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Association between folate, vitamin B_6 and vitamin B_{12} intake and depression in the SUN cohort study

A. Sánchez-Villegas,*,† J. Doreste,* J. Schlatter,‡ J. Pla,‡ M. Bes-Rastrollo† & M. A. Martínez-González†

*Department of Clinical Sciences, University of Las Palmas de Gran Canaria, Las Palmas de Gran Canaria, Las Palmas, Spain
†Department of Preventive Medicine and Public Health, University of Navarra, Pamplona, Spain
‡Department of Psychiatry, University Hospital, University of Navarra, Pamplona, Spain

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Correspondence
Dr Almudena Sánchez-Villegas, School of Health Sciences, Department of Clinical Sciences, University of Las Palmas de Gran Canaria, PO Box 550, Las Palmas de Gran Canaria, Las Palmas, Spain.
Tel.: +34 928 453454
Fax: +34 928 453475
E-mail: asanchez@dcc.ulpgc.es

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Abstract

Background: An association between low blood levels of folate, vitamins B_6 and B_{12} and a higher prevalence of depressive symptoms has been reported in several epidemiological studies. The present study aimed to assess the association between folate, vitamins B_6 and B_{12} intake and depresion prevalence in the SUN cohort study.
Methods: The study comprised a cross-sectional analysis of 9670 participants. A validated semi-quantitative food frequency questionnaire was used to ascertain vitamin intake. The association between the baseline intake of folate, vitamins B_6 and B_{12} categorized in quintiles and the prevalence of depression was assessed. The analyses were repeated after stratifying by smoking habits, alcohol intake, physical activity and personality traits.
Results: Among women, odds ratios (OR) [95% confidence interval (CI)] for the third to fifth quintile for vitamin B_{12} intake were 0.58 (0.41–0.84), 0.56 (0.38–0.82) and 0.68 (0.45–1.04), respectively. Among those men with a low level of anxiety and current smokers, a significant positive association between low folate intake and the prevalence of depression was found. The OR (95% CI) for the first quintile of intake was 2.85 (1.49–5.45) and 2.18 (1.08–4.38), respectively, compared to the upper quintiles of intake (Q2–Q5) considered as a group.
Conclusion: Low folate intake was associated with depression among currently smoking men and men with low anxiety levels. Low intake of vitamin B_{12} was associated with depression among women. No significant associations were found for vitamin B_6 intake.

Introduction

Depression is a major public health problem in developed countries. Recently, the World Health Organization has estimated that unipolar depressive disorder remains one of the leading causes of total disability adjusted life years (DALYs) worldwide (WHO, 2003). It accounts for 8% of total DALYs in the Americas and for 6.1% in Europe (Üstün *et al.*, 2004). Given the global burden of depression, awareness of how populations can possibly prevent the development of depression via changes in diet are of potentially great public health significance. As depression has also been associated with a higher incidence of cardiovascular disease and dietary exposures play a major role in the genesis of cardiovascular disease, it is of paramount importance to assess the associations between dietary exposures and the risk of depression.

In this context, an association between low blood levels of B-vitamins or high serum homocysteine levels and a higher prevalence of depressive symptoms has been reported in numerous cross-sectional (Hvas *et al.*, 2004a; Ramos *et al.*, 2004; Tolmunen *et al.*, 2004b; Sachdev *et al.*, 2005) and case–control studies (Lee *et al.*, 1998; Tiemeier *et al.*, 2002). Moreover, low blood folate levels have been associated with a poor response to antidepressant treatment (Fava *et al.*, 1997). Thus, several clinical trials have analysed whether the co-administration of vitamin B_{12}, methylfolate or folic acid with the antidepressant treatment enhanced its effect (Godfrey *et al.*, 1990; Coppen & Bailey, 2000; Alpert *et al.*, 2002; Hvas *et al.*, 2004b). Although a small randomized trial did not find an improvement in depression after the administration of vitamin B_{12} as an adjuvant (Hvas *et al.*, 2004b), other studies reported beneficial effects on antidepressant treatment response for methylfolate or folic acid (Godfrey *et al.*, 1990; Coppen & Bailey, 2000; Alpert *et al.*, 2002).

There are mechanistic reasons to expect that B-vitamins or folate may be involved in neurochemical pathways related to the risk of depression. Vitamin B_{12} and folate are involved in single-carbon transfer reactions needed for the production of serotonine and other monoamine neurotransmitters and catecholamines (Coppen *et al.*, 1989). Thus, deficiency of either vitamin might cause an impaired methylation in the central nervous system and result in neurological or psychiatric disease. Vitamin B_{12} and B_6 serve as cofactors for enzymes involved in the conversion of homocysteine to methionine and cysteine, respectively. Moreover, methylfolate is required for the synthesis of methionine from homocysteine as well through methyltetrahydrofolate reductase. On the other hand, methionine is a precursor of *S*-adenosylmethionine, which acts in several methylation reactions including those involving serotonin and other monoamine neurotransmitters (Coppen *et al.*, 1989) and has been reported to have antidepressant properties (Mischoulon & Fava, 2002). Thus, a decreased intake of B-vitamins may result in an accumulation of homocysteine and in a decreased synthesis of monoamines in brain, likely contributing to mechanisms related with the origins of depression.

Homocysteinic acid and cysteine sulfinic acid, metabolites of homocysteine, may inhibit the *S*-adenosymethionine methilation reactions. In addition, its metabolites might also have an excitotoxic effect on the *N*-methyl-D-aspartate glutamate receptors in the central nervous system (Parnetti *et al.*, 1997). Moreover, it has been suggested that hyperhomocysteinemia itself could aggravate depression. Further effects of hyperhomocysteinemia in the brain could be mediated by vascular effects (Klerk *et al.*, 2002).

In Mediterranean countries, where a high consumption of plant-based foods is observed, there is also a wider between-subjects variability in the intake of natural folate and other B-vitamins, thus enabling a better identification of the potential associations of these nutrients with disease risk (Parodi, 1997; Alonso *et al.*, 2004; Agudo *et al.*, 2007). However, there is a scarcity of epidemiological studies conducted in Mediterranean countries assessing the nutritional determinants of mental diseases. The association of B-vitamins and folate intake with the medical diagnosis of depression was assessed in the SUN cohort. The SUN Project (Seguimiento Universidad de Navarra – Follow-up University of Navarra) is a prospective cohort study designed to establish the association between several dietary habits or lifestyle-related characteristics and the occurrence of several diseases and chronic conditions, including depression (Martínez-González *et al.*, 2002).

Materials and methods

Study population

The SUN cohort was designed in collaboration with the Harvard School of Public Health using a similar methodology to that of large American cohorts, such as the Nurses' Health Study or the Health Professionals Follow-up Study.

Information is collected using self-administered questionnaires sent by postal mail every 2 years. The recruitment of participants started in December 1999 and is permanently on-going as this is a dynamic cohort study. All participants are university graduates. Up to July 2005, the baseline questionnaires of 10 557 participants were coded, processed and ready for statistical analysis. Those participants who reported extremely low or high values for total energy intake [less than 2512.1 kJ day^{-1} (600 kcal day^{-1}) in men and 1674.7 kJ day^{-1} (400 kcal day^{-1}) in women or more than 17 584.6 kJ day^{-1} (4200 kcal day^{-1}) in men and 14 653.8 kJ day^{-1} (3500 kcal day^{-1}) in women] (n = 315), and subjects with a diagnosis of depression at an age prior to the age reported in the baseline questionnaire (n = 572) were excluded. Finally, data from 9670 participants (4211 men and 5459 women) remained available for the analysis.

The study was approved by the Human Research Ethical Committee at the University of Navarra. Voluntary completion of the first self-administered questionnaire was considered to imply informed consent.

Assessment of dietary exposure

The dietary exposure was ascertained through a semi-quantitative food frequency questionnaire (136 food items) previously validated in Spain (Martín-Moreno *et al.*, 1993).

Nutrient scores were calculated as frequency × nutrient composition of specified portion size where frequencies were measured in nine categories (6+ per day/4–6 per day/2–3 per day/1 per day/5–6 per week/2–4 per week/1 per week/1–3 per month/never or almost never) for each food item. Nutrient intake scores were computed using *ad hoc* computer software specifically developed for this aim. A trained dietitian updated the nutrient data bank using the latest available information included in food composition tables for Spain (Mataix, 2003; Moreiras, 2003).

B-vitamin intakes were adjusted for total energy intake using the residuals method (Willett & Stampfer, 1998). Finally, the continuous variables were categorized in quintiles.

Assessment of nondietary variables

The baseline assessment also included other questions (totalling 46 items for men and 54 items for women). Socio-demographic (e.g. gender, age, marital status and employment status), anthropometric (e.g. weight and height), lifestyle and health-related habits (e.g. smoking status and physical activity during leisure time), psychological characteristics (e.g. self-perceived personality traits) and medical history variables (e.g. prevalence of chronic diseases and medication use) were collected.

The physical activity questionnaire included information about 17 activities, including walking, jogging, bicycling, static bicycling, swimming, racquet sports, football, aerobic, judo, trekking, skiing, sailing or gardening**.** To quantify the volume of activity during leisure time, an activity metabolic equivalent (MET) index was computed by assigning a multiple of resting metabolic rate (MET score) to each activity (Ainsworth *et al.*, 2000), and the time spent in each of the activities was multiplied by the MET score specific to each activity, and then summed over all activities obtaining a value of overall weekly MET-h. In the validation study, there was a highly significant correlation between objectively measured physical activity through an accelerometer and the overall weekly MET-h assessed using this questionnaire ($r = 0.51$, $P < 0.001$) (Martínez-González *et al.*, 2005).

Participants were classified as having a cardiovascular disease if they reported any of the following conditions: myocardial infarction, stroke, atrial fibrillation, paroxysmal tachycardia, coronary artery bypass grafting or other revascularization procedures, heart failure, aortic aneurism, pulmonary embolism, or peripheral venous thrombosis.

A participant was considered to present an incapacitating disease if he/she reported a medical diagnosis of asthma, emphysema, or rheumatoid arthritis.

The participants answered questions about personality and behaviour features, such as the level of competitiveness, level of anxiety and level of dependence, with values in the range 0–10. The three personality scores were included in the analysis.

Assessment of the outcome

Depression was assessed though a question included in the baseline questionnaire. Participants responded to the question: Have you ever been diagnosed of depression by a health professional? The question also specified the age of diagnosis grouped in four categories (<25, 25–49, 50–64, ≥65 years).

The baseline questionnaire also collected information regarding the use of regular antidepressant medication.

Recent depression was defined as a positive answer to either the question regarding physician-diagnosed depression or a participant reporting the use of antidepressant medication.

Participants were grouped according to their age in four categories (<25, 25–49, 50–64, ≥65 years). Subjects with a physician-diagnosed depression at an age (four categories) before the age category of that participant were considered as old cases and were excluded from the analyses.

Statistical analysis

A cross-sectional analysis of the cohort was carried out. All the analyses were performed for men and women separately. Nonconditional logistic regression models were fit to assess the relationship between the intake of B-vitamins and the prevalence of depression in this cohort. Odds ratios (OR) and their 95% confidence intervals (CI) were calculated considering the lowest quintile for the intake of folate, vitamin B_6 and vitamin B_{12} as the reference categories. Tests of linear trend across increasing quintiles of intake were conducted by assigning the medians of intake in each quintile and treating the intake as a continuous variable. Potential confounders included in the multivariate model were: age (continuous), body mass index (<25, 25–29.9 and ≥ 30 kg m^{-2}), physical activity during leisure-time (no exercise, 0.1–8.0 MET-h per week, 8.01–17.0 MET-h per week, 17.01–28.0 MET-h per week, >28.0 MET-h per week), marital status (single, married, widowed, separated or other), smoking (never, past and current smokers), unemployment (no/yes), severe diseases (cancer, cardiovascular or incapacitating disease), energy intake (continuous), omega-3 fatty acids intake (continuous), alcohol intake (continuous), and personality traits such as competitiveness, anxiety and dependence (continuous, score in the range 0–10). Moreover, each specific vitamin model was additionally adjusted for the intake of the other two vitamins.

Finally, product-terms to assess interactions between B-vitamin intake and the main characteristics of the participants (smoking, alcohol intake, physical activity during leisure time and personality traits) were included in the final model.

Moreover, OR for depression by quintile categories of vitamin B_{12} and folate intakes were measured after stratifying for the effect modifiers found in the logistic regression models in addition to those reported in the scientific literature.

$P < 0.05$ (two-tailed) was considered statistically significant.

Results

Table 1 shows the main characteristics of the male participants according the lowest and highest quintile of energy-adjusted intake of B-vitamins. Folate and vitamin B_6 intake was higher among older subjects, ex-smokers, married men and participants with severe diseases. Men belonging to the highest quintile of B-vitamin intake reported a total lower energy intake and they were physically more active. Men with a higher intake of folate, vitamin B_6 and vitamin B_{12} showed also a higher intake of omega-3 fatty acids and vitamin E but a lower intake of caffeine (Table 1).

Among women (Table 2), those belonging to the highest quintile of baseline intake of B-vitamins reported a lower energy and caffeine intake and they were physically more active. In addition, they reported a higher intake of omega-3 fatty acids. Folate and vitamin B_6 intakes were

Table 1 Characteristics of the male participants of SUN Cohort study according to extreme quintiles of energy-adjusted B-vitamin intake (n = 4211)

	Folate		Vitamin B_6		Vitamin B_{12}	
	Q1	Q5	Q1	Q5	Q1	Q5
Age (years)*	37.7 (11.9)	47.2 (13.5)	38.3 (11.2)	46.3 (13.9)	42.2 (12.6)	41.5 (13.6)
Body mass index (kg m^{-2})*	25.1 (3.2)	25.7 (2.9)	25.1 (3.2)	25.7 (3.1)	25.1 (3.0)	25.7 (3.1)
Smoking status†						
Ex-smoker	25.2	43.6	26.8	42.1	32.9	30.6
Current smoker	29.5	17.9	30.6	17.5	22.3	24.2
Severe diseases†						
Cancer	1.8	3.7	1.5	3.6	2.4	2.7
Cardiovascular	3.3	8.1	3.2	7.9	4.9	5.3
Incapacitating diseases‡	10.1	12.6	9.5	11.3	10.0	9.0
Marital status†						
Single	49.9	28.6	45.5	28.7	38.2	41.9
Married	47.8	67.0	51.5	67.8	58.6	55.9
Widowed	0.6	1.6	0.5	1.2	1.0	0.6
Separated	0.9	2.2	1.2	1.8	1.6	1.2
Other	0.8	0.6	1.3	0.5	0.7	0.4
Unemployed†	3.2	2.5	3.7	2.3	3.0	2.6
Folate intake (μg day^{-1})§*	302.8 (17.8)	608.3 (150.2)	251.6 (75.0)	550.9 (181.4)	357.9 (177.1)	424.6 (152.7)
Vitamin B_6 intake (mg day^{-1})§*	2.0 (0.4)	3.5 (0.8)	1.8 (0.3)	3.7 (0.7)	2.2 (0.7)	3.2 (0.8)
Vitamin B_{12} intake (mg day^{-1})§*	8.5 (4.1)	11.0 (6.2)	7.2 (3.2)	13.5 (7.0)	4.9 (1.2)	17.2 (5.6)
Vitamin E intake (mg day^{-1})§*	6.4 (3.1)	7.7 (3.5)	6.7 (3.5)	7.5 (3.2)	6.7 (3.7)	7.4 (3.2)
Caffeine intake (mg day^{-1})*	44.5 (39.4)	38.1 (41.1)	50.8 (44.8)	37.5 (40.3)	46.1 (44.5)	41.5 (41.4)
Omega-3 fatty acids intake (g day^{-1})§*	1.0 (0.6)	1.4 (0.9)	0.6 (0.3)	1.8 (1.1)	0.5 (0.3)	1.8 (1.1)
Alcohol intake (g day^{-1})*	10.9 (14.5)	9.5 (12.9)	10.7 (13.4)	10.4 (13.1)	10.1 (14.0)	11.2 (13.4)
Energy intake (kJ day^{-1})*	11 064.9 (3258.1)	10 571.3 (3004.5)	11 387.7 (3127.5)	10 637.0 (2955.5)	11 527.1 (3083.6)	10 533.6 (3061.4)
Leisure-time physical activity (MET-h per week)*¶	18.8. (22.5)	24.7 (31.8)	18.7 (21.7)	26.0 (31.8)	20.6 (25.9)	23.2 (26.9)
Personality scores**						
Competitiveness	7.1 (1.8)	7.2 (1.9)	7.0 (1.8)	7.3 (1.8)	7.1 (1.8)	7.3 (1.7)
Anxiety	6.0 (2.3)	6.2 (2.3)	5.9 (2.3)	6.2 (2.3)	6.0 (2.3)	5.9 (2.3)
Dependence	3.6 (2.9)	3.5 (3.0)	3.6 (2.9)	3.6 (3.1)	3.6 (2.9)	3.7 (2.9)

*Data are the mean (SD).
†Data are percentage.
‡Asthma, emphysema, rheumatoid arthritis.
§Energy-adjusted intake.
¶Metabolic equivalents.
**Ordinal, 0–10 points.

Table 2 Characteristics of the female participants of SUN Cohort study according to extreme quintiles of energy-adjusted B-vitamin intake (*n* = 5459)

	Folate		Vitamin B_6		Vitamin B_{12}	
	Q1	Q5	Q1	Q5	Q1	Q5
Age (years)*	33.2 (10.0)	36.2 (11.8)	33.9 (10.1)	36.4 (12.3)	35.6 (10.7)	34.2 (10.8)
Body mass index (kg m^{-2})*	21.7 (2.9)	22.1 (2.9)	21.6 (3.0)	22.2 (3.0)	21.7 (2.9)	22.2 (3.2)
Smoking status†						
Ex-smoker	19.6	25.9	20.6	24.0	23.3	21.9
Current smoker	32.6	24.4	31.9	23.1	28.1	29.4
Severe diseases†						
Cancer	2.7	5.0	2.2	4.2	3.8	3.6
Cardiovascular	1.9	3.0	2.0	3.5	2.5	2.6
Incapacitating diseases‡	7.9	8.5	7.8	7.9	7.1	7.4
Marital status†						
Single	57.4	56.6	55.4	54.4	54.4	57.1
Married	40.2	38.9	42.0	41.3	42.3	39.3
Widowed	0.7	1.4	0.9	1.5	1.3	0.6
Separated	1.4	2.7	1.4	2.5	1.8	2.6
Other	0.3	0.4	0.4	0.3	0.3	0.5
Unemployed†	7.1	7.2	7.0	7.8	6.7	8.5
Folate intake (μg day^{-1})§*	239.1 (46.7)	656.1 (137.9)	271.4 (81.6)	603.3 (173.3)	389.1 (174.9)	454.3 (153.1)
Vitamin B_6 intake (mg day^{-1})§*	2.0 (0.4)	3.5 (0.8)	1.8 (0.3)	3.8 (0.6)	2.3 (0.7)	3.1 (0.8)
Vitamin B_{12} intake (mg day^{-1})§*	7.8 (3.6)	10.3 (6.0)	6.8 (3.2)	10.1 (6.3)	4.5 (1.1)	16.0 (5.1)
Vitamin E intake (mg day^{-1})§*	7.2 (4.3)	7.3 (3.5)	7.3 (4.5)	7.2 (3.5)	7.4 (4.5)	7.3 (3.0)
Caffeine intake (mg day^{-1})*	43.5 (39.7)	43.2 (40.7)	45.8 (40.2)	40.5 (39.3)	45.9 (40.4)	42.3 (38.9)
Omega-3 fatty acids intake (g day^{-1})§*	0.7 (0.5)	1.3 (0.8)	0.6 (0.3)	1.6 (1.0)	0.5 (0.3)	1.6 (1.0)
Alcohol intake (g day^{-1})*	3.7 (5.8)	3.4 (5.2)	3.7 (5.6)	3.5 (5.4)	3.8 (6.2)	4.0 (5.7)
Energy intake (kJ day^{-1})*	10 226.3 (2606.3)	9859.5 (2404.1)	10 407.1 (2512.1)	9996.4 (2377.7)	10 464.1 (2505.8)	9720.1 (2381.9)
Leisure-time physical activity (MET-h per week)*¶	10.4 (14.7)	18.8 (22.0)	10.8 (14.0)	18.2 (20.7)	13.7 (16.8)	15.3 (19.8)
Personality scores**						
Competitiveness	6.7 (1.8)	7.1 (1.8)	6.8 (1.7)	7.1 (1.8)	6.9 (1.7)	6.9 (1.8)
Anxiety	6.1 (2.2)	6.1 (2.2)	6.1 (2.2)	6.1 (2.2)	6.1 (2.2)	6.1 (2.2)
Dependence	3.8 (2.7)	3.6 (2.8)	3.8 (2.7)	3.7 (2.8)	3.7 (2.8)	3.9 (2.8)

*Data are the mean (SD).
†Data are percentage.
‡Asthma, emphysema, rheumatoid arthritis.
§Energy-adjusted intake.
¶Metabolic equivalents.
**Ordinal, 0–10 points.

higher among older women, ex-smokers, separated women and women with prevalent severe diseases.

Two hundred twenty-nine cases of depression in men and 363 cases in women were observed.

Tables 3 and 4 show the association between the intake of vitamins (B_{12}, B_6 and folate) and the prevalence of depression for men and women.

Subjects in the lowest intake of the different vitamins were considered as the reference category for all comparisons.

No statistically significant associations between B-vitamin intake and the prevalence of depression among men were found, although some point estimates for ORs suggested an inverse association with folate intake. The multivariate adjusted OR (95% CI) for depression was 1 (reference), 0.81 (0.51–1.28), 0.77 (0.48–1.25), 0.72 (0.43–1.20) and 1.01 (0.57–1.79), respectively, for successive quintiles of energy-adjusted folate intake (Table 3). When the third, fourth and fifth quintile were merged as a single group, the results did not achieve statistical significance [OR=0.77 (0.50–1.19) for the three upper quintiles as a single category]. The linear trend for these categories of folate intake among men was not statistically significant (*P* for trend = 0.30).

A significant inverse association between energy-adjusted vitamin B_{12} intake and prevalence of depression was found among women. The OR (95% CI) for successive quintiles of intake was 0.65 (0.46–0.91), 0.58 (0.41–0.84), 0.56

Table 3 Association between baseline folate, vitamin B_6 and B_{12} intake and prevalence of depression in 4211 men of the SUN Project

	Q1	Q2	Q3	Q4	Q5	*P* for trend
Quintiles of vitamin B_{12} intake						
Median intake	5.2	7.2	8.9	11.1	15.7	
Number of cases	39	40	57	51	42	
Crude OR (95% CI)	1	1.01 (0.64–1.59)	1.47 (0.97–2.23)	1.31 (0.85–2.01)	1.07 (0.68–1.67)	0.72
Adjusted OR (95% CI)*§†	1	0.98 (0.61–1.58)	1.50 (0.95–2.36)	1.31 (0.81–2.14)	1.14 (0.66–1.98)	0.60
Quintiles of vitamin B_6 intake						
Median intake	1.9	2.3	2.6	2.9	3.5	
Number of cases	42	37	41	50	59	
Crude OR (95% CI)	1	0.86 (0.55–1.36)	0.98 (0.63–1.53)	1.19 (0.78–1.82)	1.45 (0.97–2.18)	0.02
Adjusted OR (95% CI)*§‡	1	0.81 (0.50–1.31)	0.94 (0.57–1.55)	1.15 (0.68–1.94)	1.41 (0.74–2.67)	0.17
Quintiles of folate intake						
Median intake	231.9	303.9	361.7	429.7	569.4	
Number of cases	45	39	41	40	64	
Crude OR (95% CI)	1	0.86 (0.55–1.33)	0.92 (0.60–1.42)	0.89 (0.57–1.37)	1.50 (1.01–2.22)	0.02
Adjusted OR (95% CI)*†‡	1	0.81 (0.51–1.28)	0.77 (0.48–1.25)	0.72 (0.43–1.20)	1.01 (0.57–1.79)	0.76

*Adjusted for age (years), body mass index (three categories), physical activity (five categories), marital status (five categories) smoking (three categories), unemployment status (no/yes), cardiovascular diseases (no/yes), cancer (no/yes), incapacitating diseases (no/yes), energy intake (kJ day^{-1}), omega-3 fatty acids intake (g day^{-1}), alcohol intake (g day^{-1}) and self-perceived personality traits (competitiveness, anxiety and dependence, ordinal, 0 through 10).
§Additionally adjusted for folate intake (μg day^{-1}).
†Additionally adjusted for vitamin B_6 intake (mg day^{-1}).
‡Additionally adjusted for vitamin B_{12} intake (mg day^{-1}).
OR, odds ratio; CI, confidence interval.

Table 4 Association between baseline folate, vitamin B_6 and B_{12} intake and prevalence of depression in 5459 women of the SUN Project

	Q1	Q2	Q3	Q4	Q5	*P* for trend
Quintiles of vitamin B_{12} intake						
Median intake	4.8	6.6	8.1	11.0	14.6	
Number of cases	96	73	65	56	73	
Crude OR (95% CI)	1	0.73 (0.53–1.00)	0.64 (0.46–0.89)	0.56 (0.40–0.79)	0.73 (0.53–1.00)	0.05
Adjusted OR (95% CI)*§†	1	0.65 (0.46–0.91)	0.58 (0.41–0.84)	0.56 (0.38–0.82)	0.68 (0.45–1.04)	0.15
Quintiles of vitamin B_6 intake						
Median intake	1.9	2.3	2.6	3.0	3.6	
Number of cases	73	62	86	73	69	
Crude OR (95% CI)	1	0.83 (0.59–1.18)	1.19 (0.86–1.64)	1.02 (0.73–1.42)	0.98 (0.70–1.38)	0.81
Adjusted OR (95% CI)*§‡	1	0.87 (0.60–1.26)	1.31 (0.89–1.92)	1.19 (0.77–1.86)	1.23 (0.70–1.86)	0.31
Quintiles of folate intake						
Median intake	248.8	332.2	396.6	473.3	618.5	
Number of cases	68	73	75	71	76	
Crude OR (95% CI)	1	1.11 (0.79–1.56)	1.16 (0.82–1.62)	1.07 (0.76–1.51)	1.19 (0.85–1.66)	0.42
Adjusted OR (95% CI)*†‡	1	1.11 (0.77–1.59)	1.10 (0.75–1.61)	0.99 (0.65–1.51)	1.04 (0.36–1.71)	0.94

*Adjusted for age (years), body mass index (three categories), physical activity (five categories), marital status (five categories) smoking (three categories), unemployment status (no/yes), cardiovascular diseases (no/yes), cancer (no/yes), incapacitating diseases (no/yes), energy intake (kJ day^{-1}), omega-3 fatty acids intake (g day^{-1}), alcohol intake (g day^{-1}) and self-perceived personality traits (competitiveness, anxiety and dependence, ordinal, 0 through 10).
§Additionally adjusted for folate intake (μg day^{-1}).
†Additionally adjusted for vitamin B_6 intake (mg day^{-1}).
‡Additionally adjusted for vitamin B_{12} intake (mg day^{-1}).
OR, odds ratio; CI, confidence interval.

(0.38–0.82) and 0.68 (0.45–1.04), respectively (Table 4). When the three upper quintiles were considered together as a group, the OR (95% CI) for depression was 0.59 (0.43–0.81). The results indicate a dose–response relationship (*P* for trend = 0.006). When the first quintile of intake was considered as a suboptimal B_{12} vitamin intake, the OR

(95% CI) of depression was 1.63 (1.22–2.17) compared to the rest of quintiles of intake (Q2–Q5).

Further adjustments for caffeine and vitamin E intake did not materially change the results (data not shown).

The analyses were also repeated without adjusting for cardiovascular disease as this could have led to an over-adjusted model (de Irala *et al.*, 2001). The results did not change.

When products-terms to assess interactions between B-vitamin intake and smoking, alcohol intake, physical activity during leisure time and personality traits of the participants were included in the model, a significant effect modification according to anxiety levels for folate intake was found among men ($P = 0.004$).

Tables 5 (men) and 6 (women) show the association between vitamin B_{12} and folate intake and the prevalence of depression according to several characteristics such as smoking, alcohol intake, physical activity during leisure time and personality characteristics of the participants.

Among males, an inverse association between energy-adjusted folate intake and the prevalence of depression was found only among current smokers and among those with low levels of anxiety. The OR (95% CI) for depression among current smokers was 0.31 (0.12–0.82), 0.25 (0.08–0.75) and 0.52 (0.17–1.57) for the third to fifth quintiles, respectively, compared to the reference quintile. Among those with low levels of anxiety, the OR (95% CI) for successive quintiles of intake was 0.41

Table 5 Association* between vitamin B_{12} and folate intake and depression among males in the SUN Project according to several characteristics

	n	Cases	Q2	Q3	Q4	Q5
Quintiles of folate intake§						
Smoking						
Never smokers	1816	67	0.48 (0.17–1.31)	1.24 (0.54–2.85)	1.61 (0.70–3.67)	1.15 (0.44–2.99)
Ex-smokers	1414	96	1.51 (0.70–3.27)	1.61 (0.74–3.48)	2.05 (0.91–4.58)	1.88 (0.77–4.62)
Current smokers	981	66	0.97 (0.41–2.33)	1.73 (0.75–3.98)	0.57 (0.21–1.56)	0.51 (0.16–1.62)
Tertiles of alcohol intake (g day^{-1})						
(1st) < 3.1	1410	82	1.52 (0.69–3.35)	1.28 (0.54–3.05)	1.91 (0.80–4.55)	1.46 (0.53–4.02)
(2nd) 3.1–10.8	1404	72	1.10 (0.44–2.76)	2.24 (0.98–5.13)	1.64 (0.65–4.12)	1.30 (0.48–3.54)
(3rd) > 10.8	1397	74	0.40 (0.16–1.03)	1.19 (0.57–2.47)	0.74 (0.32–1.70)	0.86 (0.34–2.16)
Physical activity (MET/h per week)						
No exercise	680	43	1.09 (0.35–3.39)	1.72 (0.59–5.06)	1.98 (0.66–5.91)	2.14 (0.61–7.45)
0.1–17.0	1627	82	1.67 (0.75–3.73)	1.85 (0.84–4.06)	1.05 (0.43–2.56)	0.94 (0.34–2.61)
>17.0	1904	104	0.62 (0.29–1.32)	1.38 (0.69–2.75)	1.55 (0.75–3.18)	1.15 (0.51–2.58)
Self-perceived anxiety level						
Low (<6)	2143	65	1.34 (0.60–3.00)	1.25 (0.53–2.92)	1.19 (0.48–2.93)	1.01 (0.36–2.84)
High (6–10)	2068	164	0.82 (0.45–1.50)	1.49 (0.87–2.57)	1.28 (0.71–2.30)	1.14 (0.59–2.23)
Quintiles of vitamin B_{12} intake‡						
Smoking						
Never smokers	1816	67	1.56 (0.67–3.67)	0.99 (0.39–2.52)	1.74 (0.71–4.28)	1.53 (0.53–4.42)
Ex-smokers	1414	96	0.51 (0.20–1.26)	1.10 (0.50–2.41)	0.70 (0.29–1.67)	1.07 (0.42–2.75)
Current smokers	981	66	0.71 (0.33–1.51)	0.31 (0.12–0.82)	0.25 (0.08–0.75)	0.52 (0.17–1.57)
Tertiles of alcohol intake (g day^{-1})						
(1st) < 3.1	1410	82	0.89 (0.41–1.91)	0.79 (0.36–1.73)	0.85 (0.37–1.96)	0.85 (0.33–2.16)
(2nd) 3.1–10.8	1404	72	0.83 (0.33–2.09)	0.86 (0.33–2.23)	1.06 (0.41–2.74)	1.87 (0.64–5.43)
(3rd) > 10.8	1397	74	0.83 (0.38–1.81)	0.78 (0.35–1.76)	0.43 (0.16–1.16)	0.82 (0.29–2.37)
Physical activity (MET/h per week)						
No exercise	680	43	2.50 (0.90–6.95)	1.13 (0.33–3.88)	2.53 (0.80–8.03)	1.92 (0.45–8.12)
0.1–17.0	1627	82	0.65 (0.31–1.39)	0.62 (0.28–1.40)	0.56 (0.24–1.32)	0.51 (0.19–1.42)
>17.0	1904	104	0.65 (0.30–1.38)	0.81 (0.40–1.64)	0.55 (0.24–1.23)	1.24 (0.54–2.86)
Self-perceived anxiety level						
Low (<6)	2143	65	0.41 (0.19–0.87)	0.19 (0.07–0.54)	0.48 (0.21–1.12)	0.46 (0.16–1.32)
High (6–10)	2068	164	1.35 (0.74–2.46)	1.44 (0.79–2.60)	1.01 (0.52–1.97)	1.68 (0.83–3.42)

*Adjusted for age (years), body mass index (three categories), physical activity (five categories), marital status (married/nonmarried) smoking (three categories), unemployment status (no/yes), cardiovascular diseases (no/yes), cancer (no/yes), incapacitating diseases (no/yes), energy intake (kJ day^{-1}), omega-3 fatty acids intake (g day^{-1}), alcohol intake (g day^{-1}), vitamin B_6 intake (mg day^{-1}) and self-perceived personality traits (competitiveness, anxiety and dependence, ordinal, 0 through 10) [Q1 = reference category].

‡Additionally adjusted for vitamin B_{12} intake (mg day^{-1}).

§Additionally adjusted for folate intake (μg day^{-1}).

MET, metabolic equivalent.

Table 6 Association* between vitamin B_{12} and folate intake and depression among females in the SUN Project according to several characteristics

	N	Cases	Q2	Q3	Q4	Q5
Quintiles of Vitamin B_{12} intake§						
Smoking						
Never smokers	2684	163	0.69 (0.42–1.13)	0.52 (0.30–0.89)	0.55 (0.31–0.99)	0.73 (0.39–1.37)
Ex-smokers	1271	85	0.88 (0.43–1.80)	1.04 (0.48–2.23)	0.59 (0.24–1.43)	1.09 (0.44–2.72)
Current smokers	1504	115	0.49 (0.25–0.93)	0.45 (0.23–0.89)	0.64 (0.33–1.24)	0.67 (0.33–1.39)
Tertiles of alcohol intake (g day^{-1})						
(1st) < 1.0	1814	155	0.63 (0.38–1.06)	0.50 (0.29–0.88)	0.46 (0.25–0.85)	0.61 (0.31–1.18)
(2nd) 1.0–3.3	1838	100	0.54 (0.28–1.03)	0.66 (0.34–1.29)	0.53 (0.24–1.16)	0.54 (0.23–1.26)
(3rd) > 3.3	1807	108	0.82 (0.43–1.57)	0.63 (0.31–1.29)	0.90 (0.45–1.82)	1.15 (0.56–2.39)
Physical activity (MET/h per week)						
No exercise	1513	116	0.74 (0.39–1.39)	1.07 (0.57–1.98)	0.68 (0.33–1.40)	0.82 (0.38–1.75)
0.1–17.0	2187	131	0.44 (0.26–0.76)	0.32 (0.17–0.59)	0.31 (0.15–0.61)	0.43 (0.20–0.93)
>17.0	1759	116	0.93 (0.50–1.74)	0.52 (0.26–1.07)	0.92 (0.47–1.80)	1.09 (0.54–2.22)
Self-perceived anxiety level						
Low (<6)	2864	107	0.47 (0.25–0.85)	0.35 (0.18–0.68)	0.39 (0.20–0.77)	0.57 (0.29–1.12)
High (6–10)	2592	256	0.79 (0.53–1.19)	0.76 (0.49–1.17)	0.72 (0.45–1.15)	0.88 (0.53–1.47)
Quintiles of folate intake‡						
Smoking						
Never smokers	2684	163	1.17 (0.69–2.00)	1.16 (0.66–2.05)	0.83 (0.44–1.56)	0.98 (0.45–2.09)
Ex-smokers	1271	85	1.82 (0.76–4.35)	1.28 (0.50–3.23)	1.77 (0.69–4.58)	1.36 (0.46–4.08)
Current smokers	1504	115	0.81 (0.42–1.55)	1.00 (0.52–1.93)	0.95 (0.45–2.03)	1.06 (0.43–2.63)
Tertiles of alcohol intake (g day^{-1})						
(1st) < 1.0	1814	155	0.94 (0.54–1.62)	0.79 (0.45–1.41)	0.81 (0.43–1.51)	0.82 (0.39–1.73)
(2nd) 1.0–3.3	1838	100	1.32 (0.59–2.95)	2.04 (0.94–4.43)	1.66 (0.70–3.93)	2.43 (0.90–6.56)
(3rd) > 3.3	1807	108	1.29 (0.68–2.46)	1.03 (0.50–2.13)	0.93 (0.43–2.01)	0.65 (0.24–1.78)
Physical activity (MET/h per week)						
No exercise	1513	116	1.23 (0.65–2.31)	1.48 (0.76–2.89)	1.27 (0.60–2.70)	1.82 (0.74–4.48)
0.1–17.0	2187	131	0.93 (0.52–1.67)	0.82 (0.45–1.52)	0.77 (0.40–1.51)	0.69 (0.31–1.56)
>17.0	1759	116	1.31 (0.64–2.65)	1.19 (0.58–2.43)	1.09 (0.51–2.34)	1.06 (0.43–2.61)
Self-perceived anxiety level						
Low (<6)	2864	107	1.20 (0.62–2.33)	0.86 (0.42–1.78)	1.00 (0.48–2.08)	1.05 (0.45–2.49)
High (6–10)	2592	256	1.03 (0.67–1.60)	1.17 (0.75–1.83)	0.95 (0.58–1.57)	1.00 (0.55–1.83)

*Adjusted for age (years), body mass index (three categories), physical activity (five categories), marital status (married/nonmarried) smoking (three categories), unemployment status (no/yes), cardiovascular diseases (no/yes), cancer (no/yes), incapacitating diseases (no/yes), energy intake (kJ day^{-1}), omega-3 fatty acids intake (g day^{-1}), alcohol intake (g day^{-1}), vitamin B_6 intake (mg day^{-1}) and self-perceived personality traits (competitiveness, anxiety and dependence, ordinal, 0 through 10) [Q1 = reference category].
§Additionally adjusted for folate intake (μg day^{-1}).
‡Additionally adjusted for vitamin B_{12} intake (mg day^{-1}).
MET, metabolic equivalent.

(0.19–0.87), 0.19 (0.07–0.54), 0.48 (0.21–1.12) and 0.46 (0.16–1.32), respectively. Indeed, when the upper quintiles of folate intake were merged and this group was considered as the reference category, a significant positive association was found between a low folate intake and the prevalence of depression. The OR (95% CI) for the first quintile of intake [as compared to the upper quintiles of intake (Q2–Q5)] was 2.18 (1.08–4.38) for smoker males and 2.85 (1.49–5.45) for men with a low level of anxiety.

Among females, an inverse association between vitamin B_{12} intake and the prevalence of depression was found among those with a low level of competitiveness [OR (95% CI) = 0.39 (0.20–0.76) 0.30 (0.15–0.61) and 0.48 (0.24–0.99) for the third to fifth quintiles, respectively].

An inverse association for vitamin B_{12} intake was also apparent among women with a low level of anxiety. The OR (95% CI) for successive quintiles of intake was 0.47 (0.25–0.85), 0.35 (0.18–0.68), 0.39 (0.20–0.77) and 0.57 (0.29–1.12), respectively.

Among women who reported moderate physical activity level (up to 17 MET-h per week), depression was among 60–70% lower among whose vitamin B_{12} intake was in the Q2–Q5 than among women in the reference category of intake. The OR (95% CI) was 0.44

(0.26–0.76), 0.32 (0.17–0.59), 0.31 (0.15–0.61) and 0.43 (0.20–0.93), respectively.

Folate intake was not statistically related to depression among women after stratifying by several characteristics (Table 6).

Discussion

Men with high intake of folate and women with high intake of vitamin B_{12} showed a lower prevalence of depression. However, the inverse association between folate and depression was only apparent among men with a low level of anxiety and among currently smoking men. No significant associations between vitamin B_6 intake and depression were found.

This is the first time that a modification of effect of B-vitamin intake on depression regarding several personality characteristics of the participants is reported.

These results regarding the role of B-vitamins on depression are consistent with other previously published studies. However, most of the previous analyses from case–control or cross-sectional studies were based on serum folate or vitamin B_{12} levels and not in the intake of these vitamins (Lee *et al.*, 1998; Tiemeier *et al.*, 2002; Ramos *et al.*, 2004; Sachdev *et al.*, 2005). Serum levels of vitamins are known to be affected by fluctuations in daily dietary intakes, smoking and alcoholic habits or physical activity (Bailey, 1990; Piyathilake *et al.*, 1994; Benton *et al.*, 1997; Jacques *et al.*, 1999; Manore, 2000; Koehler *et al.*, 2001; Mennen *et al.*, 2002; Mannino *et al.*, 2003; Tolmunen *et al.*, 2004a,b; Chiuve *et al.*, 2005; Real *et al.*, 2005). Therefore, these results provide important new evidence about this association.

The evidence about the effect of dietary intake of B-vitamins on depression is sparse. Only the Kuopio Ischaemic Heart Disease Study, a population-based study, analysed the association between B-vitamin intake and the risk of depression among healthy middle-aged men. Both the cross-sectional and follow-up analyses revealed a protective role of folate intake on depression among men (Tolmunen *et al.*, 2003, 2004a) as has consistently been found in the SUN cohort.

It has been reported that serum folate is more sensitive to nutritional intake than vitamin B_{12}. Indeed, in 1998, the US Government implemented the fortification of grain and cereal products with folic acid at a concentration of 1.4 mg g^{-1} product. This action resulted in a substantial decline in plasma homocysteine and an increase in serum folate levels (Jacques *et al.*, 1999).

An association between folate intake and depression was not found among women. The percentage of subjects with suboptimal levels of folate intake was higher in men than in women. Thus, a possible explanation for the lack of effect of folate intake among females is that very few women in this cohort were exposed to suboptimal levels of folate intake. It is very likely that, once folate intake is sufficient to prevent subclinical folate deficiency, no further benefit is obtained by additional increases in folate intake, as observed in some studies regarding cardiovascular disease risk (Hernández-Díaz *et al.*, 2002). These findings are compatible with this possibility, suggesting that only low levels of folate intake may increase the risk of depression. Once a minimal safety level is attained, further increases in intake do not lead to further reduction in depression risk. This explanation is very likely because these data suggested a threshold effect. This would also explain why the estimated ORs for the fifth quintile were not the lowest, but they were even higher than those for the third or fourth quintiles. A higher prevalence of psychiatric symptoms among subjects with folate deficiency has been reported (Coppen *et al.*, 1989; Fava *et al.*, 1997). Indeed, Tolmunen *et al.* (2004a) reported an inverse association between folate intake and depression in a cohort where only 24.6% of the participants reached the Finnish recommendations regarding folate intake (300 μg day^{-1}).

However, a recent study carried out after the implementation of folic acid fortification in the US indicated that, despite folic acid fortification, low plasma folate concentrations were associated with depressive symptoms (Ramos *et al.*, 2004).

Alcohol intake has been suggested to be an effect modifier of the relationship between folate intake and plasma homocysteine (Koehler *et al.*, 2001; Chiuve *et al.*, 2005). Ethanol could affect the absorption and metabolism of folate, leading to a reduction of the potential beneficial effect of folate intake on depression. However, these results did not change after stratifying by alcohol intake categorized in tertiles.

Smoking has also been associated with a diminished bioavalability of several B-vitamins, ascorbic acid, retinol, α-tocopherol and carotenoids (Benton *et al.*, 1997). Although a statistically significant modification of the effect of folate intake on depression by smoking (P for the product term = 0.104 in men and P for the product term = 0.538 in women) was not found, an inverse association between folate intake and depression was found only among current male smokers. A biological plausibility exists for a selective deficit of folate among smokers because cigarette smoking increases folate requirements by interfering with folate utilization and/or metabolism, also leading to a lower circulating folate concentration in smokers than in nonsmokers (Bailey, 1990; Piyathilake *et al.*, 1994). Therefore, within a constant amount of folate intake, the prevalence of biological folate deficiency will be higher for smokers. This mechanism may explain why an association between low folate intake and a higher risk of depression was found only among current smokers.

The analysis was stratified according to physical activity because exercise could increase the need for these micronutrients through different mechanisms, including a decreased absorption of vitamins, an increased turnover of the nutrients, an increase in mitochondrial enzymes that require these nutrients, or through an increased need for the nutrients for tissue maintenance and repair (Manore, 2000; Mennen *et al.*, 2002; Real *et al.*, 2005). Thus, whereas moderate exercise could exert a beneficial effect on homocysteinaemia (as well as B-vitamin intake), and subsequently on depression, vigorous exercise would have a detrimental one. This fact could explain the lack of association between B-vitamin intake and prevalence of depression among sedentary subjects and among those with vigorous physical activity.

The study may have several limitations. The cross-sectional design of the study does not allow any cause–effect relationships to be proved. Depression itself could lead to a decreased appetite and result in a lower intake of B-vitamins. The exclusion of subjects who reported a former diagnosis of depression strengthens the probability that a low dietary intake of B-vitamins is a cause of depression rather than a consequence of it.

On the other hand, participants might modify their dietary habits after they developed some diet-related or incapacitating diseases such as cardiovascular disease or cancer and these changes could have attenuated the associations between B-vitamin intake and depression. The data were adjusted for a history of cardiovascular disease and cancer as well as incapacitating diseases such as arthritis rheumatoid or asthma and it is unlikely that the presence of diseases could bias the results.

Imprecise dietary measurement could potentially have influenced the observed associations. However, random errors in dietary assessment measures might have accounted for a lack of association but not for the reverse one (Hu *et al.*, 1999). Moreover, the semiquantitative food-frequency questionnaire used in this project was previously validated in Spain (Martín-Moreno *et al.*, 1993). Thus, true associations between B-vitamin intake and depression should be greater than those reported in the present analysis.

A social desirability bias could have led to a misreport in the self-reporting of a diagnosis of depression. However, several previous cohort studies of highly educated subjects have demonstrated the validity of self-reported data (Choi *et al.*, 2005). An adequate validity for other self-reported health conditions among the highly educated participants of this cohort has been demonstrated previously (Alonso *et al.*, 2005; Bes-Rastrollo *et al.*, 2005). Moreover, potential misclassifications would mainly bias the association towards the null value.

In summary, the results obtained in the present study suggest that a subclinical deficiency of cobalamin in women and a subclinical deficiency of folate among male smokers may contribute to a higher risk of depression. However, further prospective studies should be conducted to confirm these findings.

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Conflict of interests, source of funding and authorship

The authors declare that they have no conflict of interest.
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