Associations of dietary folate, vitamin B6 and B12 intake with cardiovascular outcomes in 115664 participants: a large UK population-based cohort

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Abstract

Background & Aims

: The evidence of relationship between dietary intake of folate, vitamin B6 and vitamin B12 and cardiovascular diseases(CVD) in UK populations is limited. We aimed to analyze the association of dietary intake of folate, vitamin B6, and vitamin B12 with CVD events [stroke, myocardial infarction(MI)] and CVD mortality.

Methods

We included 115664 participants, aged 40–70 years, with no CVD events or cancer at baseline, enrolled between 2006 and 2010 and followed up to the end of 2018. Dietary intake was measured with an online 24-hour dietary assessment. Multivariable Cox proportional hazards models were used to estimate hazard ratios (HR) and 95% confidence intervals (CI) for the associations.

Results

After multivariate adjustment, higher dietary folate intake was associated with a lower risk of CVD events [HR per standard deviation(SD) 0.95, 95% CI 0.93 to 0.98], stroke (HR per SD 0.95, 95% CI 0.91 to 0.99), MI(HR per SD 0.94, 95% CI 0.91 to 0.98),CVD mortality(HR per SD 0.90, 95% CI 0.83 to 0.97). Each tablespoons/day higher intake of raw vegetable intake, pieces/day higher intake of fresh fruit intake and bowls/week higher intake of cereal intake were associated with higher intakes of of folate every 0.02,0.06 and 0.05 SD, respectively. E-value analysis suggested robustness to unmeasured confounding.

Conclusion

Each increase in folate intakes was related to 5% lower risks of total CVD events and 10% lower risks of CVD mortality. Our findings support that strengthening dietary folate intake as a primary prevention strategy for CVD events and CVD mortality.

Introduction

Vitamin B6 (pyridoxine), vitamin B9 (folate), and vitamin B12 (cobalamin) are essential cofactors of homocysteine metabolism in the body[1–2]. Abundant clinical studies have shown that increased accumulation of homocysteine will significantly increase the risk of coronary heart disease, stroke, and peripheral vascular disease[3]. Furthermore, a randomized controlled trial showed that supplementation with folic acid alone or with vitamins B6 and B12 reduced homocysteine concentrations in the blood [3].

Emerging evidence suggests that folate, vitamin B6 and vitamin B12 may play an important role in reducing the risk of cardiovascular disease (CVD) events[4]. Several cross-sectional and prospective cohort studies showed that both dietary intake of folate and vitamin B6 were associated with lower risk CVD events [5–6]. However, findings from observational studies have been inconsistent or controversial [7–8]. Evidence for an association between dietary intake of these B vitamins and the risk of CVD events in different populations remains limited and insufficient[9–10]. In addition, previous publications did not analyse the relationship between dietary factors and dietary intake of B vitamins[4–6].

Therefore, in this prospective cohort study, we examined the association between dietary intake of folate, vitamin B6, and vitamin B12 and CVD events [stroke, myocardial infarction (MI)] and CVD mortality in over 500000 participants in the UK Biobank. We further investigated the relationship between dietary intake of folate, vitamin B6, and vitamin B12 and dietary factors.

Methods

Study design and population

The detailed study design and methods of the UK Biobank study have been described elsewhere [11−12]. During 2006−2010, the UK Biobank recruited more than 500,000 community-dwelling participants aged 40−70 years who were recruited between April 2007 and December 2010. Participants provided relevant health information through touch screen questionnaires and face-to-face interviews. Data from 502505 participants can be used in our research. Participants without available measurements of folate, vitamin B6, and vitamin B12 intake were excluded. Participants with CVD or cancer (n = 40024) at baseline were excluded from the analysis. We further excluded participants whose baseline demographic and lifestyle factors measured data were of poor quality (n = 54898). Our final analysis included 115664 participants **(Supplementary Fig. 1)**.

Ascertainment of exposure

Dietary intake was assessed with a 24-hour dietary recall questionnaire that included consumption of approximately 200 commonly consumed foods and drinks. The Oxford Cancer Epidemiology Department of the UK Biobank evaluated the validity of the developed questionnaire. More details can be found elsewhere [13]. The questionnaire contains detailed questions about the food and beverage intake consumed in the previous 24 hours. Participants needed to answer the questionnaire regarding what food and drinks they had consumed yesterday. By multiplying the number of servings of each food or beverage by the quantity consumed, the quantity of each food and beverage consumed in the previous 24 hours can be calculated. Detailed information on the measurements is provided on the UK Biobank website (https://biobank.ndph.ox.ac.uk/showcase/showcase/docs/DietWebQ.pdf).

We evaluated several possible confounding variables using the baseline questionnaire, including sociodemographic factors (age, sex, ethnicity, and employment), lifestyle habits (smoking status, physical activity, drinking status), body mass index, and blood samples (HDL, LDL, triglyceride, cholesterol). Baseline disease history (diabetes, hypertension and cancer) was collected through self-reporting. Details of these assessments were available on the UK Biobank website (www.ukbiobank.ac.uk).

Ascertainment of CVD events and CVD mortality

CVD mortality and CVD events (myocardial infarction and stroke) were used as the primary outcomes of this study. We used the reported UK Biobank algorithms to determine CVD events. We collected information about CVD events and CVD mortality through certified death records and cumulative medical records diagnosed by hospitals (until March 2018). Admission and diagnosis data of the records were used to ascertain incidents. CVD events with the ICD-10 (International Classification of Diseases, 10th revision). Stroke was defined as ICD-10 codes I60-I64. Myocardial infarction was defined as codes I21, I22, I23, I24.1, or I25.2. CVD mortality was defined as codes I00-I99.

Statistical analysis

Baseline characteristics are presented as the mean ± SD (standard deviation) for continuous variables, medians (interquartile range) for asymmetrical continuous variables and percentages for categorical variables. Participants were divided into different groups according to their dietary intake of folate, vitamin B6 and vitamin B12. T tests and chi-square tests were used to compare baseline characteristics between participants in the lowest to highest quartiles of vitamin intake. Linear regression was used to estimate β coefficients and 95% (confidence interval, CI) the relationship between dietary factors and dietary intake of folate, vitamin B6, and vitamin B12.

The proportional hazard assumption was evaluated by tests based on Schoenfeld residuals[14]. We used a Cox proportional hazard model to estimate the hazard ratio (HR) and 95% CI for incident CVD, stroke, and MI. Vitamins were divided into four groups (from the lowest group 1 to the highest group 4) for comparison and compared for each one standard deviation. We established three sets of models models, adjusting for potential confounders: model 1 adjusted for age(years) and sex (male or female), and ethnic(white, black, asian, mixed, or other ethnic groups); model 2, further adjusted for physical activity (< 250 min/week,250−550 min/week,>550 min/week), smoking status(never, former, current), drinking status (never, former, current), employment (no, yes), coffee(no, yes), HDL cholesterol (continuous), LDL cholesterol (continuous) and total cholesterol; and model 3, further adjusted body index based on model2. Based on model 3, we used Cox proportional hazards models with restricted cubic splines (three knots) to evaluate and visualize the relationship of the dietary intake of folate with CVD events and CVD mortality[15].

We also conducted sensitivity analyses to confirm the robustness of our results. First, participants who had CVD events within two years of follow-up were excluded. Second, we considered other potential confounding factors, including waist-to-hip ratio, intake of vitamin D, vitamin supplements, diabetes and hypertension. Third, we removed participants with baseline diabetes and hypertension. Finally, we calculated the E-values to explore the possibility of unmeasured confounding between dietary intake of folate, vitamin B6 and vitamin B12 and CVD events[16]. All statistical analyses were performed using SAS statistical software version 9.1 (SAS Institute, Inc., Cary, NC, USA).

Results

Baseline characteristics

Table 1 shows the baseline characteristics of the study population stratified by quartiles of dietary intake of folate, vitamin B6, and vitamin B12. Baseline mean dietary intake of folate, vitamin B6, and vitamin B12 among participants without CVD events was 310.65 (126.73) ug/d, 2.21 (0.84) mg/d and 6.65(5.55) ug/d, respectively, compared with 312.13 (129.73) ug/d, 2.25(0.90) mg/d and 6.76(6.02) ug/d in participants who developed CVD. Participants with higher folate intake and higher vitamin B6 intake were more likely to be male, slightly older, had lower body mass index, were more likely to be physically active, were less likely to be current smokers, but more likely to be current drinkers, and had lower HDL cholesterol. Participants with higher vitamin B12 intake were more likely to have higher HDL cholesterol and higher serum total cholesterol. **(supplementary table S1-S3)**

Table 1
Baseline characteristics of participants in the UK Biobank study

	N	Folate(ug/d)	Vitamin B6(mg/d)	VitaminB12(ug/d)
		mean(SD)	mean(SD)	mean(SD)
Case status				
Non-cases	112262	310.65(129.73)	2.21(0.84)	6.56(5.55)
CVD events	3402	312.13(133.52)	2.25(0.90)	6.76(6.02)
Stroke	1402	311.91(133.30)	2.23(0.88)	6.73(6.14)
Myocardial infarction	2184	311.48(134.79)	2.26(0.91)	6.76(6.31)
CVD mortality	433	305.48(132.03)	2.24(0.88)	6.87(5.95)
Sex				
Female	60806	298.78(125.41)	2.10(0.79)	6.35(5.33)
Male	54858	323.92(133.33)	2.32(0.89)	6.79(5.82)
Age(years)				
<50	30924	302.23(133.82)	2.16(0.87)	6.24(5.47)
50−60	41105	308.40(128.94)	2.19(0.84)	6.48(5.51)
>60	43635	318.87(127.32)	2.26(0.83)	6.87(5.67)
Body mass index(kg/m^2)				
<25	45497	311.69(128.65)	2.16(0.81)	6.46(5.39)
25−30	48493	310.69(128.67)	2.23(0.85)	6.61(5.60)
>30	21674	308.65(134.81)	2.26(0.91)	6.68(5.83)
Ethnic				
White	105050	313.41(128.39)	2.21(0.83)	6.54(5.49)
Mixed	3689	304.51(146.75)	2.23(1.03)	6.72(6.31)
Asian or Asian british	5109	305.44(136.62)	2.12(0.88)	6.57(6.18)
Black or Black british	589	287.51(140.53)	1.99(0.89)	6.57(6.53)
Other	1227	301.60(158.87)	2.17(1.10)	7.03(6.58)
Employment				
No	39670	318.12(128.93)	2.26(0.84)	6.81(5.62)
Yes	75994	306.83(130.14)	2.18(0.85)	6.43(5.54)
Physical activity(min/week)				
<250	71559	303.80(125.21)	2.16(0.82)	6.48(5.44)
250−550	25203	318.73(132.45)	2.25(0.86)	6.71(5.64)
>550	18902	326.11(141.09)	2.29(0.91)	6.70(5.91)

HDL-C = high density lipoprotein cholesterol; LDL-C = low density lipoprotein cholesterol; SD = standard deviation

	N	Folate(ug/d)	Vitamin B6(mg/d)	VitaminB12(ug/d)
		mean(SD)	mean(SD)	mean(SD)
Smoking Status				
Never	66767	311.14(127.08)	2.21(0.84)	6.55(5.46)
Former	40061	312.79(132.50)	2.22(0.85)	6.60(5.68)
Current	8836	297.85(137.311)	2.13(0.88)	6.47(5.86)
Drinking Status				
Never	3271	304.74(142.38)	2.13(0.92)	6.02(5.38)
Former	3124	310.02(149.97)	2.18(0.93)	6.18(5.56)
Current	109269	310.90(128.82)	2.21(0.84)	6.58(5.57)
Coffee				
No	50279	314.73(138.46)	2.19(0.89)	6.20(5.47)
Yes	65385	313.61(134.39)	2.22(0.86)	6.51(5.54)
HDL-C (mmol/L)				
<1.4	61994	313.95(132.16)	2.23(0.87)	6.53(5.58)
>1.4	53670	306.95(126.99)	2.17(0.81)	6.60(5.55)
LDL-C (mmol/L)				
<3.7	70026	312.52(131.03)	2.21(0.85)	6.53(5.58)
≥3.7	45638	307.91(127.93)	2.20(0.84)	6.60(5.55)
Triglyceride (mmol/L)				
<1.69	75719	308.23(128.77)	2.18(0.83)	6.60(5.56)
≥1.69	39945	315.36(131.71)	2.25(0.87)	6.50(5.58)
Cholesterol (mmol/L)				
<5.89	69650	313.08(131.75)	2.22(0.86)	6.53(5.55)
≥5.89	46014	307.10(126.80)	2.18(0.83)	6.62(5.59)
HDL-C = high density lipoprotein cholesterol; LDL-C = low density lipoprotein cholesterol; SD = standard deviation				

Association of dietary intake of folate, vitamin B6, and vitamin B12 with dietary factors

In our analyses, after adjusting for all confounding factors, we found a positive correlation between sex (female compared with male), physical activity (compared with < 250 min/week), and dietary intake of folate, vitamin B6, and vitamin B12, while employment (yes compared with no) and current drinking status (compared with never drinking) were inversely associated with dietary intake of folate, vitamin B6, and vitamin B12 (supplemental table S4).

Dietary factors of cooked vegetable intake and fresh fruit intake, raw vegetable intake, cereal intake, and oily fish intake were all positively associated with intake of folate, vitamin B6, and vitamin B12 (Fig. 1). Dietary factors of beef intake and pork intake were positively associated with both intakes of vitamin B6 and vitamin B12. Each tablespoons/day higher intake of cooked vegetable intake was associated with 0.04,0.03 higher one SD of dietary folate and vitamin B6 intakes. For every additional

bowl/week of cereal intake, the dietary folate intake increased by 0.05 SD. More details were described in supplemental table S4.

Association of dietary intake of folate, vitamin B6, and vitamin B12 with CVD events and CVD mortality

During a mean follow-up of 8.67 years, 3402 CVD events, including 1402 strokes, 2184 myocardial infarctions, and 433 deaths from CVD occurred. In the multivariate model, higher levels of folate intake were associated with a lower hazard of CVD events; HR for each SD (HR = 0.95; 95% CI, 0. 93 to 0.98; P for trend = 0.001) in model 3. Comparing the HR of folate in groups 2, 3, and 4 with group 1 were 0.99 (0.89 to 1.01), 0.92 (0.82 to 1.02), and 0.88 (0.80 to 0.96), respectively (Table 2).

Table 2
Multivariable-adjusted ratios for dietary intakes of folate, vitamin B6, and B12 with the risk of CVD events

	Hazard ratio (95% confidence interval)					P-trend
	group 1	group 2	group 3	group 4	For each standard deviation increase	
Folate(median, ug/d)	167.45	221.52	321.41	432.81		
CVD events						
Incident rate per 100,000 person-years	331.94/100,000	351.66/100,000	393.33/100,000	280.04/100,000		
model 1	1.0(ref)	0.95(0.86,1.05)	0.86(0.78,0.96)	0.82(0.75,0.90)	0.94(0.91,0.96)	< 0.001
model 2	1.0(ref)	0.98(0.88,1.09)	0.90(0.81,1.00)	0.87(0.79,0.95)	0.95(0.92,0.98)	0.001
model 3	1.0(ref)	0.99(0.89,1.10)	0.92(0.82,1.02)	0.88(0.80,0.96)	0.95(0.93,0.98)	0.001
Stroke						
Incident rate per 100,000 person-years	140.21/100,000	139.79/100,000	162.83/100,000	116.42/100,000		
model 1	1.0(ref)	0.91(0.77,1.08)	0.85(0.72,1.01)	0.83(0.71,0.95)	0.94(0.90,0.98)	0.007
model 2	1.0(ref)	0.93(0.79,1.09)	0.88(0.75,1.04)	0.85(0.74,0.99)	0.95(0.91,0.99)	0.028
model 3	1.0(ref)	0.94(0.80,1.11)	0.90(0.76,1.06)	0.86(0.75,0.99)	0.95(0.91,0.99)	0.037
MI						
Incident rate per 100,000 person-years	213.71/100,000	231.39/100,000	248.82/100,000	177.23/100,000		
model 1	1.0(ref)	0.96(0.84,1.10)	0.86(0.75,0.99)	0.79(0.71,0.89)	0.92(0.89,0.96)	< 0.001
model 2	1.0(ref)	0.99(0.87,1.14)	0.91(0.73,1.04)	0.84(0.75,0.95)	0.94(0.91,0.97)	0.001
model 3	1.0(ref)	1.01(0.89,1.15)	0.92(0.81,1.06)	0.85(0.76,0.96)	0.94(0.91,0.98)	0.001
CVD mortality						
Incident rate per 100,000 person-years	47.14/100,000	45.80/100,000	45.38/100,000	34.40/100,000		
model 1	1.0(ref)	0.90(0.68,1.20)	0.74(0.55,0.99)	0.68(0.53,0.88)	0.88(0.81,0.95)	0.001
model 2	1.0(ref)	0.93(0.70,1.24)	0.79(0.58,1.06)	0.73(0.56,0.94)	0.90(0.83,0.97)	0.007

CVD = cardiovascular disease; MI = myocardial infarction

Model 1: adjusted for age ,sex and ethnic(white, mixed, asian or asian british, black or black british, other); model 2: model 1 + physical activity(< 250min/week, 250−550 min/week, > 550min/week), smoking status(never, former, current), employment(no, yes), coffee(no, yes), high density lipoprotein cholesterol (continuous) ,low density lipoprotein cholesterol (continuous) and total cholesterol (continuous); model 3: model 2 + adiposity (body mass index and waist circumference)

Hazard Ratios (HRs) at each category Q2-Q4 (compared with Q1) and per 1-standard deviation (SD) of each vitamin, estimated from Cox regression models. P value for trend was calculated as the trend per group.

	Hazard ratio (95% confidence interval)					P-trend
	group 1	group 2	group 3	group 4	For each standard deviation increase	
model 3	1.0(ref)	0.95(0.71,1.25)	0.80(0.59,1.07)	0.74(0.57,0.95)	0.90(0.83,0.97)	0.009
Vitamin B6(median,mg/d)	1.21	1.62	2.33	3.03		
CVD events						
Incident rate per 100,000 person-years	326.55/100,000	320.52/100,000	337.30/100,000	382.32/100,000		
model 1	1.0(ref)	0.89(0.80,0.99)	0.88(0.79,0.98)	0.88(0.80,0.96)	0.97(0.94,0.99)	0.019
model 2	1.0(ref)	0.92(0.82,1.02)	0.92(0.82,1.02)	0.92(0.84,1.01)	0.98(0.95,1.01)	0.133
model 3	1.0(ref)	0.92(0.82,1.02)	0.92(0.82,1.02)	0.91(0.83,0.99)	0.98(0.95,1.00)	0.070
Stroke						
Incident rate per 100,000 person-years	137.68/100,000	135.16/100,000	135.59/100,000	154.83/100,000		
model 1	1.0(ref)	0.87(0.74,1.03)	0.85(0.72,1.00)	0.87(0.75,1.00)	0.96(0.92,1.01)	0.096
model 2	1.0(ref)	0.89(0.75,1.05)	0.87(0.74,1.03)	0.89(0.77,1.03)	0.97(0.93,1.02)	0.185
model 3	1.0(ref)	0.88(0.77,1.02)	0.88(0.74,1.04)	0.88(0.76,1.02)	0.97(0.92,1.01)	0.136
MI						
Incident rate per 100,000 person-years	209.90/100,000	202.35/100,000	217.82/100,000	247.53/100,000		
model 1	1.0(ref)	0.86(0.75,0.98)	0.86(0.75,0.98)	0.85(0.76,0.95)	0.96(0.92,0.99)	0.018
model 2	1.0(ref)	0.89(0.78,1.01)	0.90(0.79,1.03)	0.89(0.80,1.00)	0.97(0.94,1.01)	0.121
model 3	1.0(ref)	0.89(0.78,1.02)	0.90(0.79,1.03)	0.88(0.79,0.99)	0.97(0.93,1.00)	0.071
CVD mortality						
Incident rate per 100,000 person-years	42.85/100,000	37.94/100,000	44.97/100,000	47.60/100,000		
model 1	1.0(ref)	0.70(0.51,0.94)	0.70(0.52,0.95)	0.82(0.64,1.04)	0.96(0.89,1.04)	0.334
model 2	1.0(ref)	0.73(0.53,0.99)	0.75(0.55,1.01)	0.86(0.67,1.11)	0.98(0.90,1.06)	0.566
model 3	1.0(ref)	0.73(0.53,0.99)	0.75(0.55,1.01)	0.85(0.67,1.09)	0.97(0.90,1.06)	0.496

CVD = cardiovascular disease; MI = myocardial infarction

Model 1: adjusted for age ,sex and ethnic(white, mixed, asian or asian british, black or black british, other); model 2: model 1 + physical activity(< 250min/week, 250−550 min/week, > 550min/week), smoking status(never, former, current), employment(no, yes), coffee(no, yes), high density lipoprotein cholesterol (continuous) ,low density lipoprotein cholesterol (continuous) and total cholesterol (continuous); model 3: model 2 + adiposity (body mass index and waist circumference)

Hazard Ratios (HRs) at each category Q2-Q4 (compared with Q1) and per 1-standard deviation (SD) of each vitamin, estimated from Cox regression models. P value for trend was calculated as the trend per group.

	Hazard ratio (95% confidence interval)					*P-*trend
	group 1	group 2	group 3	group 4	For each standard deviation increase	
Vitamin B12(median,ug/d)	1.83	3.03	5.89	11.78		
CVD events						
Incident rate per 100,000 person-years	337.42/100,000	326.03/100,000	339.68/100,000	357.77/100,000		
model 1	1.0(ref)	0.92(0.83,1.03)	0.91(0.82,1.01)	0.90(0.82,0.99)	0.97(0.94,1.00)	0.051
model 2	1.0(ref)	0.93(0.83,1.03)	0.92(0.82,1.02)	0.92(0.84,1.01)	0.98(0.95,1.01)	0.134
model 3	1.0(ref)	0.93(0.83,1.03)	0.91(0.82,1.01)	0.91(0.83,1.00)	0.98(0.95,1.00)	0.089
Stroke						
Incident rate per 100,000 person-years	138.94/100,000	142.19/100,000	134.08/100,000	146.70/100,000		
model 1	1.0(ref)	0.98(0.83,1.16)	0.92(0.78,1.09)	0.90(0.74,1.04)	0.96(0.92,1.01)	0.091
model 2	1.0(ref)	0.99(0.84,1.16)	0.93(0.79,1.10)	0.91(0.79,1.05)	0.97(0.92,1.01)	0.150
model 3	1.0(ref)	0.98(0.83,1.16)	0.93(0.78,1.09)	0.90(0.78,1.05)	0.97(0.92,1.01)	0.119
MI						
Incident rate per 100,000 person-years	220.32/100,000	201.87/100,000	222.24/100,000	228.03/100,000		
model 1	1.0(ref)	0.89(0.77,1.01)	0.87(0.77,0.99)	0.88(0.79,0.99)	0.97(0.93,1.00)	0.079
model 2	1.0(ref)	0.88(0.77,1.01)	0.88(0.77,1.01)	0.90(0.80,1.01)	0.98(0.94,1.01)	0.176
model 3	1.0(ref)	0.88(0.77,1.01)	0.87(0.77,0.99)	0.89(0.80,1.00)	0.97(0.94,1.01)	0.124
CVD mortality						
Incident rate per 100,000 person-years	41.28/100,000	39.65/100,000	43.92/100,000	48.90/100,000		
model 1	1.0(ref)	0.88(0.65,1.19)	0.92(0.68,1.24)	0.86(0.66,1.11)	0.96(0.88,1.04)	0.313
model 2	1.0(ref)	0.89(0.66,1.21)	0.94(0.70,1.26)	0.88(0.68,1.14)	0.97(0.89,1.05)	0.428
model 3	1.0(ref)	0.89(0.66,1.20)	0.92(0.69,1.24)	0.87(0.67,1.13)	0.96(0.89,1.05)	0.380

CVD = cardiovascular disease; MI = myocardial infarction

Model 1: adjusted for age ,sex and ethnic(white, mixed, asian or asian british, black or black british, other); model 2: model 1 + physical activity(< 250min/week, 250−550 min/week, > 550min/week), smoking status(never, former, current), employment(no, yes), coffee(no, yes), high density lipoprotein cholesterol (continuous) ,low density lipoprotein cholesterol (continuous) and total cholesterol (continuous); model 3: model 2 + adiposity (body mass index and waist circumference)

Hazard Ratios (HRs) at each category Q2-Q4 (compared with Q1) and per 1-standard deviation (SD) of each vitamin, estimated from Cox regression models. P value for trend was calculated as the trend per group.

For stroke, participants with higher folate intake had a significantly lower risk of stroke. HRs comparing groups 2, 3, and 4 of folate with group 1 were 0.99 (0.89 to 1.10), 0.92(0.82 to 1.02), and 0.88 (0.80 to 0.96); HR for each SD of dietary folate intake was 0.95(0.91 to 0.99) (model 3). For MI, the HR for each SD of dietary folate intake was 0.94 (0.91, 0.98) (model 3). For CVD mortality, the inverse association with dietary folate intake was still significant in models 1, 2 and 3. The HR for each SD was 0.90(0.83,0.97) (model 3). However, in the multivariate model, intake of vitamin b6 and vitamin B12 was not significantly associated with the risk of CVD events, stroke, myocardial infarction, or CVD mortality (Table 2).

We used restricted cubic splines to model the relationship between dietary folate intake and CVD risk. The results showed that dietary folate intake was not associated with CVD events at low concentrations, while at moderate to high concentrations (> 300 μg/d), the relevant amounts were associated with CVD events (Fig. 2). The associations of dietary intake of folate with stroke and myocardial infarction are presented in supplemental figures S2-4.

Sensitivity analyses

After excluding participants with CVD events within two years of follow-up, the results were unchanged (supplemental table S5). Additionally, after excluding the participants who had baseline diabetes and hypertension, the results remained unchanged. In addition, after further adjusting for the waist-to-hip ratio, intake of vitamin D, and vitamin supplements, the results remained unchanged. Furthermore, we calculated an E value (E-values = 1.29) to evaluate the impact on unmeasured confounding.

Discussion

In this large prospective study, we found inverse associations between folate intake and the risk of incidence of CVD events (stroke, MI) and CVD mortality. Each increase in folate intake was associated with a 5% lower risk of total CVD events, a 5% lower risk of stroke, a 6% lower risk of MI and a 10% lower risk of CVD mortality. In addition, each tablespoon/day higher intake of cooked vegetables and intake of raw vegetables was associated with 0.04 and 0.02 higher dietary folate intake by one SD, respectively.

Comparison with other studies

Our results are consistent with a number of prospective cohort studies of Americans and Europeans[17–18]. In a Nurses' Health Study including 80082 women with no history of CVD events, cancer, hypercholesterolemia, and intake of folate were associated with a lowered risk for coronary heart disease[10]. In a meta-analysis involving 13 randomized controlled trials, using folic acid alone or a combination of folic acid and a small amount of cyanocobalamin (≤ 0.05 mg/day) reduced the risk of future stroke by 25% in countries without folate food fortification [19]. Studies have shown that cereal products fortified with folate in the United States can reduce neural tube defects (NTDs)[20]. Taken together, our study supports the fortification of food with folic acid. In addition, our research also analysed the relationship between vitamin B intake and dietary factors. Dietary intake of folate was related to consumption of fruit, vegetables, cereal and oily fish in our study. Our findings provide evidence to increase fruit, vegetable, cereal and oily fish intake for the prevention of CVD events.

At present, the relationship between dietary vitamin B6 and vitamin B12 intake and CVD events is inconclusive. A cohort study showed that high dietary intake of vitamin B6 but not vitamin B12 was associated with a lower risk of stroke and coronary heart disease among Japanese patients[21]. Our findings indicate that there is no significant relationship between the intake of vitamin B6 and vitamin B12 and the incidence of stroke or MI. This may be due to the differences in study design, ethnicities, follow-up time, etc. among different studies. Therefore, further prospective studies with larger sample sizes in different populations are needed to further estimate this association.

Biological plausibility

The association we observed may involve folic acid intake reducing homocysteine concentrations in the blood. Some studies have shown that homocysteine is considered an independent vascular risk factor for sulfur-containing amino acids[22–23]. Deficiency of folic acid, vitamin B6 and vitamin B12 will lead to the occurrence of hyperhomocysteinemia [24]. Folate intake is inversely correlated with blood homocysteine, high levels of which may cause vascular damage. The metabolic process of

abnormally rising homocysteine concentrations will produce a large amount of oxygen free radicals and other substances, which will damage the morphology of endothelial cells[24]. Accompanied by dysfunction, it will eventually lead to abnormal function of the fibrinolytic system and the occurrence of coagulation[25]. In addition, it will also produce a large amount of fibrinogen, which greatly enhances platelet activity and aggregation, produces a large number of blood clots, and causes hypertension, cerebral infarction, etc. [26–27]. Homocysteine requires folic acid and vitamins as specific enzymes and cofactors in the normal metabolic conversion process. Deficiency of folic acid and vitamins leads to abnormal increases in homocysteine levels in the body, which may cause vascular damage[28–29].

Strengths and Limitations

This study has several strengths. First, our study is based on a large, prospective cohort of more than 500,000 participants, which provided a large number of outcome events and the adequacy of information on lifestyle habits, diet, and other covariates, which enabled us to conduct adequate analyses and dose–response analysis. Second, we could conduct sensitivity analyses that could help to minimize confounding factors. Third, we performed several sensitivity analyses to confirm the robustness of our study. In addition, through a Cox proportional hazard model with folate dietary intake modeled as a continuous exposure variable, we tested the threshold of potential benefits of vitamin B dietary intake to provide more clinically relevant information. Finally, we calculated the E-value to quantify the potential impact of unmeasured confounding factors.

Of course, there are some potential limitations in our study. First, we cannot exclude the influence of the remaining other constituents of vitamin supplements. However, we considered that the participants taking vitamin supplements may have a potential impact on the results, but after excluding those taking vitamin supplements, our sensitivity analysis correlation results did not change. Second, participants might change their diets after they develop some intermediate diseases, which may affect our results. To reduce the bias from this source, we excluded participants with a history of cancer and CVD events at baseline. The third limitation is selection bias. We observed that the participants included in the study had lower BMI and age and higher physical activity and vitamin B intake (supplemental table S6), although these differences were not significant. Therefore, we cannot rule out the possibility that the observed associations are influenced by healthy lifestyle factors in participants with high vitamin B intake, while we carefully adjusted for potential confounding factors in our analysis.

Conclusions

In summary, higher intakes of folate were inversely associated with a lower risk of total CVD events, stroke, MI and CVD mortality. Our research suggests that higher cooked vegetable intake, fresh fruit intake, raw vegetable intake, cereal intake and oily fish intake may help reduce the risk of CVD events. The public health significance of this result is that the consumption of even a moderate increase in folic acid-rich foods may play a role in the prevention of CVD[30]. Although we also demonstrated associations with high dietary folate intake and CVD, the mechanism of folate in the prevention of CVD, as well as the optimal dose and combination, need to be further studied.

Declarations

Consent for publication

Not applicable.

Conflict of interest statement

There is no conflict of interest in this article. The work has not been published and is not being considered for publication elsewhere.

Ethics approval

UK Biobank has received ethics approval from the National Health Service National Research Ethics Service

Data availability statement

Data may be obtained from a third party and are not publicly available. We used UK Biobank data to analyse and report the findings.Data access policy can be obtained from https://www. ukbiobank. ac. uk/.
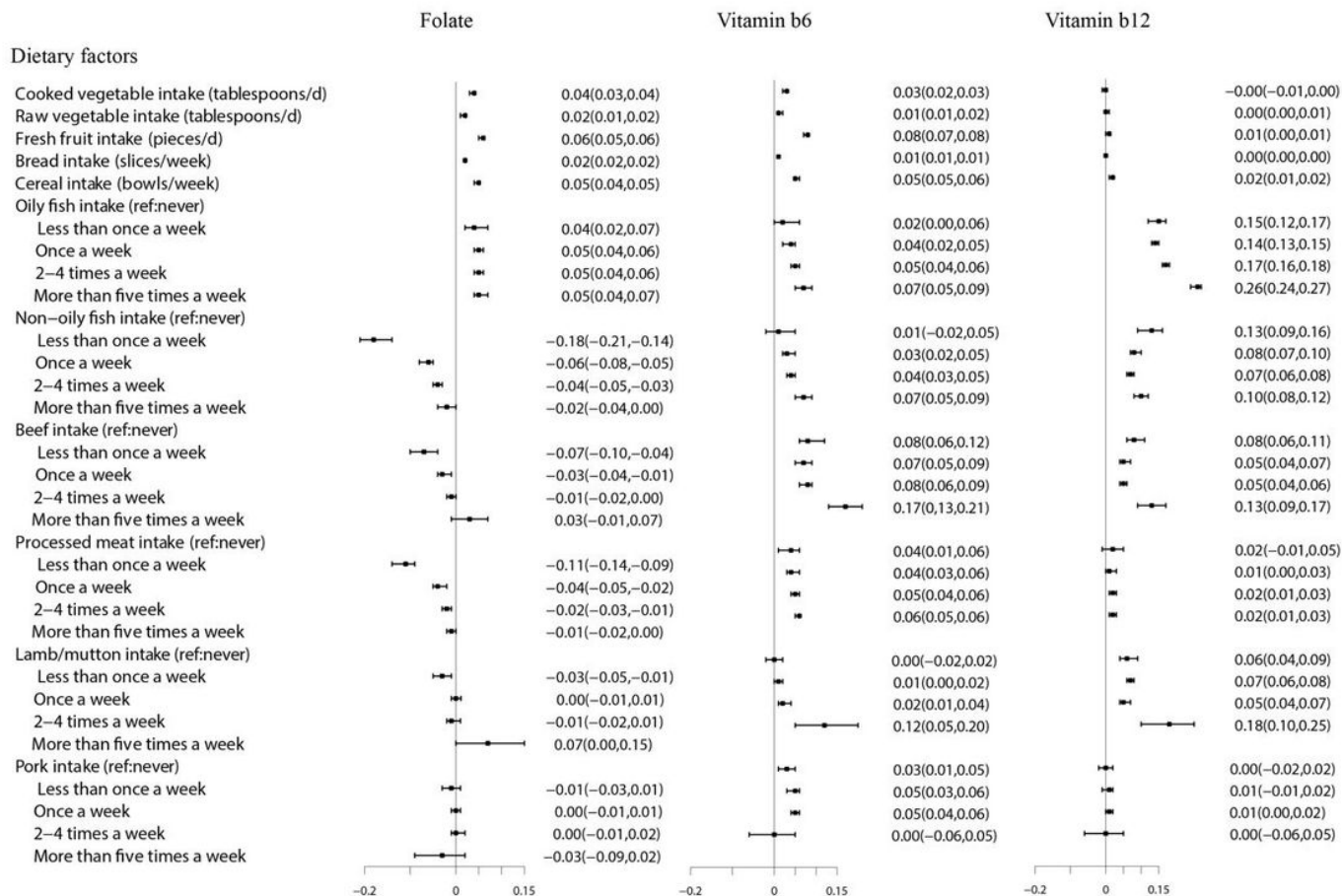
Authors' contributions

Bo-Ya Zhang and performed the data analyses and wrote the manuscript;Hao-Yu Dong contributed significantly to manuscript preparation;Xu Ying and Xu duo contributed significantly to analysis HongPeng Sun and LiYuan Han helped perform the analysis with constructive discussions. All authors reviewed the manuscript.

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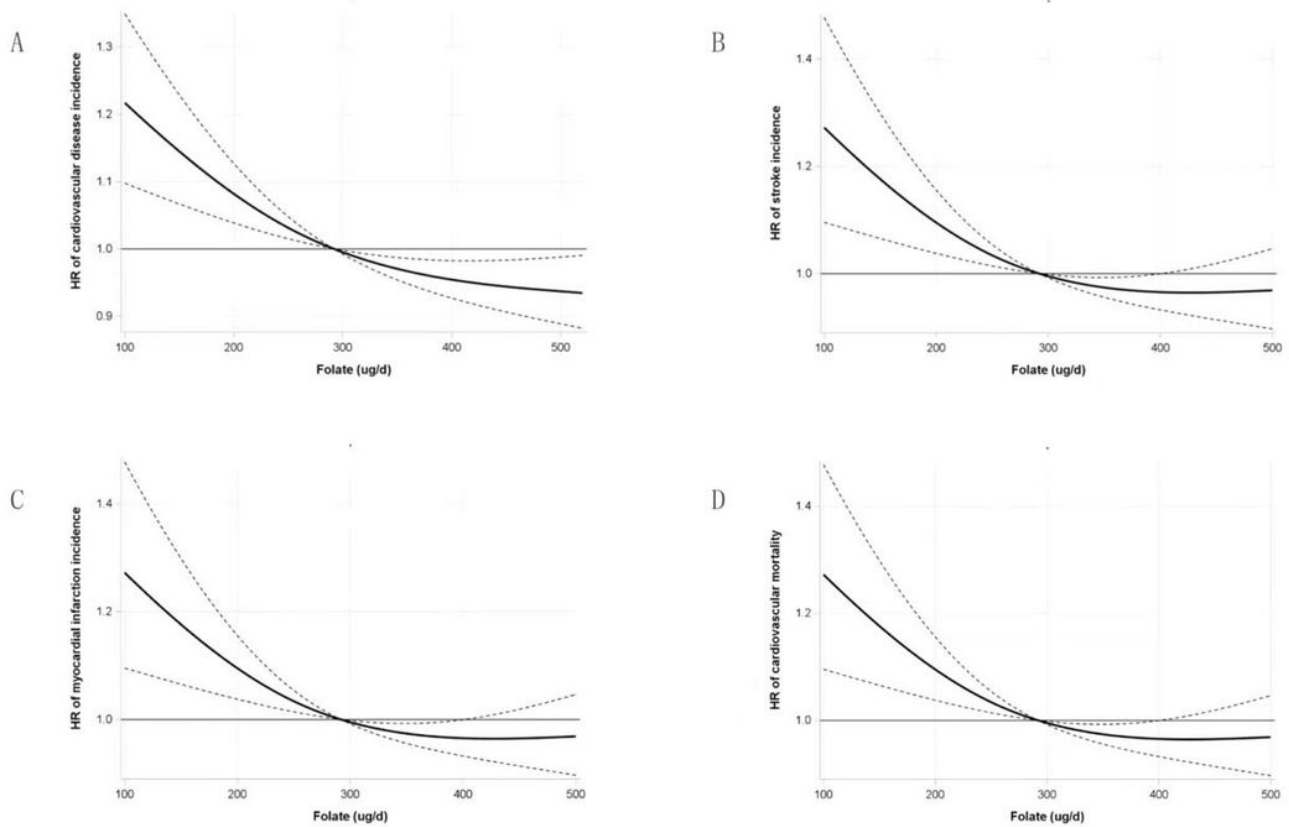
Figures

	Folate	Vitamin b6	Vitamin b12
Dietary factors			
Cooked vegetable intake (tablespoons/d)	0.04(0.03,0.04)	0.03(0.02,0.03)	−0.00(−0.01,0.00)
Raw vegetable intake (tablespoons/d)	0.02(0.01,0.02)	0.01(0.01,0.02)	0.00(0.00,0.01)
Fresh fruit intake (pieces/d)	0.06(0.05,0.06)	0.08(0.07,0.08)	0.01(0.00,0.01)
Bread intake (slices/week)	0.02(0.02,0.02)	0.01(0.01,0.01)	0.00(0.00,0.00)
Cereal intake (bowls/week)	0.05(0.04,0.05)	0.05(0.05,0.06)	0.02(0.01,0.02)
Oily fish intake (ref:never)			
Less than once a week	0.04(0.02,0.07)	0.02(0.00,0.06)	0.15(0.12,0.17)
Once a week	0.05(0.04,0.06)	0.04(0.02,0.05)	0.14(0.13,0.15)
2–4 times a week	0.05(0.04,0.06)	0.05(0.04,0.06)	0.17(0.16,0.18)
More than five times a week	0.05(0.04,0.07)	0.07(0.05,0.09)	0.26(0.24,0.27)
Non−oily fish intake (ref:never)			
Less than once a week	−0.18(−0.21,−0.14)	0.01(−0.02,0.05)	0.13(0.09,0.16)
Once a week	−0.06(−0.08,−0.05)	0.03(0.02,0.05)	0.08(0.07,0.10)
2–4 times a week	−0.04(−0.05,−0.03)	0.04(0.03,0.05)	0.07(0.06,0.08)
More than five times a week	−0.02(−0.04,0.00)	0.07(0.05,0.09)	0.10(0.08,0.12)
Beef intake (ref:never)			
Less than once a week	−0.07(−0.10,−0.04)	0.08(0.06,0.12)	0.08(0.06,0.11)
Once a week	−0.03(−0.04,−0.01)	0.07(0.05,0.09)	0.05(0.04,0.07)
2–4 times a week	−0.01(−0.02,0.00)	0.08(0.06,0.09)	0.05(0.04,0.06)
More than five times a week	0.03(−0.01,0.07)	0.17(0,13,0.21)	0.13(0.09,0.17)
Processed meat intake (ref:never)			
Less than once a week	−0.11(−0.14,−0.09)	0.04(0.01,0.06)	0.02(−0.01,0.05)
Once a week	−0.04(−0.05,−0.02)	0.04(0.03,0.06)	0.01(0.00,0.03)
2–4 times a week	−0.02(−0.03,−0.01)	0.05(0.04,0.06)	0.02(0.01,0.03)
More than five times a week	−0.01(−0.02,0.00)	0.06(0.05,0.06)	0.02(0.01,0.03)
Lamb/mutton intake (ref:never)			
Less than once a week	−0.03(−0.05,−0.01)	0.00(−0.02,0.02)	0.06(0.04,0.09)
Once a week	0.00(−0.01,0.01)	0.01(0.00,0.02)	0.07(0.06,0.08)
2–4 times a week	−0.01(−0.02,0.01)	0.02(0.01,0.04)	0.05(0.04,0.07)
More than five times a week	0.07(0.00,0.15)	0.12(0.05,0.20)	0.18(0.10,0.25)
Pork intake (ref:never)			
Less than once a week	−0.01(−0.03,0.01)	0.03(0.01,0.05)	0.00(−0.02,0.02)
Once a week	0.00(−0.01,0.01)	0.05(0.03,0.06)	0.01(−0.01,0.02)
2–4 times a week	0.00(−0.01,0.02)	0.05(0.04,0.06)	0.01(0.00,0.02)
More than five times a week	−0.03(−0.09,0.02)	0.00(−0.06,0.05)	0.00(−0.06,0.05)

Figure 1

Association of dietary factors with folate, vitamin B6 and vitamin B12 intakes in the UK Biobank study.

The beta (95%CI) of the x-axis values represented differences in folate, vitamin B6 and vitamin B12 (in standard deviation) per 1-standardised unit in each dietary factors. Linear regression was used to estimate the beta coefficients and 95% confidence intervals.

Figure 2

Associations of dietary intakes of folate with incident CVD events in the UK Biobank study.

The solid line and the dotted area represented estimates of hazard ratios and the 95% confidence intervals,respectively. Covariates included age, sex and ethnic(white, mixed, asian or asian british, black or black british, other), physical activity(<250min/week, 250-550 min/week, >550min/week), smoking status(never, former, current), employment(no, yes), coffee(no, yes), high density lipoprotein cholesterol (continuous) ,low density lipoprotein cholesterol (continuous) and total cholesterol(continuous),adiposity (body mass index and waist circumference)

Supplementary Files

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