List of supplemental materials

Supplemental Methods

Supplemental Methods: Associations of folate intake with risk of all-cause mortality in an independent, prospective cohort: National Health and Nutrition Examination Surveys (NHANES) 1999–2018.

Population and study design

The NHANES is a series of cross-sectional surveys conducted periodically before 1994; beginning in 1999, it became a continuous program, with every 2 years representing 1 cycle. To select participants representative of the civilian, non-institutionalized US population, NHANES excluded all persons in supervised care or custody in institutional settings, all active-duty military personnel, active-duty family members living overseas, and any other U.S. citizens residing outside the 50 states and the District of Columbia. Non-institutional group quarters (such as college and university residence halls) were included in the survey. NHANES used a complex, multistage, four-stage sampling design: first, selection of the primary sampling units (i.e., mostly individual counties); second, selection of segments within the counties; third, selection of dwelling units or households within segments; and fourth, selection of individuals within a household. Oversampling of certain population subgroups was also done to increase the reliability and precision of health status indicator estimates for these particular subgroups, and therefore, sampling weights and the complex survey design must be taken into account in the data analysis. A household interview and a physical examination at a mobile examination center were completed once per individual. Detailed survey operation manuals, consent documents, and brochures of each period are available on the NHANES website (1). NHANES was approved by the National Center for Health Statistics Institutional Review Board and all participants signed an informed consent.

In this study, we analyzed data from the continuous NHANES (1999-2018)

datasets. We restricted our analysis to CKD participants (an eGFR <60 mL/min/1.73 m^2, or the presence of urine albumin: creatinine ratio [UACR] ≥30 mg/g) (n=9027). Those with missing information on death status (n=8) or missing dietary information (n=571), or those with implausible total energy intake (male with <800 kcal/d or >4200 kcal/d or female with <600 kcal/d or >3500 kcal/d) (n=349), or those receiving dialysis (n=73) were further excluded. Therefore, a total of 8026 participants were enrolled in our present analysis for dietary folate intake (Supplemental Figure 2). In addition, a subset of those CKD participants in the NHANES 2011-2018 had information on serum folate concentrations, and thus, 2792 participants were enrolled in the analysis for serum folate (Supplemental Figure 2).

Measurements of folate intake

Dietary data was collected via 24-hour dietary recalls conducted by trained interviewers. From 1999 to 2002, one recall was conducted in person at the mobile examination center; from 2003 to 2018, a second recall, administered over the phone, was added approximately 3 to 10 days after the first recall. For participants of NHANES database, dietary intake data were used to estimate the types and amounts of foods and beverages consumed during the 24-hour period before the interview (midnight to midnight), and to estimate the energy, nutrition, and other food components consumed from these foods and beverages. The US Department of Agriculture (USDA) Automated Multiple-Pass Method was used to enhance complete and accurate recall of all foods and beverages consumed during the previous day and reduce respondent burden across all NHANES cycles. In this study, dietary intake was estimated using the average of data from two 24-h dietary recall.

Measurements of serum folate forms

Specimen donors were recommended fast prior to specimen collection, but fasting is

not required. Details on fasting before blood draw were collected via questionnaire before blood draw. Serum samples were analyzed for 5-mTHF and UMFA using LC-MS/MS by the CDC laboratory. Since UMFA measurements in 2011– 2014 were biased about 25% higher due to issues with folic acid calibrator solubility, the UMFA calibration bias has been corrected mathematically in NHANES 2011–2014 prior to data release and UMFA results produced during NHANES 2015–2018 are based on a modified procedure that avoided the calibration bias. (3-4)

Covariates

Detailed information on covariates were collected through standardized questionnaires during interviews, including age, sex, race/ethnicity, education, marital status, smoking status, alcohol drinking, physical activity, and self-reported baseline history of diabetes mellitus, hypertension, and CVD.

Serum and urine samples were collected during the physical examination. Serum creatinine was measured using the kinetic rate Jaffe method, and all serum creatinine measurements were recalibrated to the standardized creatinine measurements obtained at the Cleveland Clinic Research Laboratory (Cleveland, Ohio) to appropriately estimate glomerular filtration rate (GFR) (5). The eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (6). Urine albumin and creatinine concentrations were measured in a random single-voided urine sample using a solid-phase fluorescent immunoassay and a Jaffe rate reaction, respectively. Hemoglobin A1c (HbA1c) was measured using high-pressure liquid chromatography.

BMI was calculated as weight in kilogram divided by squared height in meter. Inactive leisure time physical activity level defined as 0 min/week moderate-to-vigorous physical activity; intermediate leisure time physical activity level defined as

1 to 149 minutes per week of moderate activities, or 1 to 74 minutes per week of vigorous activities, or 1 to149 minutes per week of a combination of moderate and vigorous activities; and ideal leisure time physical activity level defined as ≥150 minutes per week of moderate activities, or ≥75 minutes per week of vigorous activities, or ≥150 minutes per week of a combination of moderate and vigorous activities. Hypertension was defined as mean systolic blood pressure ≥140 mmHg and/or mean diastolic blood pressure ≥90 mmHg, or having a self-reported history of hypertension, or undergoing antihypertensive treatment. Diabetes was defined as having a self-reported history of diabetes, undergoing glucose-lowering therapy, or HbA1c ≥ 6.5% (HbA1c is available for a full sample while plasma glucose is available in participants aged 12 years and over in the morning examination session only.).

Ascertainment of death

The primary outcome was all-cause mortality. Mortality status of the NHANES participants was ascertained by probabilistic matching with the National Death Index through December 31, 2019. All participants with sufficient identifying data were eligible for mortality status, and any survey participant record that did not meet the minimum data requirements was ineligible for record linkage.

Statistical analysis

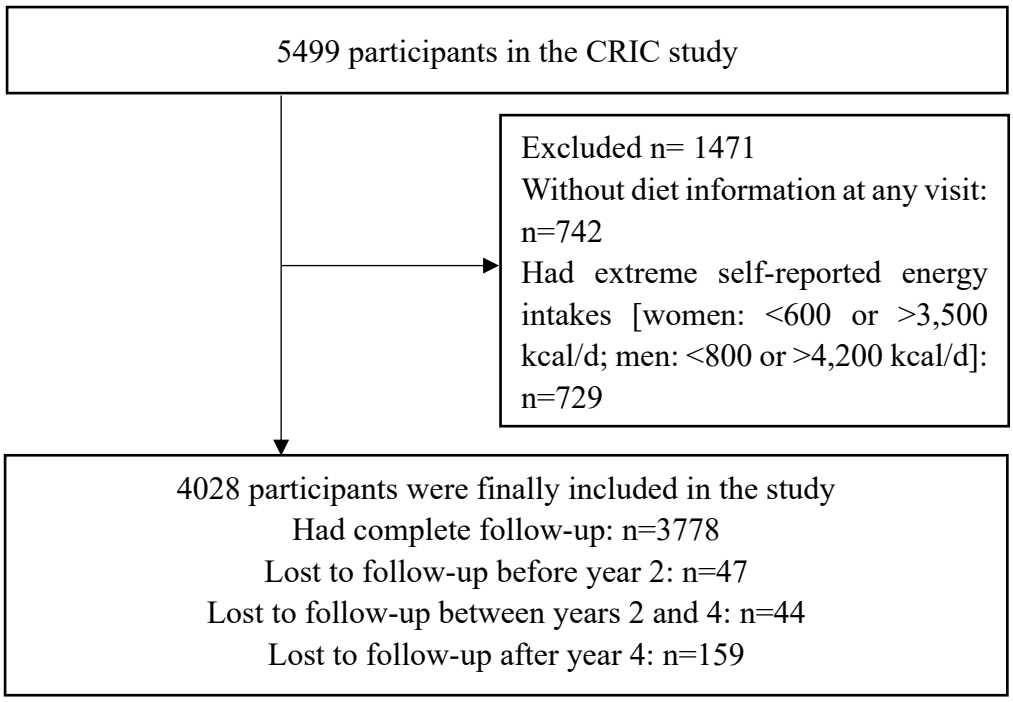
All statistical analyses accounted for complex survey design factors for NHANES, including sample weights, stratification, and clustering, following the NHANES analytic and reporting guidelines (5). Other analysis strategy was similar to that in CRIC. That is, restricted cubic spline Cox regression was used to explore the shape of the dose-response relation of folate intake and all-cause mortality, and Cox proportional hazards models were used to estimate hazard ratio (HR) and 95% confidence interval (CI), without and with adjustment for covariates including age, sex, race, education,

marital status, smoking status, alcohol status, physical activity, BMI, systolic blood pressure, diastolic blood pressure, HbA1c, eGFR, UACR, diabetes, hypertension, cardiovascular disease, folic acid supplementation use and total energy intake.

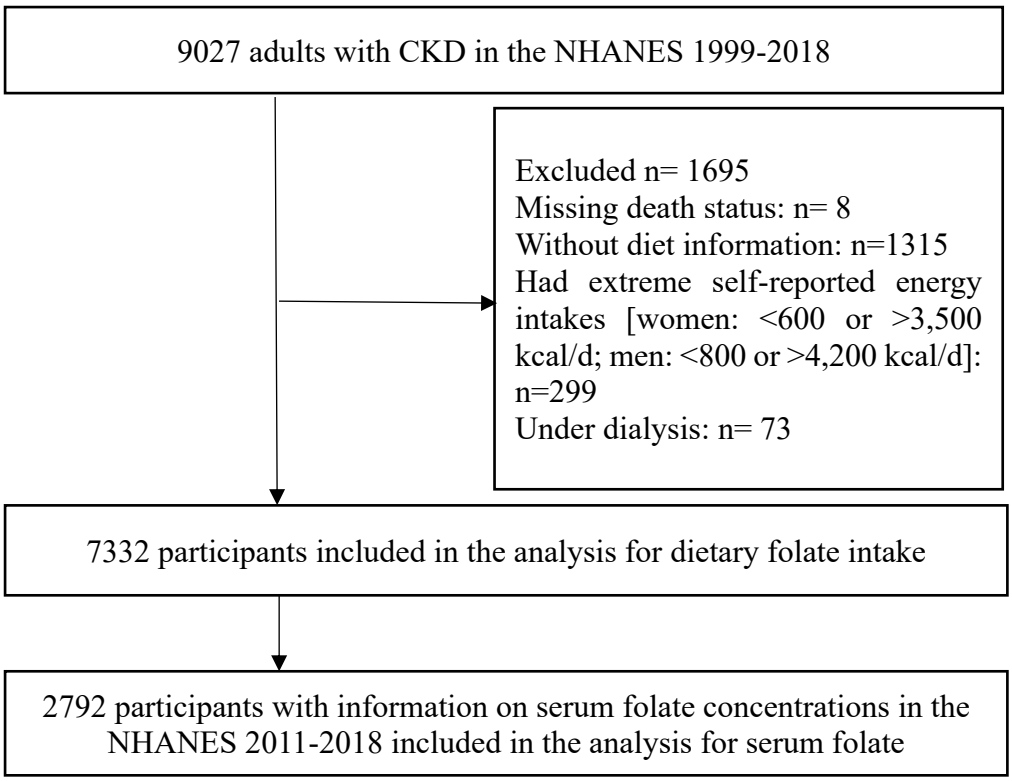
Reference

1. Centers for Disease Control and Prevention. About the National Health and Nutrition Examination Survey. https://www.cdc.gov/nchs/nhanes/ about_nhanes.htm.
2. Murphy D, McCulloch CE, Lin F, Banerjee T, Bragg-Gresham JL, Eberhardt MS, et al. Trends in Prevalence of Chronic Kidney Disease in the United States. *Ann Intern Med*. 2016;165(7):473-481.
3. 24. Fazili Z, Sternberg MR, Paladugula N, Pfeiffer CM. Two international Round Robin studies showed good comparability of 5-methyltetrahydrofolate, but poor comparability of folic acid measured in serum by different HPLC-MS/MS methods. *J Nutr* 2017;147:1815-25.
4. 25. Centers for Disease Control and Prevention. National Health and Nutrition Examination Survey. 2011-2012 Data documentation, codebook, and frequencies. Folate forms – Total & Individual Serum (FOLFMS_G). Available from: https://wwwn.cdc.gov/Nchs/Nhanes/2011-2012/FOLFMS_G.htm.
5. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF 3rd, Feldman HI, et al A new equation to estimate glomerular filtration rate. *Ann Intern Med*. 2009;150(9):604-12.
6. Centers for Disease Control and Prevention. About the National Health and Nutrition Examination Survey. https://www.cdc.gov/nchs/nhanes/ about_nhanes.

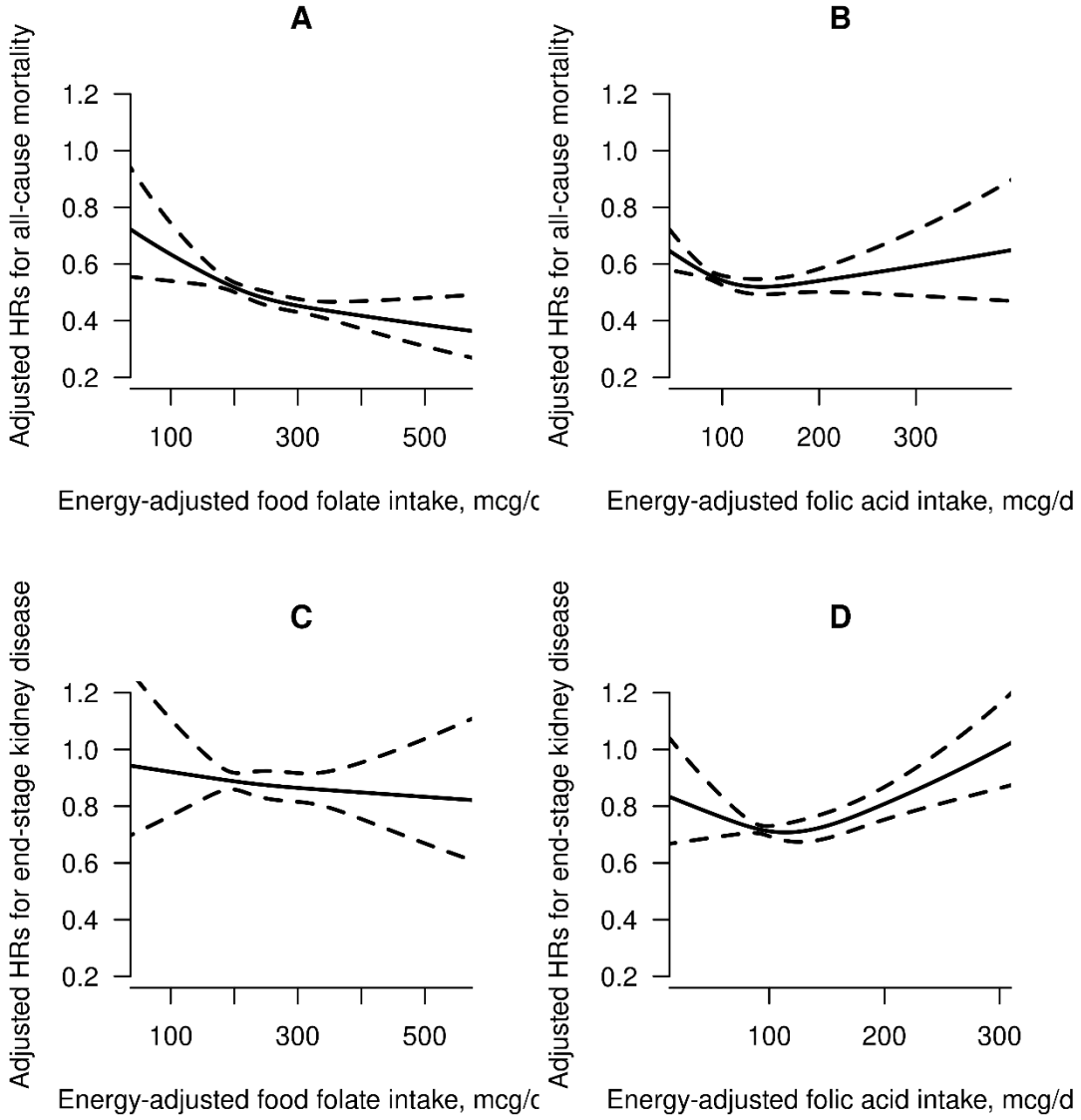
htm.



Supplemental Figure 1. Flow chart of study participants in CRIC study.



Supplemental Figure 2. Flow chart of study participants in NHANES.



Supplemental Figure 3. Relationship of energy-adjusted dietary intake of food folate and synthetic folic acid intake from fortified foods with the risk of all-cause mortality (A, B) and end-stage kidney disease (C, D) in CRIC study based on restricted cubic splines*.

*Results were adjusted for age, sex, race, clinical site, education, marital status, smoking status, alcohol status, physical activity, body mass index, systolic blood pressure, diastolic blood pressure, glucose, estimated glomerular filtration rate, urinary protein, diabetes mellitus, hypertension, cardiovascular disease at baseline, as well as folic acid supplementation use, cumulative averages of total energy intake, and mutually adjusted for energy-adjusted dietary intake of food folate and synthetic folic acid from fortified foods.

Supplemental Table 1. Baseline characteristics of the included and excluded population in CRIC study.

Characteristics	Included	Excluded
N	4028	1471
Age, years	61 (54,67)	62 (54,68)
Male, No. (%)	2174 (54)	926 (63.0)
Non-Hispanic White, No. (%)	1724 (42.8)	698 (47.5)
Education, n (%)		
Less than high school	587 (14.6)	472 (32.1)
High school graduate	736 (18.3)	283 (19.3)
Some college	1231 (30.6)	380 (25.9)
College graduate or higher	1473 (36.6)	335 (22.8)
Married, n (%)	2203 (54.7)	715 (48.6)
Drinker, n (%)	932 (23.1)	252 (17.1)
Smoker, n (%)	509 (12.6)	188 (12.8)
Physical activity, METs/week	168.3 (114.3,246.6)	143.6 (88.9,228.1)
Disease history, No. (%)		
Diabetes	1894 (47.0)	922 (62.7)
Hypertension	3442 (85.5)	1313 (89.3)
Cardiovascular disease	1325 (32.9)	531 (36.1)
Body mass index, kg/m^2	31 (26.9,36.2)	31.7 (27.4,37)
Systolic blood pressure, mmHg	124 (112,138.7)	130.7 (117.3,144.7)
Diastolic blood pressure, mmHg	70 (62,78.7)	71.3 (62.7,80.7)
Glucose, mg/dL	99 (88,126)	111 (92,152.5)
Estimated glomerular filtration rate, mL/min/1.73 m^2	47.5 (36.8,57.8)	50.3 (38.1,61.4)
Urinary protein, g/24 hour	0.2 (0.1,0.7)	0.4 (0.1,1.4)
Energy intake, kcal/d	4028	1471

* Values are medians [IQRs] for continuous variables and n (%) for categoric variables.

Supplemental Table 2. The relationship of dietary total folate intake with risk of all-cause mortality and ESKD in CRIC study.

Total folate, mcg/d	N	Cases (Incidence rate*)	Adjusted Model 1†		Adjusted Model 2†	
			HR (95%CI)	*P value*	HR (95%CI)	*P value*
All-cause mortality						
Quartiles						
Q1 (<241.7)	1007	297(3.5)	ref		ref	
Q2 (241.7-327.8)	1007	297(3.2)	0.86(0.73, 1.01)	0.071	0.90(0.76, 1.07)	0.225
Q3 (327.8 -<439.0)	1007	262(2.8)	0.76(0.64, 0.90)	0.001	0.76(0.63, 0.92)	0.005
Q4 (≥439.0)	1007	299(3.3)	0.92(0.78, 1.08)	0.304	0.79(0.61, 1.01)	0.055
P for trend			0.162		0.014	
ESKD						
Quartiles						
Q1 (<241.7)	1007	226(3.0)	ref		ref	
Q2 (241.7-327.8)	1007	226(2.8)	0.87(0.72, 1.05)	0.141	0.83(0.69, 1.01)	0.068
Q3 (327.8 -<439.0)	1007	229(2.7)	0.85(0.71, 1.03)	0.093	0.80(0.65, 0.99)	0.045
Q4 (≥439.0)	1007	257(3.3)	1.00(0.83, 1.19)	0.959	0.90(0.68, 1.18)	0.430
P for trend			0.985		0.301	

*Incidence rates per 100 person years.

† Model 1: adjusted for age and sex; model 2: adjusted for age, sex, race, clinical site, education, marital status, smoking status, alcohol status, physical activity, body mass index, systolic blood pressure, diastolic blood pressure, glucose, estimated glomerular filtration rate, urinary protein, diabetes mellitus, hypertension, cardiovascular disease at baseline, as well as folic acid supplementation use and cumulative averages of total energy intake.

Supplemental Table 3. Sensitivity analysis for the relationship of dietary intake of food folate and synthetic folic acid intake from fortified foods with risk of all-cause mortality in CRIC study.

	Food folate		Synthetic folic acid	
	Events	Adjusted HR (95%CI)*	Events	Adjusted HR (95%CI) *
Sensitivity analyses 1: further adjusting for consumption of vitamin A, vitamin B6, vitamin B12, vitamin C, vitamin E				
Quartiles				
Q1	317	ref	272	ref
Q2	285	0.82(0.69, 0.97)	299	0.98(0.82, 1.15)
Q3	256	0.76(0.63, 0.93)	285	0.87(0.73, 1.05)
Q4	297	0.77(0.59, 1.01)	299	0.92(0.74, 1.15)
P for trend		0.022		0.271
Sensitivity analyses 2: performing time-varying Cox proportional hazards models using time-varying exposure and covariates				
Quartiles				
Q1	360	ref	300	ref
Q2	288	0.84(0.71, 0.99)	298	1.03(0.87, 1.22)
Q3	245	0.83(0.69, 1.01)	280	0.96(0.80, 1.15)
Q4	262	0.76(0.60, 0.96)	277	0.88(0.72, 1.08)
P for trend		0.025		0.191
Sensitivity analyses 3: performing Cox proportional hazards models with age as the underlying timescale				
Quartiles				
Q1	317	ref	272	ref
Q2	285	0.89(0.75, 1.05)	299	0.92(0.77, 1.09)
Q3	256	0.76(0.63, 0.92)	285	0.85(0.71, 1.02)
Q4	297	0.80(0.64, 1.00)	299	0.87(0.71, 1.07)
P for trend		0.018		0.138

*Adjusted for age, sex, race, clinical site, education, marital status, smoking status, alcohol status, physical activity, body mass index, systolic blood pressure, diastolic blood pressure, glucose, estimated glomerular filtration rate, urinary protein, diabetes mellitus, hypertension, cardiovascular disease at baseline, as well as folic acid supplementation use, cumulative averages of total energy intake, and mutually adjusted for dietary intake of food folate and synthetic folic acid from fortified foods.

Supplemental Table 4. The relationship of dietary intake of food folate and synthetic folic acid intake from fortified foods with risk of cardiovascular or non-cardiovascular death in CRIC study.

	Food folate		Synthetic folic acid	
	Events	**Adjusted HR (95%CI)***	**Events**	**Adjusted HR (95%CI)***
Cardiovascular death				
Quartiles				
Q1	105	ref	90	ref
Q2	87	0.81(0.60, 1.09)	98	0.89(0.67, 1.20)
Q3	86	0.68(0.49, 0.95)	83	0.82(0.59, 1.13)
Q4	87	0.66(0.44, 1.00)	94	0.82(0.57, 1.17)
P for trend		0.028		0.233
Non-cardiovascular death				
Quartiles				
Q1	128	ref	101	ref
Q2	118	0.84(0.64, 1.09)	121	1.01(0.77, 1.33)
Q3	116	0.76(0.57, 1.02)	116	0.93(0.70, 1.24)
Q4	104	0.63(0.44, 0.91)	128	1.08(0.78, 1.48)
P for trend		0.013		0.798

*Adjusted for age, sex, race, clinical site, education, marital status, smoking status, alcohol status, physical activity, body mass index, systolic blood pressure, diastolic blood pressure, glucose, estimated glomerular filtration rate, urinary protein, diabetes mellitus, hypertension, cardiovascular disease at baseline, as well as folic acid supplementation use, cumulative averages of total energy intake, and mutually adjusted for dietary intake of food folate and synthetic folic acid from fortified foods.

Supplemental Table 5. HR (95%CI) for all-cause mortality associated with food folate intake in various subgroups in CRIC study*.

	Quartiles of food folate intake, mcg/d				P for interaction
	Q1	Q2	Q3	Q4	
Age, years					0.771
<60	ref	0.97(0.73,1.29)	0.72(0.53,0.97)	0.81(0.59,1.11)	
⩾60	ref	0.85(0.69,1.04)	0.76(0.61,0.95)	0.78(0.61,1.00)	
Sex					0.796
Male	ref	0.92(0.73,1.14)	0.72(0.57,0.92)	0.78(0.59,1.02)	
Female	ref	0.83(0.65,1.06)	0.78(0.60,1.02)	0.80(0.60,1.07)	
White					0.493
No	ref	0.90(0.72,1.12)	0.84(0.66,1.07)	0.82(0.63,1.06)	
Yes	ref	0.87(0.68,1.11)	0.65(0.50,0.85)	0.76(0.57,1.01)	
CRIC Enrollment Phase					0.484
1	ref	0.90(0.76,1.07)	0.76(0.63,0.92)	0.81(0.65,1.02)	
3	ref	0.66(0.34,1.30)	0.57(0.28,1.16)	0.47(0.23,0.96)	
Body mass index, kg/m^2					0.317
<30	ref	0.77(0.61,0.99)	0.65(0.49,0.85)	0.67(0.50,0.90)	
⩾30	ref	0.98(0.78,1.22)	0.83(0.65,1.05)	0.88(0.68,1.15)	
eGFR, mL/min/1.73 m^2					0.888
<45		0.86(0.70,1.05)	0.73(0.58,0.92)	0.80(0.62,1.03)	
⩾45		0.91(0.69,1.21)	0.76(0.56,1.01)	0.74(0.54,1.02)	
Diabetes					0.812
No	ref	0.92(0.71,1.19)	0.82(0.62,1.07)	0.84(0.62,1.13)	
Yes	ref	0.85(0.69,1.06)	0.70(0.55,0.88)	0.75(0.58,0.97)	
Hypertension					0.795
No	ref	0.82(0.44,1.51)	0.74(0.40,1.37)	0.60(0.31,1.14)	
Yes	ref	0.89(0.75,1.06)	0.74(0.61,0.90)	0.80(0.64,1.01)	
Cardiovascular disease					0.393
No	ref	0.87(0.69,1.11)	0.80(0.61,1.04)	0.88(0.67,1.16)	
Yes	ref	0.89(0.71,1.12)	0.70(0.55,0.89)	0.70(0.53,0.92)	
Folic acid supplementation					0.268
No	ref	0.94(0.73,1.21)	0.78(0.59,1.03)	0.69(0.51,0.93)	

Yes	ref	0.84(0.68,1.05)	0.72(0.57,0.90)	0.84(0.65,1.09)

*Adjusted for age, sex, race, clinical site, education, marital status, smoking status, alcohol status, physical activity, body mass index, systolic blood pressure, diastolic blood pressure, glucose, estimated glomerular filtration rate, urinary protein, diabetes mellitus, hypertension, cardiovascular disease at baseline, as well as folic acid supplementation use and cumulative averages intake of total energy and synthetic folic acid from fortified foods.

Supplement Table 6. Baseline characteristics of study participants according to quartiles of dietary food folate intake in NHANES [*]

	Quartiles of food folate intake, mcg/d				P value
	Q1 (<124.5)	**Q2 (124.5-171.5)**	**Q3 (171.5-<235.1)**	**Q4 (≥235.1)**	
N	1825	1831	1843	1833	
Age, years	65(47,77)	67(51,78)	65(50,76)	61(46,73)	<0.001
Male, No. (%)	631 (29.0)	799 (37.3)	932 (46.9)	1046 (53.7)	<0.001
Non-Hispanic White, No. (%)	855 (66.8)	987 (72.5)	990 (72.6)	896 (72.0)	<0.001
Education, n (%)					<0.001
Less than high school	759 (34.2)	552 (23.4)	532 (21.0)	473 (17.0)	
High school or equivalent	448 (28.4)	468 (27.5)	446 (26.1)	382 (21.9)	
College or above	568 (37.3)	768 (49.1)	823 (52.9)	950 (61.1)	
Married, n (%)	878 (51.4)	905 (52.6)	1034 (61.8)	1092 (62.9)	<0.001
Current drinker, n (%)	753 (46.7)	863 (54.3)	972 (62.6)	1079 (69.9)	<0.001
Current smoker, n (%)	341 (21.9)	298 (17.5)	257 (13.9)	277 (14.7)	<0.001
Physical Activity[†], n (%)					<0.001
Inactive	1272 (67.7)	1149 (56.4)	1070 (52.9)	944 (45.9)	
Intermediate	240 (13.1)	309 (20.3)	319 (18.9)	352 (21.5)	
Ideal	311 (19.2)	371 (23.3)	452 (28.1)	535 (32.6)	
Disease history, No. (%)					
Diabetes	509 (24.6)	582 (29.3)	531 (25.4)	527 (24.1)	0.032
Hypertension	1287 (68.9)	1327 (69.8)	1276 (68.3)	1209 (63.0)	0.071
Cardiovascular disease	550 (24.4)	540 (25.4)	508 (24.2)	500 (22.8)	<0.001
Body mass index, kg/m^2	28.7(24.3,33.3)	29.2(24.9,33.8)	28.6(25.1,33.5)	28.6(24.8,34.5)	0.058
Systolic blood pressure, mmHg	130(116,148.7)	130(116,146.7)	130.7(116,145.3)	128(115.3,142.7)	<0.001

Diastolic blood pressure, mmHg	68.7(59.3,77.3)	68(60,76.7)	70.7(61.3,78.7)	71.3(62.7,80)	<0.001
Glucose, mg/dL	5.7(5.3,6.2)	5.8(5.4,6.3)	5.7(5.3,6.3)	5.6(5.3,6.2)	0.628
Estimated glomerular filtration rate, mL/min/1.73 m^2	63(49.5,96.9)	59.5(50,94.9)	63.8(53.2,93.9)	74.8(54.9,101.5)	<0.001
Urinary albumin: creatinine ratio, mg/g	42.8(13.9,90.9)	40.2(12.5,97.3)	38.5(12.3,86)	42.8(13,91.7)	0.781
Energy intake, kcal/d	1301.5(1030,1621)	1621(1347,1971)	1874(1550,2272)	2250(1820,2747)	<0.001
Folic acid intake, mcg/d	101(60.5,168)	128(76,197)	149(89.5,245)	144(86.5,259)	<0.001
Folic acid supplementation use, No. (%)	483 (29.8)	570 (34.0)	662 (39.7)	706 (41.0)	<0.001

* Values are medians [IQRs] for continuous variables and n (%) for categoric variables, and all estimates accounted for complex survey designs.
†Inactive leisure time physical activity level defined as 0 min/week moderate-to-vigorous physical activity; intermediate leisure time physical activity level defined as 1 to 149 minutes per week of moderate activities, or 1 to 74 minutes per week of vigorous activities, or 1 to149 minutes per week of a combination of moderate and vigorous activities; and ideal leisure time physical activity level defined as ≥150 minutes per week of moderate activities, or ≥75 minutes per week of vigorous activities, or ≥150 minutes per week of a combination of moderate and vigorous activities.