



Intakes of Folate, Vitamin B₆, and Vitamin B₁₂ in Relation to Diabetes Incidence Among American Young Adults: A 30-Year Follow-up Study

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OBJECTIVE

To prospectively examine intakes of folate, vitamin B₆, and vitamin B₁₂ in relation to diabetes incidence in a large U.S. cohort.

RESEARCH DESIGN AND METHODS

A total of 4,704 American adults aged 18–30 years and without diabetes were enrolled in 1985–1986 and monitored until 2015–2016 in the Coronary Artery Risk Development in Young Adults (CARDIA) study. Dietary assessment was conducted by a validated dietary history questionnaire at baseline, in 1992–1993, and in 2005–2006. The cumulative average intakes of folate, vitamin B₆, and vitamin B₁₂ were used in the analyses. Incident diabetes was ascertained by plasma glucose levels, oral glucose tolerance tests, hemoglobin A_{1c} concentrations, and/or antidiabetic medications.

RESULTS

During 30 years (mean 20.5 ± 8.9) of follow-up, 655 incident cases of diabetes occurred. Intake of folate, but not vitamin B₆ or vitamin B₁₂, was inversely associated with diabetes incidence after adjustment for potential confounders. Compared with the lowest quintile of total folate intake, the multivariable-adjusted hazard ratios (95% CI) in quintiles 2–5 were 0.85 (0.67–1.08), 0.78 (0.60–1.02), 0.82 (0.62–1.09), and 0.70 (0.51–0.97; $P_{\text{trend}} = 0.02$). Higher folate intake was also associated with lower plasma homocysteine ($P_{\text{trend}} < 0.01$) and insulin ($P_{\text{trend}} < 0.01$). Among supplement users, folate intake was inversely associated with serum C-reactive protein levels ($P_{\text{trend}} < 0.01$).

CONCLUSIONS

Intake of folate in young adulthood was inversely associated with diabetes incidence in midlife among Americans. The observed association may be partially explained by mechanisms related to homocysteine level, insulin sensitivity, and systemic inflammation.

The high prevalence of type 2 diabetes mellitus (T2DM) is a severe public health concern worldwide (1). Hyperhomocysteinemia has emerged as a risk factor for T2DM due to its association with insulin resistance (IR) (2). Hyperhomocysteinemia may be caused by genetic defects (3), renal dysfunction (3), or dietary factors, including low intakes of folate, vitamin B₆, and B₁₂ (4). These B vitamins play a pivotal role in homocysteine (Hcy) degradation by acting as a prerequisite substrate donor (folate) or

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as essential coenzymes (vitamin B₆ and B₁₂) (3,5). Additionally, these B vitamins are vital components of one-carbon metabolism that contributes to DNA methylation, which is critical for the pathogenesis of diabetes (6).

Previously, several randomized clinical trials reported that folic acid (or folate) supplementation improved IR (7–9) and reduced the levels of inflammatory mediators (7,10), which may beneficially affect T2DM risk. However, prospective cohort studies that directly relate long-term consumptions of folate, vitamin B₆, or vitamin B₁₂ to incident diabetes are limited. Two studies found an inverse association between dietary intake of folate in relation to diabetes risk among Korean women aged ≥40 years, with an average follow-up of 4 years (11), and among Japanese women aged 40–79 years within the 5-year period (12). However, the diabetes cases in these two studies were self-reported and were not validated with medical records.

Therefore, we prospectively examined intakes of folate and vitamins B₆ and B₁₂ in relation to diabetes incidence, ascertained by clinical examinations and antidiabetic medication in a large U.S. cohort with 30 years of follow-up, using data from the Coronary Artery Risk Development in Young Adults (CARDIA) study. To elucidate the underlying mechanisms, we examined whether these B vitamin intakes are associated with available markers of IR and systemic inflammation.

RESEARCH DESIGN AND METHODS

Study Design

The CARDIA study is an ongoing longitudinal study that monitors participants from young adulthood to midlife. Briefly, 5,115 American adults aged 18–30 years were initially enrolled from four sites in the U.S. (Birmingham, AL; Chicago, IL; Minneapolis, MN; and Oakland, CA) from 1985 to 1986 (year 0, baseline) (13,14). Up to now, there have been eight follow-up examinations at years 2, 5, 7, 10, 15, 20, 25, and 30, with follow-up rates of 91%, 86%, 81%, 79%, 74%, 72%, 72%, and 71% correspondingly. Specific information about the study design and enrollment protocol has been published (14).

From 5,115 CARDIA participants, 1 person dropped out after recruitment. After excluding 50 participants who reported an extreme total energy intake (≤600

or ≥6,000 kcal/day for women; ≤800 or ≥8,000 kcal/day for men) (15), 7 who had no information on vitamin B intakes, 112 who had diabetes or whose diabetes status could not be determined at baseline, and 241 women who were pregnant at any examination, 4,704 individuals (92% of 5,115) remained in the analyses (each nutrient had slightly different sample size). All participants provided written informed consent. The institutional review board at each study center approved the study.

Dietary Assessment

The detailed information of dietary assessment in the CARDIA study has been described previously (16). Briefly, dietary data were collected at baseline, year 7, and year 20 examinations by a validated interviewer-administered CARDIA Diet History Questionnaire (16,17). Nutrients intakes were calculated through Nutrition Data System for Research (Nutrition Coordinating Center at the University of Minnesota). The intakes of folate, vitamin B₆, and vitamin B₁₂ in this study were defined as the sum of both dietary and supplemental intakes. Folate intake was assessed at baseline and at the year 7 and year 20 visits, while intakes of vitamin B₆ and vitamin B₁₂ were measured at follow-ups of year 7 and year 20. The A Priori Diet Quality Scores at baseline, year 7, and year 20 were calculated to measure the overall quality of diet (18). All values of nutrients in the analyses, if not specified, were presented as the cumulative averages by the time of incident diabetes, last follow-up, death, or data freeze, whichever came first, to reduce the measurement errors caused by within-person variation and to best represent the nature of long-term dietary habits.

Assessment of Covariates

Information on covariates was collected by verified self-administered questionnaires or in clinical examinations at baseline and at each follow-up. Important covariates include age, sex, race, education, height, weight, smoking status, alcohol consumption, physical activity, blood pressures, blood biochemical index, and parental history of diabetes. Years of education through examination year 30 were quantified. Smoking status was classified into three groups based on self-report: never, former, or current smokers. Alcohol consumption was presented as

milliliters per day (19). Physical activity during the prior year was estimated by the interview-based CARDIA Physical Activity History Questionnaire and was calculated in exercise units (20). Participants' weight and height were assessed when they were in light clothes with bare feet during clinical examinations. BMI was computed as weight in kilograms divided by the square of height in meters. Resting systolic and diastolic blood pressures were measured by adopting a random-zero sphygmomanometer from baseline to examination year 15 and the Omron HEM907XL sphygmomanometer at examination years 20 to 30. After a fasting blood draw, plasma levels of HDL cholesterol (HDL-c) and triglyceride (TG) were assayed using enzymatic methods (21). LDL cholesterol (LDL-c) concentrations were determined adopting the Friedewald equation (22). These characteristics were presented, if not specified, as cumulative averages from baseline to the time of incident diabetes, last follow-up, death, or data freeze, whichever came first. Parental history of diabetes was identified as the father or mother having diabetes.

Assessment of Glucose, Insulin, Hemoglobin A_{1c}, Inflammatory Markers, and Hcy

Fasting plasma glucose and insulin concentrations were measured initially by a nonspecific insulin assay (the hexokinase ultraviolet method) at baseline and by a new radioimmunoassay at examination years 7, 10, 15, 20, 25, and 30. These two measurements were recalibrated to ensure comparability of plasma glycemic parameters across examinations, which were described in details previously (23). HOMA of IR (HOMA-IR) was computed as glucose (mmol/L) × insulin (μIU/mL)/22.5 (13,23). HOMA β-cell function (HOMA-β) was calculated as [20 × insulin (μIU/mL)]/[glucose (mmol/L) – 3.5]% (8). Postprandial 2-h plasma glucose concentrations were assessed from a standard 2-h oral glucose tolerance test (OGTT) at examination years 10, 20, 25, and 30. Hemoglobin A_{1c} (HbA_{1c}) was assayed using a Tosoh G7 high-performance liquid chromatography instrument at examination years 20, 25, and 30.

Serum C-reactive protein (CRP) was determined at years 7, 15, 20, and 25 using a nephelometry-based high throughput assay (24). Interleukin-6 (IL-6) was

measured at year 20 with a high-sensitivity ELISA (25). Fibrinogen was assessed via the Clauss method at year 5 and by the BNII nephelometer and calibrated with standard normal plasma at year 20 (26). Serum Hcy concentrations were measured at years 0, 7, and 15 using a fluorescence polarization immunoassay in a subcohort of 1,496 participants (3).

Ascertainment of Diabetes

At each follow-up examination, participants with one or more of the following criteria were defined to have incident diabetes: 1) fasting plasma glucose ≥ 7.0 mmol/L (126 mg/dL); 2) nonfasting plasma glucose ≥ 11.1 mmol/L (200 mg/dL); 3) postprandial 2-h plasma glucose ≥ 11.1 mmol/L (200 mg/dL) from an OGTT; 4) HbA_{1c} $\geq 6.5\%$ (48 mmol/mol); or 5) reported use of antidiabetic medications, which were verified by medication names (27). The type of diabetes could not be clearly differentiated, because some individuals were young at diagnosis and treated with insulin. Therefore, we adopted the term “diabetes” rather than “T2DM,” although, presumably, the vast majority of the cases should be T2DM.

Statistical Analysis

All analyses were performed by using SAS 9.4 software (SAS Institute, Cary, NC). Two-sided *P* values ≤ 0.05 were considered statistically significant. Participants were divided into quintiles according to the distribution of their B vitamin intakes, and the differences of baseline characteristics across folate intake quintiles were compared by using ANOVA, χ^2 test, or the Kruskal-Wallis test as appropriate. Cox proportional hazards regression models were used to examine the associations between B vitamin intakes and diabetes incidence. Each participant contributed follow-up time from baseline to the examination date when incident diabetes was identified, when the participant was censored, or the end of the follow-up period at examination year 30, whichever came first. Multivariable-adjusted hazard ratios (HRs) and corresponding 95% CIs were calculated in three sequential models. Model 1 was adjusted for geodemographics (age, sex, race, and study center across U.S.) and total energy intake. Model 2 was additionally adjusted for socioeconomic (education) and major lifestyle indicators (smoking status, alcohol consumption,

physical activity, and BMI). Model 3 (final model) was further adjusted for relevant clinical characteristics (clinically measured glucose at baseline, the HDL-c-to-LDL-c ratio, and TG) and medical history (family history of diabetes and supplement use of each B vitamin). Linear trend was examined using the continuous variables of the B vitamin intake with the values >99 th percentile excluded. The assumption for using Cox model was assessed by examining the coefficient of interaction between nutrient intake quintiles and survival time.

Several sensitivity analyses were performed to test the robustness of our findings. First, the associations were examined separately among supplement users and nonusers of folic acid, vitamin B₆, or vitamin B₁₂. Second, to account for the potential influence of total energy intake, nutrient density of B vitamins was examined as the exposures in a sensitivity analysis. Third, we additionally adjusted for the overall dietary quality using the A Priori Diet Quality Score in the final model. Fourth, since the regulations on folate fortification took effect on 1 January 1998, calculating the cumulative average of folate intake using measurements before and after the fortification may bias the results, although individual dietary habits should be stable over time. Therefore, we calculated the cumulative average of folate in a sensitivity analysis by using the following method: if the survival time ends before examination year 20 (2005), the cumulative folate is the average of baseline (1985) and year 7 (1992) measurements depending on the actual survival time; if the survival time ends after year 20, the cumulative folate is the measurement at year 20. In another sensitivity analysis, we examined the association between folate intake at year 20 and after diabetes incidence by excluding those who had incident diabetes or who were censored before year 20.

In addition, we stratified the data in accordance to some preidentified factors, including age, sex, race, and fasting glucose at baseline, to examine whether they modified the associations of interest. Possible interactions were examined by testing the corresponding multiplicative interaction terms in the models using likelihood ratio detection.

To explore the underlying mechanisms of action, we examined the associations

between B vitamin intakes and blood markers of Hcy, IR (fasting glucose level, insulin level, HOMA-IR score, and HOMA- β function index), and systemic inflammation (CRP, fibrinogen, and IL-6). Because fasting Hcy, glucose, insulin, CRP, and fibrinogen were measured multiple times during follow-up, generalized linear regression models with exchangeable correlation structure were adopted. IL-6 was assayed only once, at year 20; thus, the ordinary linear regression model was used. All models were adjusted for the covariates in the final model. The distributions of these markers at each examination year were right-skewed. However, since mean differences were reported by comparing higher quintiles of folate intake to the lowest quintile, the values of these markers without transformation were used in the models for easy interpretation.

RESULTS

The current study includes 4,704 participants, with 52% being female and 51% being Black. The mean \pm SD age at baseline was 24.9 ± 3.6 years. Because racial diversities of existing risk factors for diabetes were found in previous CARDIA studies (28), the baseline characteristics of this study population were presented separately for Black and White participants (Table 1). The median levels of folate intake across quintiles were 212.5, 336.3, 449.2, 584.5, and 877.0 $\mu\text{g/day}$ in Blacks ($n = 2,412$) and 221.8, 341.8, 451.3, 595.6, and 889.2 $\mu\text{g/day}$ in Whites ($n = 2,291$). Participants with higher folate intake were more likely to have higher total energy intake, have better overall dietary quality, be older and males, have a higher education level (in Whites), be never smokers (in Whites), consume more alcohol, exercise more, have lower BMI, have higher HDL-c-to-LDL-c ratio (in Whites), and higher TG in Blacks, but lower TG in Whites. Additionally, they were less likely to have family history of diabetes (in Blacks) and more likely to use folic acid supplements.

During 30 years (mean 20.5 ± 8.9) of follow-up, 655 incident cases of diabetes occurred. Folate intake was inversely associated with the incidence of diabetes. Compared with the lowest quintile of folate intake, the fully adjusted HR in the highest quintile was 0.70 in the final model (95% CI 0.51, 0.97; $P_{\text{trend}} = 0.02$) (model 3 in Table 2). Regarding the association between intake of vitamin

Table 1—Baseline characteristics of the study population by quintiles of folate intake: the CARDIA study, 1985–2015††
Black men and women (n = 2,412) White men and women (n = 2,291)

Characteristics	Black men and women (n = 2,412)					P value	White men and women (n = 2,291)					P value
	Q1	Q2	Q3	Q4	Q5		Q1	Q2	Q3	Q4	Q5	
Range (μg/day)	n = 620 <281.6	n = 528 281.6–393.4	n = 467 393.4–508.4	n = 427 508.4–694.0	n = 370 ≥694.0	NA	n = 320 <281.6	n = 413 281.6–393.5	n = 474 393.5–508.6	n = 514 508.6–693.5	n = 570 ≥693.5	NA
Median level (μg/day)	212.5	336.3	449.2	584.5	877.0	NA	221.8	341.8	451.3	595.6	889.2	NA
Total energy (kcal/day)	1,996.6 ± 687.8	2,687.4 ± 878.6	3,253.9 ± 1,259.1	3,512.1 ± 1,400.5	3,818.8 ± 1,470.6	<0.05	1,930.9 ± 613.9	2,354.4 ± 711.4	2,565.1 ± 841.5	2,812.6 ± 1,011.4	3,096.6 ± 1,173.7	<0.05
A Priori Diet Quality Score	54.0 ± 7.8	55.9 ± 9.0	56.2 ± 8.9	58.4 ± 9.2	60.1 ± 9.6	<0.05	59.7 ± 9.5	64.8 ± 9.4	66.5 ± 9.9	68.1 ± 9.9	71.3 ± 9.8	<0.05
Age at baseline (years)	24.4 ± 3.9	24.3 ± 3.8	23.9 ± 3.9	24.6 ± 3.8	24.6 ± 3.5	<0.05	25.0 ± 3.5	25.4 ± 3.4	25.5 ± 3.4	25.5 ± 3.3	25.7 ± 3.3	<0.05
Female (%)	70.3	57.0	45.0	46.1	45.1	<0.05	66.3	50.1	46.6	44.6	48.4	<0.05
Education (years)	13.7 ± 2.3	13.8 ± 2.3	14.0 ± 4.6	13.9 ± 2.6	14.1 ± 2.4	0.21	14.6 ± 2.8	15.4 ± 2.7	15.8 ± 2.6	15.8 ± 2.7	15.9 ± 2.7	<0.05
Smokers (%)						0.44						<0.05
Never	58.6	56.1	61.0	55.0	57.5		44.8	57.5	60.3	59.0	61.3	
Former	15.1	15.7	12.2	13.2	15.3		24.1	23.0	26.7	27.2	26.8	
Current	26.3	28.2	26.9	31.8	27.1		31.0	19.6	13.0	13.9	11.9	
Alcohol consumption (mL/day)	2.0 (0, 8.3)	4.4 (0.3, 15.1)	4.8 (0.3, 16.2)	6.2 (0.8, 17.9)	4.2 (0.5, 16.7)	<0.05	4.1 (0.7, 10.9)	8.2 (2.4, 16.0)	6.7 (1.3, 16.7)	8.5 (2.5, 20.2)	8.5 (2.4, 19.1)	<0.05
Physical activity (EU)	211.0 (125.5,324.2)	238.9 (152.6,425.2)	335.4 (193.5,527.3)	350.0 (215.6,527.6)	392.9 (263.7,606.0)	<0.05	276.8 (185.8,407.7)	315.8 (211.3,442.4)	348.2 (238.1,514.0)	383.3 (253.0,531.8)	436.3 (304.0,597.7)	<0.05
BMI (kg/m ²)	28.9 ± 7.0	28.1 ± 6.7	27.7 ± 5.9	27.9 ± 6.3	27.2 ± 5.7	<0.05	26.2 ± 5.4	26.1 ± 4.9	26.0 ± 4.9	25.8 ± 4.5	25.1 ± 4.5	<0.05
Glucose at baseline (mmol/L)	4.49 ± 0.46	4.50 ± 0.50	4.49 ± 0.46	4.53 ± 0.46	4.50 ± 0.46	0.62	4.58 ± 0.47	4.58 ± 0.46	4.57 ± 0.46	4.63 ± 0.46	4.59 ± 0.41	0.32
SBP (pascal)	0.85 ± 0.08	0.85 ± 0.07	0.85 ± 0.07	0.86 ± 0.08	0.85 ± 0.07	0.17	0.82 ± 0.08	0.82 ± 0.07	0.82 ± 0.07	0.82 ± 0.07	0.82 ± 0.07	0.92
DBP (pascal)	0.54 ± 0.07	0.54 ± 0.06	0.54 ± 0.06	0.54 ± 0.06	0.54 ± 0.06	0.78	0.52 ± 0.06	0.52 ± 0.06	0.52 ± 0.06	0.52 ± 0.05	0.52 ± 0.05	0.24
HDL-c-to-LDL-c ratio	0.55 ± 0.24	0.56 ± 0.29	0.55 ± 0.23	0.56 ± 0.25	0.58 ± 0.28	0.23	0.51 ± 0.31	0.49 ± 0.21	0.51 ± 0.25	0.51 ± 0.21	0.56 ± 0.26	<0.05
TG (mmol/L)	0.88 ± 0.46	0.90 ± 0.43	0.90 ± 0.46	0.95 ± 0.47	0.92 ± 0.47	<0.05	1.10 ± 0.68	1.16 ± 0.73	1.12 ± 0.68	1.15 ± 0.71	1.07 ± 0.68	<0.05
Family history of diabetes (yes, %)	20.7	16.7	17.8	15.9	11.4	<0.05	12.8	12.8	9.9	9.1	9.8	0.24
Folic acid supplement use (yes, %)	28.2	47.2	52.9	62.8	74.1	<0.05	31.3	49.6	69.0	75.3	87.0	<0.05

DBP, diastolic blood pressure; EU, exercise units; NA, not applicable; Q, quintile; SBP, systolic blood pressure. *Results were presented as means ± SDs, medians (interquartile ranges), or proportions. All mean or median values, if not specified, were based on the values of cumulative averages by the time of incident diabetes, last follow-up, death, or data freeze, whichever came first. †P values are for any differences across quintiles of folate intake (ANOVA, Kruskal-Wallis test, or χ^2 test, as appropriate).

B₆ or vitamin B₁₂ and the incident diabetes, a significant inverse association was observed only for vitamin B₆ in model 1 (quintile 5 vs. 1: HR 0.64 [95% CI 0.48, 0.85]; $P_{\text{trend}} = 0.06$) (Table 2). The proportional hazards assumptions were met for all nutrients in the final model ($P = 0.07$ for folate, $P = 0.25$ for B₆, and $P = 0.17$ for B₁₂).

The results were generally consistent in all sensitivity analyses. First, the observed association between folate intake and diabetes incidence was slightly attenuated in the sensitivity analysis when examined separately among supplement users or nonusers (Supplementary Table 1). Second, the associations were not materially altered when nutrient density of B vitamins, instead of absolute intake, was examined as the exposures (Supplementary Table 2). Third, the additional adjustment for the A Priori Diet

Quality Score in the final model did not appreciably change the association between B vitamin intakes and diabetes incidence (Supplementary Table 2). Fourth, when we separately calculated the cumulative average folate intake for those who were monitored before and after folic acid fortification in 1998, the inverse association between folate and diabetes incidence remained. When examining the intake of folate at year 20 with following diabetes incidence, this inverse association was attenuated, presumably due to insufficient statistical power or number of cases (Supplementary Table 2).

The associations between B vitamin intakes and diabetes incidence were not significantly modified by age at baseline (median <26 vs. ≥26 years), sex, race (Black vs. White), or fasting glucose at baseline (<0.0555 vs. ≥0.0555 mmol/L

[100 mg/dL]). However, the inverse association between folate intake and diabetes incidence seemed to be more pronounced among older participants and Whites, probably because these individuals had a higher level of folate intake (Table 3).

To explore the possible mechanisms behind the potential benefits of folate on diabetes, we examined the associations between folate intake and serum Hcy, biomarkers of IR (fasting plasma glucose level, insulin level, HOMA-IR score, and HOMA-β function index), and systemic inflammation (CRP, fibrinogen, and IL-6). Folate intake was significantly inversely associated with the levels of Hcy (quintile 5 vs. 1: mean difference -1.90 [95% CI $-2.32, -1.48$]; $P_{\text{trend}} < 0.01$) (Table 4) and insulin (quintile 5 vs. 1: mean difference -8.47 [95% CI $-12.90, -4.05$]; $P_{\text{trend}} < 0.01$) (Table 4). Folate intake was

Table 2—Multivariable-adjusted HRs (95% CIs) of incident diabetes by quintiles of B vitamin intake levels: the CARDIA study, 1985–2015*†‡§

	Quintiles of nutrient intake					P_{trend}
	Q1	Q2	Q3	Q4	Q5	
Folate ($n = 4,703$)						
Range ($\mu\text{g/day}$)	<281.6	281.6–393.4	393.4–508.4	508.4–693.5	≥693.5	NA
Median ($\mu\text{g/day}$)	216.4	338.9	450.0	591.2	885.7	NA
No. of cases/participants	147/940	150/941	125/941	132/941	101/940	NA
Model 1	1	0.80	0.66	0.66	0.48	<0.01
	[Ref.]	(0.63, 1.01)	(0.52, 0.86)	(0.51, 0.86)	(0.36, 0.64)	
Model 2	1	0.93	0.81	0.84	0.68	<0.01
	[Ref.]	(0.74, 1.18)	(0.63, 1.05)	(0.64, 1.09)	(0.50, 0.92)	
Model 3 (final model)	1	0.85	0.78	0.82	0.70	0.02
	[Ref.]	(0.67, 1.08)	(0.60, 1.02)	(0.62, 1.09)	(0.51, 0.97)	
B₆ ($n = 3,894$)						
Range (mg/day)	<1.7	1.7–2.4	2.4–3.2	3.2–4.6	≥4.6	NA
Median (mg/day)	1.4	2.0	2.7	3.8	6.8	NA
No. of cases/participants	143/779	112/777	123/779	111/779	98/780	NA
Model 1	1	0.74	0.84	0.75	0.64	0.06
	[Ref.]	(0.58, 0.96)	(0.65, 1.09)	(0.57, 0.99)	(0.48, 0.85)	
Model 2	1	0.86	0.96	0.94	0.82	0.69
	[Ref.]	(0.66, 1.10)	(0.74, 1.25)	(0.71, 1.24)	(0.61, 1.11)	
Model 3 (final model)	1	0.89	1.07	1.06	1.04	0.40
	[Ref.]	(0.68, 1.15)	(0.80, 1.42)	(0.76, 1.47)	(0.71, 1.51)	
B₁₂ ($n = 3,894$)						
Range ($\mu\text{g/day}$)	<4.1	4.1–6.1	6.1–8.7	8.7–13.8	≥13.8	NA
Median ($\mu\text{g/day}$)	3.0	5.1	7.3	10.6	21.0	NA
No. of cases/participants	120/778	131/779	121/780	104/778	111/779	NA
Model 1	1	1.05	0.96	0.86	0.88	0.49
	[Ref.]	(0.82, 1.35)	(0.74, 1.25)	(0.65, 1.14)	(0.66, 1.16)	
Model 2	1	1.08	1.02	0.99	1.08	0.46
	[Ref.]	(0.84, 1.39)	(0.79, 1.34)	(0.75, 1.31)	(0.81, 1.44)	
Model 3 (final model)	1	1.04	1.08	1.14	1.29	0.053
	[Ref.]	(0.80, 1.35)	(0.81, 1.44)	(0.83, 1.56)	(0.91, 1.84)	

NA, not applicable; Q, quintile; Ref., reference. *Cox proportional hazards regression models were used. Linear trend was examined by using the continuous variables that excluded the values above the 99th percentile. †Model 1 was adjusted for age, sex (female or male), race (White or Black), study center, and total energy intake (continuous). ‡Model 2 was additionally adjusted for education levels (<12, 12–15.9, ≥16 years), smoking status (never, former, or current smoker), alcohol consumption (0, 0.1–11.9, 12–23.9, ≥24 mL/day), physical activity levels (quintiles), and BMI (continuous). §Model 3 was additionally adjusted for glucose levels at baseline (continuous), HDL-c-to-LDL-c ratio (quintiles), TG (quintiles), family history of diabetes (parental, yes or no), and supplement use of B vitamin of interest (yes or no).

Table 3—Multivariable-adjusted HRs (95% CIs) between folate intake levels and incidence of diabetes stratified by prespecified factors: the CARDIA study, 1985–2015 (n = 4,703)*

	Folate intake, mean ± SD	Tertiles of folate intake levels			<i>P</i> _{trend}
		Tertile 1	Tertile 2	Tertile 3	
Range (μg/day)	NA	<354.8	354.8–563.0	≥563.0	NA
Median (μg/day)	NA	260.7	450.0	744.2	NA
Age					
<Median 26 years	515.1 ± 483.9	0 [Ref.]	0.79 (0.58, 1.08)	0.93 (0.64, 1.35)	0.18
≥Median 26 years	546.6 ± 506.4	0 [Ref.]	1.10 (0.83, 1.45)	0.73 (0.52, 1.02)	0.04
<i>P</i> for interaction	NA		0.79		
Sex					
Female	508.3 ± 590.5	0 [Ref.]	0.98 (0.74, 1.29)	0.87 (0.63, 1.22)	0.04
Male	554.1 ± 361.6	0 [Ref.]	0.92 (0.67, 1.26)	0.74 (0.51, 1.06)	0.27
<i>P</i> for interaction	NA		0.27		
Race					
Blacks	470.7 ± 315.9	0 [Ref.]	0.95 (0.74, 1.21)	0.97 (0.72, 1.30)	0.37
Whites	592.8 ± 624.8	0 [Ref.]	0.77 (0.53, 1.10)	0.54 (0.35, 0.83)	0.011
<i>P</i> for interaction	NA		0.38		
Baseline glucose					
<0.0555 mmol/L (n = 4,603)	530.8 ± 496.8	0 [Ref.]	0.91 (0.74, 1.12)	0.83 (0.64, 1.06)	0.03
≥0.0555 mmol/L (n = 101)	503.8 ± 403.2	0 [Ref.]	1.87 (0.42, 8.24)	1.52 (0.24, 9.66)	0.67
<i>P</i> for interaction	NA		0.35		

NA, not applicable; Ref., reference. *All models were constructed using Cox proportional hazards regression model with adjustment for covariates in model 3, Table 2. *P*_{trend} was examined by using the continuous folate intake variable that excluded the values >99th percentile.

also inversely associated with CRP concentrations among supplement users (quintile 5 vs. 1: mean difference -0.82 [95% CI $-1.31, -0.32$]; *P*_{trend} < 0.01) (Table 4). The inverse associations with fasting glucose, HOMA-IR, HOMA-β, fibrinogen, and IL-6 were generally consistent, although statistically nonsignificant (Supplementary Table 3).

CONCLUSIONS

In this large prospective cohort study, folate intake (including both dietary and supplemental resources) was inversely associated with the incidence of diabetes among American adults during a 30-year follow-up. Presumably, this inverse association is at least in part explained by or through the pathways of folate with insulin sensitivity or inflammation.

Our findings are supported by one earlier case-control study demonstrating that both dietary intake and serum level of folic acids were lower in Omani

patients with T2DM than those in healthy control subjects (29), and two prospective cohort studies that found dietary folate consumption was inversely associated with incident diabetes in Korean or Japanese women (11,12). However, the diabetes cases in these two cohort studies were self-reported and were not validated with medical records. In the current study, incident diabetes cases were ascertained by plasma glucose levels (both fasting and nonfasting), oral glucose tolerance tests, HbA_{1c} concentrations, and antidiabetic medications during the 30-year follow-up. In addition, we examined the associations between folate intake and blood markers of insulin resistance and systemic inflammation to explore the underlying mechanisms of action. Moreover, unlike the previous studies conducted exclusively among older adults in Asian populations with relatively short follow-up (~5 years) (11,12), the current study is unique that it monitored American young adults for 30 years.

Findings from randomized clinical trials have not demonstrated the efficacy of B vitamin supplementation on T2DM risk. For example, one clinical trial manifested that a combined daily administration of folic acid (2.5 mg), vitamin B₆ (50 mg), and vitamin B₁₂ (1 mg) exerted no effects on T2DM risk among U.S. women with a history of cardiovascular disease (CVD) or CVD risk factors over a median follow-up of 7.3 years (4). Similarly, another trial demonstrated that daily supplementation of folic acid (0.8 mg) to enalapril failed to decrease the risk of new-onset diabetes among adults with hypertension over a median duration of 4.5 years (30). However, the findings from clinical trials may not be consistent with those from observational studies, because they did not consider the intakes of nutrients from diet, which is a major source of B vitamins.

It has been hypothesized that nutrient supplementation may only benefit individuals with insufficient dietary intakes. This hypothesis may explain the null associations of vitamin B₆ and vitamin B₁₂ with diabetes incidence found in the current study. In this study population, the median intake in the lowest quintiles of vitamin B₆ (1.4 mg/day) and vitamin B₁₂ (3.0 μg/day) exceeded the Recommended Dietary Allowances (RDA) for young adults in the U.S. (RDA of vitamin B₆: 1.3 mg/day; of vitamin B₁₂: 2.4 μg/day) (31). Thus, unlike folate, the vast majority of this cohort did not experience inadequacy of vitamin B₆ and B₁₂, and the benefits of these two B vitamins on diabetes risk may not be as pronounced as folate. In addition, clinical trials may not be practically feasible for examining the long-term influence of B vitamin intakes on diabetes risk. In contrast, longitudinal cohort studies, such as the present one that monitored a cohort of 4,704 Black and White men and women for 30 years, provides a unique opportunity to observe the potential long-term benefits of B vitamins. Moreover, the published clinical trials were conducted among older adults with CVD or CVD risk factors who were prone to develop diabetes. Thus, B vitamin supplementation or elevated dietary intake of B vitamins may not benefit these populations as much as the apparently healthy individuals.

The positive associations of folate with diabetes risk found in this study may be explained by its inverse relation to

Table 4—Multivariable-adjusted MDs (95% CI) in homocysteine, insulin, and CRP according to quintiles of folate intake levels: the CARDIA study, 1985–2015*†‡§

	Quintiles of folate intake ($\mu\text{g}/\text{day}$)					P_{trend}
	Q1	Q2	Q3	Q4	Q5	
Hcy ($\mu\text{mol}/\text{L}$)						
All participants ($n = 1,496$)‡						
Range	<234.4	234.4–329.1	329.1–440.4	440.4–607.5	≥ 607.5	NA
Median	179.5	282.1	380.3	508.3	797.0	NA
MD (95% CI)	0	–0.25	–0.68	–1.21	–1.90	<0.01
[Ref.]		(–0.74, 0.24)	(–1.11, –0.24)	(–1.63, –0.80)	(–2.32, –1.48)	
Supplement nonusers ($n = 438$)						
Range	<190.8	190.8–265.0	265.0–335.9	335.9–440.5	≥ 440.5	NA
Median	146.	227.7	295.7	379.6	527.4	NA
MD (95% CI)	0	–1.02	–1.16	–1.37	–1.67	<0.01
[Ref.]		(–1.68, –0.35)	(–1.97, –0.36)	(–2.22, –0.53)	(–2.58, –0.75)	
Supplement users ($n = 1,058$)						
Range	<259.7	259.7–370.0	370.0–495.3	495.3–691.0	≥ 691.0	NA
Median	203.5	319.5	430.4	566.8	878.3	NA
MD (95% CI)	0	–0.32	–0.80	–1.52	–1.97	<0.01
[Ref.]		(–0.84, 0.19)	(–1.36, –0.25)	(–2.06, –0.98)	(–2.53, –1.41)	
Insulin (pmol/L)						
All participants ($n = 4,366$)‡						
Range	<263.8	263.8–370.3	370.3–488.7	488.7–668.6	≥ 668.6	NA
Median	203.1	319.4	427.9	565.3	845.5	NA
MD (95% CI)	0	–1.15	–3.53	–6.79	–8.47	<0.01
[Ref.]		(–5.29, 2.98)	(–7.46, 0.41)	(–10.71, –2.88)	(–12.90, –4.05)	
Supplement nonusers ($n = 1,583$)						
Range	<209.0	209.0–284.2	284.2–365.5	365.5–488.4	≥ 488.4	NA
Median	166.0	248.9	323.4	416.5	600.2	NA
MD (95% CI)	0	–0.45	–2.46	–4.13	–14.04	0.08
[Ref.]		(–8.64, 7.75)	(–13.34, 8.43)	(–13.88, 5.63)	(–24.14, –3.94)	
Supplement users ($n = 2,783$)						
Range	<313.1	313.1–427.3	427.3–549.3	549.3–745.7	≥ 745.7	NA
Median	235.8	368.8	484.7	633.9	938.4	NA
MD (95% CI)	0	–1.92	–4.58	–5.59	–7.67	<0.01
[Ref.]		(–6.49, 2.66)	(–9.01, –0.16)	(–10.04, –1.14)	(–12.89, –2.45)	
CRP (mg/L)						
All participants ($n = 4,277$)‡						
Range	<279.8	279.8–387.3	387.3–502.1	502.1–681.6	≥ 681.6	NA
Median	216.7	334.7	442.4	576.7	866.6	NA
MD (95% CI)	0	–0.09	0.15	0.03	–0.21	<0.01
[Ref.]		(–0.38, 0.21)	(–0.20, 0.50)	(–0.36, 0.42)	(–0.64, 0.22)	
Supplement nonusers ($n = 1,498$)						
Range	<222.8	222.8–299.3	299.3–379.9	379.9–501.2	≥ 501.2	NA
Median	179.7	262.3	337.3	433.3	608.8	NA
MD (95% CI)	0	–0.02	0.17	0.45	0.56	0.85
[Ref.]		(–0.56, 0.52)	(–0.52, 0.85)	(–0.30, 1.20)	(–0.27, 1.39)	
Supplement users ($n = 2,779$)						
Range	<328.1	328.1–442.3	442.3–562.6	562.6–758.0	≥ 758.0	NA
Median	252.7	385.7	497.4	645.6	945.9	NA
MD (95% CI)	0	–0.26	–0.13	–0.26	–0.82	<0.01
[Ref.]		(–0.62, 0.10)	(–0.55, 0.30)	(–0.80, 0.29)	(–1.31, –0.32)	

MD, mean difference; NA, not applicable; Q, quintile; Ref., reference. *All models were constructed using generalized linear regression model with identity linkage under exchangeable correlation structure assumption. P_{trend} was examined by using the continuous folate intake variable that excluded the values above the 99th percentile. †Models were adjusted for age, sex, race, study center, total energy intake, education levels, smoking status, alcohol consumption, physical activity, BMI, HDL-c-to-LDL-c ratio, TG, and family history of diabetes. ‡The model was additionally adjusted for supplement use of folic acid. §The median (interquartile range, $\mu\text{mol}/\text{L}$) of Hcy is 9.6 (7.9–11.8) at year 0, 9.4 (7.8–11.5) at year 7, and 8.5 (7.2–10.2) at year 15. The median (interquartile range, pmol/L) of insulin is 71.8 (57.4–93.3) at year 0, 78.9 (64.6–114.8) at years 7 and 10, 86.1 (64.6–122.0) at year 15, 100.5 (71.8–143.5) at year 20, 63.4 (38.3–102.8) at year 25, and 74.1 (46.6–118.4) at year 30. The median (interquartile range, mg/L) of CRP is 1.1 (0.5–3.1) at year 7, 1.4 (0.6–3.8) at year 15, 1.1 (0.5–3.1) at year 20, and 1.4 (0.6–3.5) at year 25.

blood Hcy, an elevated level of which is causally linked to the development of T2DM (2). Folate deficiency has been reported to severely hamper biosynthesis and secretion of insulin in pancreatic

β -cells (32,33) and impair glucose tolerance in spontaneously hypertensive rats (32). Prenatal high-dose folic acid administration appears to decrease hyperglycemia caused by a gestational high-fat and

high-sucrose diet in offspring rats (34). Dietary high-dose folic acid supplementation was also reported to ameliorate IR in rats with metabolic syndrome (35) and mice fed a high-fat diet (36). In human

studies, randomized clinical trials found that folic acid supplementation was associated with a significant decrease of the circulating insulin (7,9,28) and CRP levels (7,9). In addition, folic acid supplementation was found to reduce oxidative stress (12,37), ameliorate endothelial dysfunction (38), and modulate DNA methylation of genes associated with insulin signaling (36), which may influence the risk of diabetes. However, all of these possible mechanisms remain to be examined in human studies. In the current study, we examined the associations between folate intake and available markers of Hcy, IR, and systemic inflammation and found folate intake was significantly associated with lower levels of Hcy, insulin, and CRP. The observed inverse association between folate intake and diabetes incidence was attenuated to nonsignificance when further adjusting for these three markers, which suggests that the positive associations of folate with diabetes may be explained by its regulation on blood Hcy, IR, and systemic inflammation.

The major strengths of our study include a longitudinal study design with 30 years of follow-up, a large community-based cohort of young adults, a relatively well-balanced sample of sex and race, and repeatedly comprehensive data collection, which together enabled us to adjust for possible confounders and identify potential effect modifiers. Another strength is the stringent quality control in the CARDIA study, which enhanced the validity of our findings. In addition, diabetes was ascertained mainly based on fasting and postprandial glucose concentrations from OGTT and HbA_{1c} assessments in addition to antidiabetic medications, which largely reduces the possibility of misclassification. Moreover, we longitudinally determined several IR and inflammatory indicators over the follow-up, which capacitated us to examine the underlying pathophysiological mechanisms of B vitamins in relation to diabetes risk.

Several limitations of this study need to be acknowledged. First, biomarkers of B vitamin status in the body were not examined in this study. However, previous CARDIA studies showed folate intake had a reasonably high correlation with serum folate levels (39). Second, although the B vitamin intakes in this study come from both foods and supplements, high B vitamin intake may be a marker of a healthy dietary pattern,

which means that other nutrients in B vitamin-rich foods may confound the observed association. However, we adjusted for the A Priori Diet Quality Score in a sensitivity analysis, and our findings remain, which, to some extent, suggest that folate protected against diabetes independent of other nutrients and/or foods. Third, any potentially unknown or unmeasured confounders cannot be completely excluded. Nevertheless, the possibility that our findings were materially explained by residual confounding should be small, considering our extensive data analyses, consistent findings in sensitivity analyses, and the supportive biological mechanisms. Finally genetic risk factors affecting B vitamin metabolism were not examined. Hence, it would be intriguing to explore the potential diet-gene interaction on diabetes (40).

In summary, findings from this longitudinal cohort study monitored over 30 years suggest that American young adults with higher folate intake are less likely to develop diabetes later in life, presumably through mechanisms related to Hcy level, IR and systemic inflammation.

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