow-up, including assessment of incident depression at both Wave 2 and Wave 3

Ethics

The study was approved by the Faculty of Health Sciences Research Ethics Committee at Trinity College Dublin, and all participants gave informed written consent. All experimental procedures adhered to the Declaration of Helsinki, and all assessments were performed by trained research nurses. Anonymized data and materials have been made publicly available at the Irish Social Science Data Archive based in University College Dublin and the

Interuniversity Consortium for Political and Social Research based in the University of Michigan and can be accessed at http://www.tilda.ie.

Folate and vitamin B12 analysis

A non-fasting blood sample was collected by venepuncture into one 10 ml EDTA tube (BD, Becton, Dickinson Limited) by a trained phlebotomist. Samples were kept chilled during transport, were centrifuged (3000 rpm for 15 minutes) and plasma aliquots were labelled and stored at -80°C until required for analysis. As detailed previously[5] plasma total B_{12} and folate concentrations were determined by microbiological assays.[25,26] The inter-assay CV for plasma B_{12} and folate were <10.9 %. B_{12} status profiles (pmol/l) were defined as follows: <185, deficient-low; 185 - <258, low-normal; >258 - 601, normal and >601 high.[27-29] Folate status profiles (nmol/l) were defined as follows: ≤10.0, deficient-low; >10 - 23.0, low-normal; >23.0 - 45.0, normal; >45.0, high.[30] Normal folate (>23.0-45.0 nmol/l) and normal B_{12} (>258-601) concentrations were used as the reference categories for comparison purposes.

Depressive Symptoms

Depressive symptoms were assessed at Wave 1 using the 20-item Centre for Epidemiological Studies Depression Scale (CES-D-20). A score ≥ 16 was used to define clinically significant depressive symptoms.[31] Participants with depressive symptoms at Wave 1 were excluded from the study. At Waves 2 and 3 the 8-item Centre for Epidemiological Studies Depression Scale (CES-D-8) was used to screen for depressive symptoms. The CES-D-8 was introduced in the TILDA study at Waves 2 and 3 and a score of ≥ 9 on this scale was used to define clinically significant depressive symptoms at these Waves.[32,33] The 8-item CES-D has been validated against the 20-item scale within the TILDA cohort and has been shown to be consistent, reliable and valid.[32] In terms of duration of follow-up, we only included participants who completed 4-year follow-up and excluded those who were lost to follow-up/attrition before this. We excluded participants at wave 1 with significant depressive symptoms. We did not exclude participants who did not meet criteria for depressive symptoms but were taking antidepressants as antidepressant medication is used for a range of problems beyond depression including chronic pain and anxiety and is therefore not necessarily suggestive of a diagnosis of depression.

Other co-variates

Cardiovascular disease was defined as self-report of prior myocardial infarct, cardiac failure, angina, hypertension or cardiac arrhythmia. Self-report was also elicited for chronic disease burden, with respondents asked specifically about a history of lung disease, osteoporosis, cancer, liver disease, age-related macular degeneration, cataracts, glaucoma, arthritis, urinary incontinence, Parkinson's disease and diabetes. Medication records were examined for antihypertensive use and antidepressant use. Medication lists were also examined directly for FA supplement use (single tablet or multi-vitamin) and for use of B_{12} (injection or single tablet or B_{12} in multi-vitamin) and coded as yes/no. Participants were a asked about physical activity within the last week and those who were inactive for the full 7 days were defined as having low physical activity, compared to moderate (active 1-3 days) and high (active 4 or more days) physical activity. Alcohol excess was assessed through the "Cut down, Annoyed, Guilty, Eye-opener" (CAGE) questionnaire, a screening tool for problematic drinking with a score equal or greater than 3 indicating an issue. This was in addition to the coding of the current smoking status of the subjects. Functional impairment was defined as impairment in one or more instrumental activities of daily living (IADL) while cognitive impairment was defined as a Mini Mental Sate Examination (MMSE) score $\leq$24. As described previously[20], vitamin D analysis included total plasma 25-hydroxyvitamin D (25(OH)D (D2 and D3)) concentrations which were quantified by using liquid chromatography-tandem mass spectrometry (LC-MS/MS) (API 4000; AB SCIEX) and batch analysed in the Biochemistry Department of St James's Hospital (which is accredited (ISO 15189)). As per Health and Medicine Division (formerly Institute of Medicine) guidelines, risk of vitamin D deficiency, insufficiency, and sufficiency were defined as <30, 30-50, and >50 nmol/L, respectively.[34]

Statistical Analysis

Normally distributed continuous variables were described as means and standard deviations and compared using student's t-test. Categorical variables were compared using the chi-square test. Proportional estimates were used to compare incidence of depression by folate and B_{12} categories. Logistic regression models reporting odds ratios (OR) with 95% confidence intervals (CI) were used to analyse the longitudinal association of folate and B_{12} categories with depression. Two models were tested: the first model was unadjusted; the second model adjusted for age, sex, body mass index, alcohol, smoking status, cardiac disease, cognitive impairment, chronic disease burden, vitamin D status and antidepressant

use. In order to confirm that results were not related to antidepressant use or folate or vitamin B_{12} supplementation, analyses were re-run excluding participants prescribed either of these medications/supplements. A P value ≤ 0.05 was considered statistically significant. Data were analysed using Stata 15 (Satatcorp).

Results

Baseline characteristics of the study population are presented in Table 1. At Wave 1, a higher proportion of those classified with incident depressive symptoms were female, obese, had a higher burden of chronic and cardiovascular disease and reported a higher use of anti-depressant medications. Those with incident depressive symptoms had a significantly lower mean concentration of plasma folate (21.4 vs. 25.1 nmol/L; P=<0.001) and a lower mean concentration of B_{12} (315.7 vs. 335.9 pmol/L; P=0.014) (unadjusted) (Table 1). The mean concentrations of the proportions and status of plasma folate and B_{12} are displayed in Table 2. There were no significant differences in the proportions of either the folate or B_{12} status category by depression status (Table 2).

In an unadjusted regression model with normal vitamin B_{12} concentration (>258-601 pmol/L) as the reference, those with deficient-low B12 status had an increased likelihood of incident depression [OR 1.55 (95% CI 1.07-2.25); t= 2.32; P=0.021] (Table 3). This finding then persisted in model 2 [OR 1.52 (95% CI 1.05-2.21); t= 2.25; P=0.025] with age, sex and education and also in the fully adjusted model [OR 1.51 (95% CI 1.01-2.27); t= 2.03; P=0.043] (Table 3). When vitamin B_{12} concentration was examined as a continuous variable (data not shown), higher concentrations (per unit increased in blood B_{12} concentrations) were associated with a decreased likelihood of incident depression symptoms [OR 0.99 (95% CI 0.97-0.99); t= -2.01; P=0.045]. In a sensitivity analysis which evaluated depressive symptoms as a continuous variable, deficient-low B12 status predicted a higher CESD score (regression coefficient (B): 0.50; P=0.037].

In terms of folate, there was no statistically significant difference in the likelihood of incident depressive symptoms by folate categories using normal folate status (>23.0-45.0 nmol/L) as the reference (Supplemental Table 1). In all models, other determinants of depressive symptoms included gender, education, chronic disease, BMI, antidepressant use at baseline, subthreshold depressive symptoms and vitamin D status as reported previously.[20] When examined by the exclusion of B12-supplement/injection users and/or anti-depressant medication users, the results remained consistent in both the unadjusted and fully adjusted

models (Table 4). For instance, when excluding both supplements and medications, those with a deficient-low B12 status had a significantly increased risk of incident depressive symptoms [OR 1.65 (95% CI 1.07-2.54); P=0.024]. The results for folate did not change when those reporting antidepressant use or FA supplement use were removed from the analysis (Supplemental Table 2).

Discussion

In this population representative study, we observed that those with deficient-low B12 status had a 51% increased likelihood of developing depressive symptoms over 4 years. These findings remained consistent even after adjustment for confounders and after removing those reporting B_{12} supplements/injections and anti-depressant users. Results from previous studies have been inconsistent regarding the association of B_{12} in relation with depression. In a study of 700 older community dwelling adults (>65 years), those with B_{12} deficiency (<148 pmol/L) had a doubled risk of depression compared to subjects with normal concentrations.[35] Another study in older adults observed a similar increased risk of depression with low B12 status[36] while a further four studies in similar cohorts observed no such association.[17,37-39] Two studies have also shown a lower risk of depression with higher dietary intakes of B_{12}.[40,41]

Similar inconsistency has been reported in studies using B_{12} injections to treat depression[42,43] while two meta-analyses investigating B_{12} in relation to depression in older adults reported contradictory results.[44,45] Prospective studies have also followed a similar pattern of inconsistency. In a two-year follow-up of 732 older Korean adults (>65 years), low B_{12} concentrations were predictive for depression.[46] However, in a 15 year follow-up of 1,012 older adults (>65 years) from the Longitudinal Aging Study Amsterdam, serum B_{12} was not associated with depression.[47] It is difficult to reconcile the conflicting results of studies to-date. Substantial differences across all studies include the use of different biomarkers to characterise B_{12} status, different tests/cut-offs to assess depression, different follow-up time periods, different confounders in statistical modelling and different food fortification or B_{12} supplement use/guidelines in the various international populations. Differences may have also occurred in the cut-points for B_{12} deficiency across studies (which adds to the comparability difficulty) and there is much debate in the literature on what cut-off to use. The current study utilised 185 pmol/L as a cut-point as it would include both frank deficiency and borderline deficiency.[27-29] Furthermore, although 148 pmol/L is commonly used clinically for

low vitamin B-12 status, there is evidence that the prevalence of vitamin B_{12} deficiency is underestimated when using <148 pmol/L as many individuals above that level can still exhibit clinical symptoms of deficiency.[27-29] However, despite all these differences there is still a biological plausibility of a link between B_{12} and depression given that B_{12} is a necessary co-factor for methionine synthesis which provides methionine, the precursor of SAM that is then needed for the formation of important brain neurotransmitters such as dopamine, norepinephrine etc. [18,19]

We observed no association of folate status with depression risk in the current study. Although mean blood folate concentrations were lower in those with incident depression, after adjusting for important co-variates, folate status had no association with depression. Other factors that influenced micronutrient status in this population included obesity, medication use, smoking, wealth, gender and geographic location.[5] Our findings are consistent with other international longitudinal studies observing no such association of folate with depression risk.[40,41] Both the Quebec Longitudinal Study on Nutrition and Aging (NuAge)[40] and the Chicago Health and Aging Study (CHAP)[41] did not observe any association of folate with depression although dietary folate intakes and not blood concentrations were assessed. Additionally, the countries where these studies are located implement mandatory FA food fortification, and it is possible that folate could be associated with depression but only at insufficient concentrations below folate intake ranges in these populations.[41] In contrast, the TUDA study did observe a positive association of folate with depression risk using red blood cell folate (instead of plasma folate) as a marker of folate status, though the study did not control for important co-variates such as vitamin D status.[17] The TUDA study also observed no such association of vitamin B_{12} with depression after adjustment for other factors. The findings between TILDA and TUDA appear inconsistent as the two study populations were exposed to a similar background of B-vitamin intake. However, there are some key differences in the cohorts. TILDA was a nationally representative recruitment of healthy free-living persons versus specific recruitment of persons with mild to moderate age-related diseases (TUDA) in hospital or GP settings.[17] Additionally, the mean age of the participants of TUDA was significantly older than TILDA and TUDA was a cross-sectional analysis vs. longitudinal analysis in TILDA. Inconsistences also exist for folate in terms of international studies in relation to the different types of blood biomarker measured, different depression assessments and different policies of FA fortification across countries, again making comparative interpretations of the data difficult.

Interestingly we observed that as age increased, the risk of incident depression decreased which has been reported previously[20]. This was unrelated to vitamin B12 and folate blood concentrations as the older participants had the lowest concentrations and the highest levels of deficiency and low status.[5]

A major strength of the current research is that it is based on a large, nationally representative population sample. Additional strengths include the longitudinal design of over four years in a well characterized cohort adjusting for a wide range of confounders including chronic disease, medications, lifestyle factors and other nutrient blood biomarkers which has not been attempted previously. Limitations include the fact that both folate and B_{12} were only measured at baseline, we did not have other biochemical measures of these micronutrients (which limits accuracy in determining blood status) and we lacked data pertaining to dietary intake of these vitamins. Depression was measured using CES-D, which is not the gold standard clinical interview while both chronic disease and CVD conditions were self-reported which could increase the risk of response bias.

Conclusion

In conclusion, we observed that low B_{12} status was associated with a significantly increased risk of depressive symptoms over four-year period in a large population representative study of older adults. No associations were observed for folate. These findings are relevant given the high occurrence of incident depression and the high levels of low-deficient status of B_{12} in older adults. These observations also provide reassurance for food policy makers that fortification of foods to increase levels of these vitamins could have the potential for benefits in prevention of this condition. However, future prospective studies and randomized trials using an agreed set of harmonized measures and blood biomarkers are needed to fully ascertain the utility of vitamin B_{12} in relation to the prevention or delay in the onset of depression in older adults.

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Table 1 Baseline Characteristics by Incident Depression status

	Incident Depression n = 384	Not Depressed N = 3465	P value
Age: mean (95% CI), y	62.4 (612-63.6)	63.0 (62.6-63.5)	0.282
Female: prop. (95% CI)	57.6 (51.9-63.2)	49.1 (47.5-50.7)	<0.001
Educational Attainment, prop. (95 % CI)			
Primary	38.6 (33.2-44.2)	27.2 (25.3-29.2)	
Secondary	41.7 (36.4-47.1)	48.0 (46.0-49.9)	
Tertiary	19.7 (16.1-23.8)	24.7 (23.0-26.5)	<0.001
Body Mass Index, prop. (95% CI)			
- BMI <24.9 kg/m^2	21.3 (17.2-25.9)	22.2 (20.6-23.8)	
- BMI 25.0 – 29.9 kg/m^2	39.8 (34.4-45.3)	45.8 (44.0-47.6)	
- BMI ≥ 30.0 kg/m^2	38.9 (33.5-44.5)	32.0 (30.2-33.7)	0.014
Current Smoker, prop. (95% CI)	22.1 (17.5-27.5)	15.8 (14.3-17.5)	0.001
CAGE Score, prop. (95 % CI)			
CAGE =1	74.0 (68.7-78.7)	80.5 (78.9-81.9)	
CAGE =2	13.9 (10.5-18.1)	11.0 (9.8-12.2)	
CAGE =/>3	12.1 (8.7-16.4)	8.5 (7.4-9.6)	0.010
I-ADL Impairment, prop. (95% CI)*	6.5 (4.1-10.2)	4.3 (3.4-5.3)	0.044
Number of days active in last week, prop. (95 % CI)			
- 0 days active in last week	76.0 (70.8-80.5)	70.5 (68.6-72.4)	
- 1-3 days	12.8 (9.6-16.7)	16.1 (14.7-17.5)	
- ≥4 days	11.2 (7.9-15.3)	13.4 (12.0-14.7)	0.083
No. of Chronic Diseases, prop. (95 % CI)†			
- 0 chronic diseases	40.6 (35.1-46.5)	50.5 (48.5-52.4)	
- 1 chronic disease	26.0 (21.0-31.1)	27.9 (26.2-29.6)	
- 2-3 chronic diseases	26.8 (21.9-32.3)	19.3 (17.7-20.8)	
- ≥ 4 chronic diseases	6.6 (4.2-10.4)	2.3 (1.7-2.9)	<0.001
Cardiovascular Disease, prop. (95 % CI) ‡	45.2 (39.9-50.6)	39.8 (38.0-41.7)	0.050
Cognitive Impairment, prop. (95 % CI)****	7.6 (4.9-11.6)	5.3 (4.4-6.5)	0.036
Antidepressant Use, prop. (95 % CI)	11.7 (8.5-15.9)	4.3 (3.6-5.2)	<0.001
Plasma folate: mean (95% CI), nmol/L	21.4 (19.8-23.0)	25.1 (23.8-26.5)	<0.001

Plasma vitamin B_{12} : mean (95% CI), pmol/L	315.8 (300.5-331.1)	335.9 (330.6-341.1)	0.014

BMI, body mass index; CAGE, Cut Down, Annoyed, Guilty, Eye Opener Alcohol Scale; I-ADL, instrumental activities of daily living; prop., proportional estimation; SD, standard deviation.

Baseline characteristics of study sample by depression diagnosis. Incident depression is 8 item CES-D score 9 at either wave 2 or wave 3 (ie, 2- or 4-year follow-up). Student t-test used for continuous variables with adjusted Wald test postestimation. Chi square analysis was used for categorical variables. *Self-reported difficulty in at least 1 instrumental ADL (ie, shopping, housekeeping, accounting, food preparation, and telephone/transportation). †Self-report of lung disease, osteoporosis, cancer, liver disease, eye disease (age-related macular degeneration, glaucoma or cataracts), arthritis, urinary incontinence, Parkinson disease, and diabetes. ‡Self-report of myocardial infarction, arrhythmia, hypertension, angina, or cardiac failure. **Mini-Mental State Examination score of ≤2

Table 2. Folate and vitamin B_{12} concentration and status by incident depression

	Incident Depression (n 384)	Not Depressed (n 3465)	P-value
	Mean concentration (95% CI)	Mean concentration (95% CI)	
Plasma folate categories (nmol/L)			
Deficient-low (<10.0)	7.7 (7.0-8.4)	7.9 (7.7-8.1)	0.535
Low normal (>10-23.0)	15.6 (15.0-16.1)	15.8 (15.6-16.0)	0.489
Normal (>23.0-45.0)	31.8 (30.5-33.1)	31.5 (31.0-31.9)	0.651
High (>45.0)	56.0 (53.0-59.1)	78.0 (67.4-88.7)	0.001
Vitamin B_{12} categories (pmol/L)			
Deficient-low (<185)	146.6 (136.8-156.4)	145.6 (141.6-149.6)	0.865
Low normal (185-<258)	225.0 (219.7-230.3)	223.3 (221.5-225.1)	0.543
Normal (>258-601)	372.7 (359.8-385.5)	376.4 (372.6-380.1)	0.587
High (>601)	686.8 (659.6-714.1)	707.5 (681.6-733.3)	0.281
Percentage prevalence*	% (95% CI)*	%, (95% CI) *	
Plasma folate categories (nmol/L)			
Deficient-low (<10.0)	18.3 (13.9-23.0)	13.9 (12.5-15.4)	
Low normal (>10-23.0)	47.5 (41.9-53.2)	49.1 (47.2-51.0)	
Normal (>23.0-45.0)	27.0 (22.4-32.1)	26.9 (25.3-28.5)	
High (>45.0)	7.2 (4.6-10.7)	10.1 (8.7-11.3)	0.810
Vitamin B_{12} categories (pmol/L)			
Deficient-low (<185)	14.9 (11.0-19.8)	10.7 (9.5-11.9)	
Low normal (185-<258)	22.5 (18.0-27.6)	18.9 (17.4-20.4)	
Normal (>258-601)	59.4 (53.8-64.7)	66.4 (64.5-68.1)	
High (>601)	3.2 (1.6-5.9)	4.0 (3.2-4.8)	0.440

*Weighted population proportion.

Incident depression is 8-item CES-D score 9 at either wave 2 or wave 3 (ie, 2- or 4-year follow-up).

Student t-test used for continuous variables with adjusted Wald test postestimation. Chi Square analysis was used for categorical variables

Table 3. Vitamin B_{12} status and risk of incident depression

	OR (95% CI) (1.0 ref)	P
Model 1 (Unadjusted)		
Total B_{12} category (pmol/L) (ref: (normal: >258-601)) (n = 2584)		
Deficient-low (<185) (n=389)	1.55 (1.07-2.25)	0.021
Low normal (185-<258) (n = 723)	1.32 (0.97-1.80)	0.070
High (>601) (n =153)	0.89 (0.44-1.80)	0.757
Model 2 (Partially adjusted)		
Total B_{12} category (pmol/L) (ref: (normal: >258-601))		
Deficient-low (<185)	1.52 (1.05-2.21)	0.025
Low normal (185-<258)	1.31 (0.96-1.78)	0.085
High (>601)	0.87 (0.43-1.74)	0.696
Age (Ref: 50-64 y)		
Age 65-74 y	0.70 (0.52-0.94)	0.022
Age ≥75 y	0.70 (0.47-1.03)	0.074
Female sex	1.49 (1.17-1.89)	0.001
Educational attainment (ref: primary)		
Secondary	0.55 (0.42-0.73)	<0.001
Tertiary	0.52 (0.38-0.72)	<0.001
Model 3 (Fully adjusted)		
Total B_{12} category (pmol/L) (ref: (normal: >258-601))		
Deficient-low (<185)	1.51 (1.01-2.27)	0.043
Low normal (185-<258)	1.20 (0.87-1.65)	0.252
High (>601)	0.86 (0.44-1.68)	0.675
Age (Ref: 50-64 y)		
Age 65-74 y	0.71 (0.51-0.97)	0.034
Age ≥75 y	0.61 (0.37-0.98)	0.042
Female sex	1.25 (0.95-1.63)	0.098
Educational attainment (ref: primary)		
Secondary	0.66 (0.49-0.89)	0.007
Tertiary	0.65 (0.46-0.91)	0.014

BMI (ref: BMI <24.9 kg/m)		
BMI 25.0-29.9 kg/m	0.99 (0.71-1.37)	0.965
BMI ≥ 30.0 kg/m	1.40 (0.98-2.00)	0.061
Current smoker	1.04 (0.86-1.24)	0.657
CAGE alcohol status (ref: CAGE 0-1)		
CAGE 2-4	1.27 (0.87-1.84)	0.206
Did not complete	1.40 (0.92-2.14)	0.109
Vitamin D status (ref: >50 nmol/L)		
<30.0 (nmol/L)	1.50 (1.03-2.17)	0.032
30-50 (nmol/L)	1.04 (0.79-1.36)	0.757
Number of days active in last wk (ref: 0 days)		
1-3 days	0.91 (0.66-1.27)	0.608
≥4 days	0.99 (0.66-1.49)	0.994
I-ADL impairment	0.86 (0.45-1.63)	0.649
CES-D wave 1 (ref: CES-D = 0-5)		
CES-D 6-10	2.43 (1.81-3.27)	<0.001
CES-D 11-15	5.56 (3.90-7.93)	<0.001
No of chronic disease (ref: 0 chronic disease)†		
1 chronic disease	1.05 (0.77-1.43)	0.743
2-3 chronic diseases	1.41 (1.01-1.98)	0.043
≥4 chronic diseases	2.66 (1.38-5.11)	0.003
Cardiovascular disease‡	1.03 (0.79-1.35)	0.822
Cognitive impairment**	1.03 (0.57-1.86)	0.903
Antidepressant use	2.09 (1.32-3.31)	0.002

BMI, body mass index; I-ADL, instrumental activities of daily living; ref, reference value.
Logistic regression models with incident depression as dependent variable.
*Self-reported difficulty in at least 1 I-ADL (ie, shopping, housekeeping, accounting, food preparation, and telephone/transportation).
†Self-report of lung disease, osteoporosis, cancer, liver disease, eye disease (age-related macular degeneration, glaucoma or cataracts), arthritis, urinary incontinence, Parkinson disease, and diabetes. ‡Self-report of myocardial infarction, arrhythmia, hypertension, angina, or cardiac failure.
**Mini-Mental State Examination score of ≤24

Table 4. Exclusion of anti-depressants and vitamin B_{12} injection/supplement use and the association of vitamin B12 status with incident depression

	Model 1	P	Model 2	P
Excluding antidepressant users (n =3,667)				
Total B_{12} category (pmol/L) (ref: (normal: >258-601))	Reference		Reference	
Deficient-low (<185)	1.63 (1.10-2.42)	0.015	1.65 (1.08-2.51)	0.019
Low normal (185-<258)	1.36 (0.99-1.88)	0.057	1.23 (0.88-1.71)	0.218
High (>601)	0.60 (0.29-1.24)	0.175	0.61 (0.28-1.35)	0.229
Excluding supplement users (n =3,735)				
Total B_{12} category (pmol/L) (ref: (normal: >258-601))	Reference		Reference	
Deficient-low (<185)	1.53 (1.05-2.25)	0.026	1.49 (0.98-2.260	0.057
Low normal (185-<258)	1.34 (0.99-1.83)	0.057	1.23 (0.89-1.70)	0.198
High (>601)	0.94 (0.46-1.92)	0.868	0.91 (0.46-1.78)	0.788
Excluding antidepressant and supplement users (n =3,559)				
Total B_{12} category (pmol/L) (ref: (normal: >258-601))	Reference		Reference	
Deficient-low (<185)	1.63 (1.09-2.44)	0.015	1.65 (1.07-2.54)	0.021
Low normal (185-<258)	1.42 (1.03-1.96)	0.032	1.28 (0.92-1.79)	0.134
High (>601)	0.63 (0.30-1.32)	0.225	0.65 (0.29-1.44)	0.291

Logistic regression models, reporting ORs with 95% CIs for vitamin B12 status regressed on incident depression. Model 1 is unadjusted; model 2 controls for age, sex, educational attainment, body mass index, smoking status, and alcohol excess, subthreshold depressive symptoms, vitamin D, functional impairment, physical activity, chronic disease burden, cardiovascular disease, cognitive impairment, and antidepressant use.