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Epidemiologic Follow-Up Study * Editorial Comment: NHANES I Epidemiologic
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Dietary Intake of Folate and Risk of Stroke in US Men and Women

NHANES I Epidemiologic Follow-Up Study

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Background and Purpose—Few population-based studies have examined the relationship between dietary intake of folate and risk of stroke and cardiovascular disease (CVD). This study examines the association between dietary intake of folate and the subsequent risk of stroke and CVD.

Methods—Study participants included 9764 US men and women aged 25 to 74 years who participated in the National Health and Nutrition Examination Survey I Epidemiologic Follow-up Study (NHEFS) and were free of CVD at baseline. Dietary intake of folate was assessed at baseline using a 24-hour dietary recall and calculated using ESHA software. Incidence data for stroke and CVD were obtained from medical records and death certificates.

Results—Over an average of 19 years of follow-up, 926 incident stroke events and 3758 incident CVD events were documented. The relative risk (RR) was 0.79 (95% confidence interval [CI], 0.63 to 0.99, $P=0.03$ for trend) for incident stroke events and 0.86 (95% CI: 0.78 to 0.95, $P<0.001$ for trend) for incident CVD events in the highest quartile of dietary folate intake (median, 405.0 $\mu\text{g}/\text{day}$) compared with those in the lowest quartile (median, 99.0 $\mu\text{g}/\text{day}$), after adjustment for established cardiovascular risk factors and dietary factors.

Conclusions—Our findings indicate an inverse relationship between dietary intake of folate and subsequent risk of stroke and CVD. Increasing dietary intake of folate from food sources may be an important approach to the prevention of CVD in the US population. (*Stroke*. 2002;33:1183-1189.)

Key Words: cardiovascular disease ■ cerebrovascular disorders ■ diet ■ folic acid ■ prospective studies

As early as 1969, homocysteine was hypothesized to affect atherosclerotic processes.¹ Since that time, substantial evidence has accumulated linking homocysteine in plasma and serum to the risk of cardiovascular diseases.²⁻⁵ Folate and cyanocobalamin (vitamin B₁₂) are important regulators of the metabolism of homocysteine in the body, and studies have shown an inverse relationship between levels of these factors and levels of homocysteine in the blood.⁶⁻⁸ In addition, randomized controlled trials have demonstrated that folate supplementation reduces blood levels of homocysteine.^{9,10}

Epidemiological studies have also identified an inverse relationship between blood concentrations of folate and cardiovascular disease end points, including stroke.¹¹⁻¹³ However, few studies have prospectively examined the relationship between dietary intake of folate and the risk of cardiovascular diseases.¹⁴ To our knowledge, no previous prospective study has reported a significant relationship between dietary intake of folate and the risk of stroke.

The importance of examining the relationship between dietary intake of folate and risk of cardiovascular disease is

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underscored by the recent mandate for fortification of grain and cereal products with folate and recommendations to further increase levels.¹⁵⁻¹⁷ Therefore, we took advantage of the large sample size and prolonged follow-up experience of participants in the NHANES I Epidemiologic Follow-up Study (NHEFS) to examine the relationship of dietary folate intake to subsequent risk of cardiovascular disease in a nationally representative sample of the US noninstitutionalized population.

Subjects and Methods

Study Population

The first National Health and Nutrition Examination Survey (NHANES I) used a multistage, stratified, probability sampling design to select a representative sample of the US civilian noninstitutionalized population aged 1 to 74 years.^{18,19} Certain population subgroups, including those with a low income, women of childbearing age (25 to 44 years), and elderly persons (65 years or older), were oversampled. The NHEFS is a prospective cohort study of NHANES I participants who were 25 to 74 years of age when the survey was

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conducted between 1971 and 1975. Of the 14 407 persons in this age range at baseline, we excluded 1353 who had a self-reported history of heart attack, heart failure, or stroke at baseline or had used medication for heart disease during the preceding 6 months, and we excluded 2893 NHANES I Augmentation Survey participants for whom the study protocol did not include collection of dietary information. Among the remaining participants, 397 (3.9%) were lost to follow-up, leaving a total of 9764 participants who contributed 161 236 person-years of experience.

Measurement

Baseline data collection included a medical history, dietary assessment, standardized medical examination, laboratory tests, and anthropometric measurements.^{18,19} The dietary assessment included a single 24-hour dietary recall collected by trained NHANES I personnel using a standardized protocol and 51 three-dimensional models to estimate portion size. Dietary intake of folate was not available in the original NHANES I nutrient database; therefore, a listing of the 3481 unique foods recorded during collection of the 24 hour dietary recalls was obtained. Each unique food was matched to a corresponding food item listed in the ESHA Food Processor nutrient database by name and available nutrient content from NHANES I documentation.²⁰ Participants' dietary intake of folate ($\mu\text{g}/24\text{ h}$), saturated fat ($\text{g}/24\text{ h}$), and total energy ($\text{kcal}/24\text{ h}$) were calculated. A reliability analysis was conducted to evaluate correspondence between NHANES I and ESHA nutrient databases. Intraclass correlation coefficients for nutrient intakes derived using NHANES I and ESHA food composition databases ranged from 0.85 to 0.98, whereas graphical methods also demonstrated good agreement for most nutrients (data not shown). Blood pressure, body weight, and height were obtained using standard protocols.¹⁸ Frozen sera were sent to the Centers for Disease Control and Prevention for measurement of serum total cholesterol levels. The baseline questionnaire on medical history included questions about selected health conditions and medications used for these conditions during the preceding 6 months. Data on education, physical activity, and alcohol consumption were obtained by means of interviewer-administered questionnaires. Information on smoking status was obtained in 6913 participants who underwent a more detailed baseline examination.^{18,19} For the remaining study participants, information on smoking status at baseline was derived from responses to questions on lifetime smoking history obtained at their follow-up interviews between 1982 to 1984 or later.^{21,22} The validity of information obtained using this approach has been documented.^{23,24}

Follow-Up Procedures

Follow-up data were collected between 1982 and 1984 and in 1986, 1987, and 1992.^{21,22,25,26} Each follow-up examination included tracking a participant or his/her proxy to a current address; performing an in-depth interview; obtaining hospital and nursing home records, including pathology reports and electrocardiograms; and, for decedents, acquiring a death certificate. Incident stroke and cardiovascular disease events were based on documentation of an event that met prespecified diagnostic criteria and occurred during the period between the participant's baseline examination and last follow-up interview. Validity of study outcome data has been documented.²⁷

Incident stroke was based on a death certificate report in which the underlying cause of death was recorded with an *International Classification of Diseases, Ninth Revision* (ICD-9) code of 430 to 438 or on one or more hospital and/or nursing home stays in which the participant had a discharge diagnosis with the above codes. Incident cardiovascular disease was based on a death certificate report in which the underlying cause of death was recorded using an ICD-9 code of 390 to 459 or on one or more hospital and/or nursing home stays in which the participant had a discharge diagnosis with the above codes. The date of record for incident events was identified by the date of first hospital admission with an established study event or date of death from a study event in the absence of hospital or nursing home documentation.

Statistical Analysis

Dietary folate intake was modeled as a categorical (quartile groups) variable in primary analyses and modeled continuously in additional analyses. In continuous analyses, dietary folate intake was log transformed because of

right skew. The distribution of each baseline characteristic was calculated by quartile of dietary folate intake (mean or percent of study participants). The statistical significance of differences was examined by analysis of variance (continuous variables) and by the χ^2 test (categorical variables). Trend tests were conducted using orthogonal coefficients. The cumulative incidence of cardiovascular disease by quartile of dietary folate intake was calculated using the Kaplan-Meier method,²⁸ and differences in cumulative rates were examined by the log-rank test for trend.²⁹ The adjusted relationship between dietary intake of folate and risk of cardiovascular disease was modeled using Cox regression analysis.³⁰ Cox proportional hazard models were stratified by birth cohort using 10-year intervals to control for calendar period and cohort effects.³¹ Age was used as the time-scale in all time-to-event analyses.³¹ Methods to estimate variance that take into account sample clustering and stratification of the NHANES I sample were used in the Cox proportional hazards models.³¹ Because few participants remained and the all-cause mortality was disproportionately high after age 85 years, all analyses were truncated at this age.

Results

In this cohort, the median folate intake was 203.7 μg per day with an interquartile range of 164.6 μg per day. Compared with those with a lower intake, participants with a higher dietary folate intake tended to be male and white (Table 1). Participants with a higher intake of folate had, on average, a slightly lower systolic blood pressure and serum total cholesterol than those with a lower dietary folate intake. Hypertension and hypercholesterolemia appeared less often, on the whole, in participants with a higher folate intake compared with those with a lower intake. In addition, participants who consumed higher levels of folate tended to perform more recreational physical activity than their counterparts who consumed lower levels of folate in their diet. Participants in the lowest quartile of folate intake had a higher body mass index than those in the upper 3 quartiles. They were also less likely to have completed high school, be consuming alcohol regularly, and be using a vitamin supplement; however, cigarette smoking did not differ across quartile of dietary folate intake. Energy and saturated fat intakes tended to be higher with progressively higher intake of dietary folate.

During the 161 236 person-years of follow-up between 1971 and 1992, 926 stroke events and 3758 cardiovascular disease events were documented. Cumulative hazard of stroke by quartile of dietary folate intake is presented in the Figure. The cumulative hazard of stroke at age 85 years from the lowest to highest quartile of dietary folate intake was 38.6%, 34.3%, 34.6%, and 32.9% ($P=0.02$ for trend). The cumulative hazard of cardiovascular disease did not clearly separate by quartile of dietary folate intake in Kaplan-Meier plots.

Relative risks (RR) and corresponding 95% confidence intervals (CI) for incidence of stroke and cardiovascular disease by quartile of dietary folate intake are presented in Table 2. In age-, race-, sex-, and energy-adjusted analyses, the risk of cardiovascular diseases was significantly and inversely related to quartile of dietary folate intake at baseline (P value for trend=0.03), whereas the risk of stroke was inversely related to quartile of dietary folate intake with borderline significance (P value for trend=0.09). After further adjustment for history of diabetes, systolic blood pressure, serum cholesterol, body mass index, recreational physical activity, level of education, regular alcohol consumption, and current cigarette smoking, dietary folate intake was significantly and inversely related to the subsequent risk of stroke and cardiovascular disease. Participants who consumed at least 300 μg of folate per day had a 20% lower risk of stroke (RR, 0.80; 95% CI, 0.64 to 0.99; P value for

TABLE 1. Baseline Characteristics of 9764 NHEFS Participants According to Quartile of Folate Intake

Variable*	Quartile of Folate Intake ($\mu\text{g}/\text{d}$)				P Value for Linear Trend
	<136.0	136.0–203.7	203.7–300.6	>300.6	
No. of participants	2440	2441	2441	2442	
Age, years	48.8 \pm 15.8	50.7 \pm 15.7	49.1 \pm 15.4	48.3 \pm 15.4	0.02
Men, %	20.6	32.6	43.8	56.1	<0.001
Whites, %	78.7	85.1	86.5	84.8	<0.001
Systolic BP, mm Hg	135.1 \pm 25.9	135.1 \pm 24.4	134.5 \pm 23.7	132.7 \pm 23.0	<0.001
Diastolic BP, mm Hg	83.5 \pm 13.4	83.2 \pm 13.1	83.2 \pm 13.2	82.9 \pm 12.5	0.15
Hypertension, [†] %	29.1	29.8	27.2	21.9	<0.001
Serum total cholesterol, mg/dL	222.0 \pm 49.9	222.5 \pm 49.0	219.7 \pm 48.4	217.1 \pm 48.2	<0.001
Hypercholesterolemia, [‡] %	33.2	33.6	30.9	27.6	<0.001
Diabetes, %	3.8	4.2	4.3	4.0	0.61
Body mass index, kg/m ²	26.1 \pm 5.6	25.8 \pm 5.3	25.3 \pm 4.8	25.4 \pm 4.7	<0.001
Low recreational physical activity, %	51.1	48.3	43.4	42.0	<0.001
Less than high school education, %	52.8	46.0	43.5	45.1	<0.001
Current cigarette smoking, %	36.7	32.2	35.4	35.4	0.90
Regular alcohol consumption, %	17.2	22.4	26.6	28.2	<0.001
Vitamin supplement use, [§] %	29.3	33.2	34.8	32.7	0.004
Took aspirin in the past 30 days, %	9.8	10.7	10.1	10.9	0.01
Dietary intake of saturated fat, g/24 h	17.8 \pm 11.0	23.4 \pm 12.5	28.8 \pm 16.2	33.0 \pm 20.1	<0.001
Dietary intake of calories, kcal/24 h	1161 \pm 503	1550 \pm 555	1900 \pm 692	2326 \pm 966	<0.001

NHEFS indicates National Health and Nutrition Examination Study I Epidemiologic Follow-Up Study; BP, blood pressure.

*Mean \pm SD or % of participants.

[†]Systolic blood pressure \geq 160 mm Hg and/or diastolic blood pressure \geq 95 mm Hg and/or use of antihypertensive medication.

[‡]Serum cholesterol \geq 240 mg/dL.

[§]Vitamin supplement use was defined as the regular or irregular use of any type of vitamin supplement.

trend=0.03) and a 13% lower risk of cardiovascular disease (RR, 0.87; 95% CI, 0.79 to 0.96; P value for trend=0.002) than those consuming less than 136 μg of folate per day.

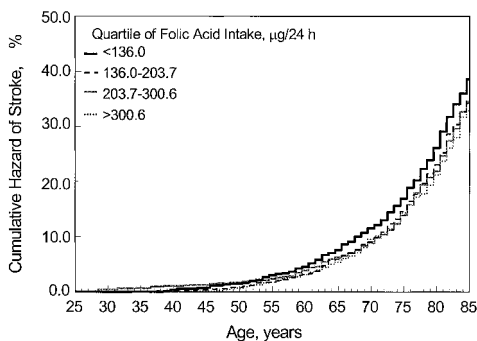
Results were consistent using the logarithm of dietary folate intake as a continuous variable (Table 3). For instance, an increase in the log of folate intake representing the interquartile range was associated with a 9% lower risk of stroke (RR, 0.91; 95% CI, 0.83 to 0.99; P=0.04) and an 8% lower risk of cardiovascular disease (RR, 0.92; 95% CI, 0.88 to 0.97; P<0.001) after adjustment for important cardiovascular disease risk factors.

The inverse associations between dietary intake of folate and incidence of stroke and cardiovascular diseases seemed consistent

across genders, levels of physical activity, and tobacco use. For example, the risk of stroke among women in the highest quartile of folate intake was 17% (RR, 0.83; 95% CI, 0.60 to 1.14) lower than that of women in the lowest quartile, whereas the corresponding estimate for men was 18% (RR, 0.82; 95% CI, 0.61 to 1.11). For smokers, the relative risk of stroke for the comparison of extreme quartiles was 0.73 (95% CI, 0.46 to 1.16), similar to that of former or nonsmokers (0.82; 95% CI, 0.65 to 1.04). Estimates of relative risk for the comparison of extreme quartiles were similar for participants with low levels of recreational physical activity and those with medium or high levels (RR, 0.84; 95% CI, 0.59 to 1.18; and RR, 0.77; 95% CI, 0.58 to 1.02; respectively). Measures of the association between dietary folate intake and cardiovascular disease were also comparable in groups based on gender, physical activity, and tobacco use (data not shown).

Discussion

This prospective study documents the presence of an independent and inverse relationship between dietary folate intake and risk of stroke and cardiovascular diseases in adults. Stroke is the third leading cause of death and the most common basis for long-term disability in the US general population.³² Given the high mortality and cost of treatment of stroke and other cardiovascular disease, primary prevention should be a central goal of plans aimed at reducing its societal burden. The findings from this study suggest that a higher intake of folate may be associated with a lower risk of



Cumulative hazard of stroke according to quartile of dietary folate intake in 9764 participants of the NHEFS cohort (log-rank test of trend P value=0.02).

TABLE 2. Relative Risk and 95% Confidence Interval for Incidence of Stroke and Cardiovascular Disease Incidence According to Quartile of Folate Intake for 9764 NHEFS Participants

Variable	Quartile of Folate Intake ($\mu\text{g}/\text{d}$)				P Value for Linear Trend
	<136.0	136.0–203.7	203.7–300.6	>300.6	
Number of participants	2440	2441	2441	2442	
Person-years	40 258	39 583	40 545	40 850	
Stroke					
Events	254	244	224	204	
RR (95% CI)					
Age, race, sex, energy-adjusted	1.00	0.89 (0.73, 1.09)	0.87 (0.72, 1.06)	0.82 (0.67, 1.02)	0.09
Multivariate*	1.00	0.93 (0.74, 1.16)	0.85 (0.71, 1.02)	0.80 (0.64, 0.99)	0.03
Cardiovascular disease					
Events	957	982	899	920	
RR (95% CI)					
Age, race, sex, energy-adjusted	1.00	0.97 (0.89, 1.05)	0.88 (0.80, 0.98)	0.90 (0.82, 1.00)	0.03
Multivariate*	1.00	0.98 (0.89, 1.07)	0.88 (0.79, 0.97)	0.87 (0.79, 0.96)	0.002

RR indicates relative risk; CI, confidence interval.

*Stratified by birth cohort and adjusted for age, race, sex, systolic blood pressure, serum cholesterol, body mass index, history of diabetes, physical activity, level of education, regular alcohol consumption, current cigarette smoking, saturated fat intake, and total energy intake; n=9244.

stroke in a representative sample of the US general population and have important public health implications.

Evidence relating homocysteine levels in blood and risk of cardiovascular disease is abundant. A meta-analysis of 29 epidemiological studies indicates that elevated levels of homocysteine are related to stroke and cardiovascular disease.^{33,34} In addition, dietary folate intake has been inversely related to plasma levels of total homocysteine in several epidemiological studies.^{6–10,33} At least 1 prospective study has suggested the presence of an inverse association between risk of stroke and serum concentrations of folate in a separate random subsample of 2006 participants in the NHEFS who underwent a detailed physical examination.¹¹ The latter study suffered from a small sample size and few events (98 strokes), which may have contributed to the lack of statistical significance in most findings. This study extends those results by examining the association between dietary folate and the risk of stroke and cardiovascular disease in a much larger sample.

Few data are currently available regarding the effect of dietary folate on risk of cardiovascular disease. In 1998, Rimm et al¹⁴ identified a significant inverse relationship between dietary intake of folate from foods and supplements and subsequent risk of coronary heart disease in a cohort of US nurses. That study did not report an

association between dietary folate and risk of stroke or the combined outcome of cardiovascular disease and was conducted in a select, highly educated, female population that may have different dietary characteristics than the US general population. Mean folate intake in that study was 366 $\mu\text{g}/\text{day}$, well above the national average of 224 $\mu\text{g}/\text{day}$.¹⁴ Our study is conducted in a representative sample of the noninstitutionalized US population, therefore our findings are highly generalizable.

Folate supplementation reduces blood levels of homocysteine, which many believe to be an important risk factor for cardiovascular disease. The mechanism by which homocysteine may increase risk of stroke and cardiovascular diseases remains unclear.³⁵ Homocysteine may promote atherogenesis by damaging the vascular matrix, increasing proliferation of endothelial cells, and facilitating oxidative injury of vascular walls.^{36,37} In addition, homocysteine may increase coagulation and disturb endothelium-dependent vasomotor regulation.³⁶ To date, most experimental studies have involved extreme concentrations of homocysteine not present in the general population; therefore, the results of these studies may have limited relevance.³⁷ In addition to regulation of homocysteine, folate itself may have vasculoprotective effects.³⁸ A recent randomized trial demonstrated that supplementation with folic acid and vitamin B₆

TABLE 3. Relative Risk and 95% Confidence Interval for Stroke and Cardiovascular Disease Incidence Associated with an Increase in Logarithm of Dietary Folate Representing the Interquartile Range*

	Age, Race, Sex, Energy-adjusted		Multivariate†	
	RR (95% CI)	P Value	RR (95% CI)	P Value
Stroke	0.93 (0.85, 1.01)	0.08	0.91 (0.83, 0.99)	0.04
Cardiovascular disease	0.95 (0.91, 0.99)	0.01	0.92 (0.88, 0.97)	0.001

*0.8 Log units or a 2.2-fold increase in folate intake.

†Stratified by birth cohort and adjusted for age, sex, race, systolic blood pressure, serum cholesterol, body mass index, history of diabetes, physical activity, education level, % of regular alcohol consumption, % of current cigarette smoking, saturated fat intake and total caloric intake; n=9244.

reduces markers of endothelial dysfunction and the progression of subclinical atherosclerosis.³⁹

It is possible that persons who consume high levels of folate in their diet may have other dietary and nondietary lifestyle factors that promote health, such as exercising regularly, not smoking, using aspirin regularly, and consuming a diet low in saturated fat. To reduce the impact of such "healthy habits," the estimates of risk in our study were adjusted for important potential confounders of cardiovascular disease like age, race, sex, education level, recreational physical activity, cigarette smoking, diabetic status, regular alcohol consumption, and total energy intake. When vitamin supplement use, often considered an indicator of a "healthy lifestyle," was added to multivariate models, risk estimates did not change. In multivariate models adjusted for established cardiovascular disease risk factors and vitamin supplement use, the relative risks of stroke and cardiovascular disease for the comparison of extreme quartiles were 0.80 (95% CI from 0.64 to 0.99; *P* for linear trend=0.04) and 0.87 (95% CI from 0.79 to 0.97; *P* for linear trend=0.003), respectively. When any aspirin use in the past 30 days was added to models in addition to established cardiovascular risk factors, the relative risks of stroke and cardiovascular disease in extreme quartiles were 0.82 (95% CI from 0.65 to 1.02; *P* for linear trend=0.05) and 0.88 (95% CI from 0.79 to 0.97; *P* for linear trend=0.004), respectively. Moreover, it is reassuring that when the study population was examined by subgroups based on gender, tobacco use, and physical activity, the inverse association between dietary intake of folate and risk of stroke was consistent in both direction and magnitude.

The present study has several limitations. One limitation of this study is the estimation of dietary folate intake using a single 24-hour dietary recall. Because of day-to-day variability in participants' food intakes, estimates from a single day of intake may not be representative of participants' usual, long-term intake and so may result in misclassification of usual folate consumption at the individual level.^{40–42} However, such random errors of measurement in folate intake would tend to bias any observed association toward the null rather than create a spurious association. Second, information on folic acid supplementation from multivitamin use was not collected in the NHANES I study. Therefore, folic acid supplements could not be taken into account in the analysis. Although approximately 30% of our sample reported the regular or irregular use of any type of vitamin supplement during their baseline examination (1971 to 1975), only 12.6% of persons reported regularly using multivitamins that may have contained folate, and they were equally distributed across levels of dietary folate intake. During the early 1970s when this data were collected, the folate content of vitamin supplements was restricted to 100 μg in a single day's preparation available without prescription.⁴³ Thus vitamin supplements may not have been a significant source of folate in this sample and so may not confound the relationship we observed. Third, folate intake was calculated using a current nutrient database that might overestimate folate intake over the whole study population because of changes in the folate content of foods. This may not affect the rank of dietary folate intake for each individual in the study population and thus not bias our finding of an inverse association between dietary folate intake and risk of stroke. In addition, some NHANES I participants may have underreported their dietary energy intake.^{44,45} However, adjustment for energy intake in our analyses may have reduced the potential impact of such underreporting.

This study maintained several strengths. Foremost, this study was conducted in a nationally representative sample of the noninstitutionalized US population; therefore these findings are broadly generalizable. In addition, temporal relationships can be established with confidence because intake of folate was estimated from dietary information collected at baseline. Further, assessment of the incidence of cardiovascular diseases occurred over an average of 19 years of follow-up, and more than 96% of the study participants were successfully tracked through 1992.

In conclusion, these findings indicate that dietary intake of folate from food sources is independently and inversely related to the risk of stroke and cardiovascular disease in a representative sample of the noninstitutionalized US population. Folate intake has been inversely related to homocysteine levels, an important risk factor for stroke and cardiovascular disease. Food fortification of folic acid was mandated in 1996 to be fully implemented by 1998.¹⁵ A fortification of 140 μg of synthetic folic acid per 100 g of enriched grain product was used to provide an additional 80 to 100 μg of folic acid per day to the diet of women of childbearing age and 70 to 120 μg to the diet of middle-aged and older adults. Assuming a causal and linear association between dietary folate intake and stroke risk, an increase of 95 $\mu\text{g}/\text{day}$ in the diet of middle-aged and older adults would be consistent with an approximate 12% reduction in stroke risk over 20 years based on our data. Given these results, increasing intake of folate from foods may be an important element in dietary interventions to reduce the incidence of stroke and cardiovascular diseases. As in all observational epidemiological studies, imperfect measurement of confounding variables and unmeasured potential confounders may bias study findings. Further studies designed to examine the relationship of dietary folate intake and risk of stroke and cardiovascular diseases are needed to shed light on this important public health issue.

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Editorial Comment

Folate and the Risk of Stroke: Fortify First and Ask Questions Later?

In this article, Bazzano and colleagues demonstrated an inverse relationship between dietary intake of folate and the risk of stroke and cardiovascular disease. This association has been reported previously in several epidemiologic studies,^{1–4} but the present study is the first to confirm its existence in a large prospective cohort of a representative sample of the US population. People whose daily diet included at least 300 μg of folate (about three fourths of the current

US Recommended Daily Allowance of 400 μg) were about 20% less likely to suffer a subsequent stroke than those whose daily diet contained less than 136 μg . This relationship persisted even after adjusting for other dietary and vascular risk factors. It is often extraordinarily difficult to explore the connection between nutrition and disease, both retrospectively and prospectively, because of numerous potential threats to validity such as inaccuracies of dietary

recall, variability in eating habits, and confounding by other dietary and environmental exposures.⁵ The current study is certainly no exception, but the authors address the limitations effectively in their discussion. Thus, along with the prior reports, Bazzano and colleagues have provided us with credible class II evidence that greater folate consumption is associated with a lower risk of subsequent stroke, and support for a level B recommendation that folate is probably effective in stroke prevention.

As with all observational studies, the question of causality lingers.⁶ The relationship is biologically plausible, as folate reduces serum levels of homocysteine.⁷ However, the mechanisms by which homocysteine increases the risk of vascular disease remain uncertain and continue to be extensively studied in vascular biology.

An alternative approach to establishing causality, which would also provide upgraded class I evidence, may come in the form of definitive randomized controlled clinical trials. To determine if folate supplementation is effective in primary or secondary prevention of vascular events, 3 major trials are currently under way in North America: the Vitamin Intervention for Stroke Prevention (VISP) trial, the Women's Antioxidant Cardiovascular Disease Study (WACS), and the Heart Outcomes Prevention Evaluation-The Ongoing Outcomes (HOPE-TOO) study. In each of these trials, patients are randomly assigned to receive either high-dose folate (2.5 to 5 mg daily) along with vitamins B₆ (pyridoxine) and B₁₂ (cobalamin) versus very low-dose folate (20 μg daily in VISP) or placebo (in WACS and HOPE-TOO). There is a potential conundrum facing these trials that may undermine their ability to answer this important clinical question. Because of the evidence for reduction of neural tube defects with folate, the US Food and Drug Administration mandated the addition of 140 μg of folic acid per 100 g of flour, rice, pasta, cornmeal, and related food products by January 1998.⁸ The 3 major vascular studies started enrolling patients after this date but had been designed before this date and estimated the expected treatment effect and respective sample sizes based on prior data that did not reflect massive vitamin fortification policies. In effect, the control arms of these studies are now exposed to much higher levels of folate than was intended. Consequently, there is a high probability that all 3 studies will be underpowered to detect differences among groups.⁹ Moreover, negative results from these otherwise well-designed randomized clinical trials may be believed to provide class I evidence that folate supplementation does not reduce the risk of vascular disease, even if the data are falsely negative due to these sample size considerations (ie, type II error).

How, then, are we to proceed in the face of this impending possible paradox, and what should we recommend? The randomized trials should go on to completion, and there is no ethical issue about withholding a "proven" therapy since even the control patients are receiving some level of folate supplementation from their diets. Even if individually negative, the combined weight (ie, future meta-analysis) of these trials may have enough power to detect a treatment effect if one does in fact exist. Further, these trials may identify relevant subgroups for whom higher doses of folate may be particularly important. Finally, these trials may determine whether there are any safety concerns with high doses of folate that have not been apparent to date. Until then, outside the context of clinical trials, we should recommend (level B rating) dietary intake of at least 300 to 400 μg of folate for patients at risk for cerebrovascular and cardiovascular disease. A list of breakfast cereals that contain 100% of the recommended amount of folate can be found at <http://www.cdc.gov/ncbddd/folicacid/cereal.htm>.

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