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Background and Purpose—Few studies have examined the association between folate intake and risk of stroke, although numerous studies have suggested that high levels of homocysteine are positively related to stroke. We aim to assess the relation between folate intake and stroke incidence among women participating in the Nurses' Health Study.

Methods—83 272 female nurses aged 34 to 59 years in 1980 and residing in 11 US states were followed-up for 18 years. Follow-up questionnaires were sent biennially to update information on diet and to identify newly diagnosed cases of stroke and other illnesses.

Results—During 1 379 614 person-years of follow-up from 1980 to 1998, we identified 1140 incident cases of stroke. Using age-adjusted and multivariable-adjusted models, no appreciable association between the intake of folate and total incidence of stroke was observed [relative risk in the multivariable-adjusted model for the highest quintile of folate intake (median=696 μg/d) compared with the lowest quintile (median=158 μg/d) was 1.01 (95% confidence interval [CI]: 0.79 to 1.29), *P* for trend=0.8]. Similar null results were found in secondary analyses on stroke subtypes (ischemic, thrombotic, embolic, subarachnoid hemorrhage, intraparenchymal hemorrhage) and in analyses that separately assessed dietary folate (excluding supplement users) and folate supplement intake.

Conclusions—Folate intake was not associated with incident stroke among women participating in the Nurses' Health Study. **(*Stroke*. 2004;35:1259-1263.)**

Key Words: stroke ■ diet ■ epidemiology

Numerous retrospective and cross-sectional studies have found significant positive associations between elevated homocysteine levels and stroke.[1–4] However, there are data to suggest that homocysteine levels may increase significantly after stroke,[5,6] and thus the possibility that "reverse causality" accounts for these observations must be considered. Prospective cohort studies, in which homocysteine levels are measured before a diagnosis of a cardiovascular disease (CVD), reduce the potential for this type of bias. The results of prospective studies have not been consistent,[7–13] with some[7–9] reporting significant positive associations between homocysteine levels and stroke whereas others do not.[10–13]

Homocysteine levels depend in part on folate status: lower intake of folate leads to higher homocysteine levels.[14,15] As a result of the observed inverse association between homocysteine levels and stroke, folate has been hypothesized to be protective against stroke incidence.[16] Studies of serum folate or folate intake in relation to incidence of stroke have been inconsistent.[17–20] Therefore, we prospectively examined the association between folate intake and the risk of stroke among 83 896 women participating in the Nurses' Health study who were followed-up for 18 years.

Subjects and Methods

Details of the Nurses' Health Study have been published elsewhere.[21] Briefly, the cohort was established in 1976 when 121 700 female registered nurses aged 30 to 55 years and residing in 11 large US states completed a mailed questionnaire on their medical history and lifestyle. Every 2 years, follow-up questionnaires have been sent to update information on potential risk factors and to identify newly diagnosed cases of CVD and other diseases.

Semiquantitative Food Frequency Questionnaire

In 1980, we collected data on usual dietary intake (61 items) using a validated semiquantitative food frequency questionnaire (FFQ) described elsewhere.[22] In 1984, the FFQ was expanded to include 116 items. Similar questionnaires were used to update diet in 1986, 1990, and 1994. The average daily intake of nutrients was calculated by multiplying the frequency of consumption of each item by the nutrient content and totaling the nutrient intake for all food items. Nutrient intake was adjusted for total energy intake using the residual approach.[23] Folic acid intake as measured on the FFQ was significantly correlated with circulating levels of folate (r=0.55)[24] and predicted homocysteine, as well as serum folate levels.[25] Food composition values for folate and other nutrients were obtained from the Harvard University Food Composition Database (November 22, 1993) derived from US Department of Agriculture sources[26] and supplemented with manufacturer information.

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Population

A total of 98 462 women returned the 1980 diet questionnaire. After excluding women who had implausibly high (>14 700 kJ/d [3500 kcal/d]) or low (<2100 kJ/d [500 kcal/d]) total energy intake, those who left 10 or more items blank, and women with cancers (excluding nonmelanoma skin cancer) or cardiovascular diseases diagnosed at baseline or before the development of stroke (during follow-up), the final population consisted of 83 896 women. We excluded women with cardiovascular or cancer diagnosis at baseline to minimize the potential bias caused by disease-induced changes in dietary habits and intake of folate.

Ascertainment of Stroke

Incident stroke occurring after the date of return of the 1980 questionnaire but before June 1, 1998 were included. Women who reported a nonfatal stroke on a follow-up questionnaire were asked for permission to review their medical records. Fatal strokes were initially ascertained by reports from relatives or postal authorities and a search of the National Death Index,[27] and were then documented by medical records and death certificates. Mortality follow-up was >98% complete.[27] Nonfatal or fatal strokes that were confirmed by telephone, letter, or death certificate, but for which no medical records were available, were regarded as probable. Confirmed and probable strokes were considered in the analyses for total stroke.

Strokes were confirmed by medical records according to the criteria of the National Survey of Stroke,[28] which requires a constellation of neurological deficits of sudden or rapid onset lasting ≥24 hours or until death. Strokes were subcategorized as subarachnoid hemorrhages, intraparenchymal hemorrhages, thrombotic, embolic, or ischemic (which includes thrombotic, embolic, and nonhemorrhagic strokes).

Data Analysis

We computed person-time of follow-up for each participant from the return date of the 1980 questionnaire to the date of stroke diagnosis, to the day of death from any cause, or January 31, 1998, whichever came first. Women who reported CVD on previous questionnaires were excluded from subsequent follow-up; thus, each participant could contribute only 1 end point.

The incidence rate for each category of folate was calculated as the number of cases with stroke divided by the person-time of follow-up. Total and dietary folate intake variables were energy-adjusted, but folate supplement intake was not. Cutoff points for total and dietary folate intake (excluding supplements) were obtained by dividing each into quintiles. We categorized folate supplement intake into 4 categories that approximately resembled the quartiles of folate supplement intake levels among our population (0.1 to 149 μg/d, 150 to 249 μg/d, 250 to 399 μg/d, ≥400 μg/d), and women who did not report supplement use served as the reference category in the analysis. To adjust for covariates, we used pooled logistic regression,[29] pooling over each 2-year period using SAS statistical software version 6.12,[30] that is asymptotically equivalent to the Cox regression model with time-dependent covariates, given short time intervals and low probability of the outcome within the interval, as in this study.

Exposure categories were updated every 2 years in all analyses. In multivariable analyses, CVD risk factors were included in the model (see Table 1 footnote). We conducted further analyses to adjust for dietary variables that are related to risk of stroke, including specific fatty acids, protein, and cereal fiber.

Because of the long follow-up period, dietary variables were updated and the cumulative average of nutrient intake was calculated from the dietary questionnaires administered in 1980, 1984, 1986, 1990, and 1994. Incident cases of stroke documented between each diet questionnaire cycle were examined in relation to the average diet calculated from all the preceding diet measures. Cumulative averaging reduces within-person variation and thus can better represent long-term intake.[31] In secondary analyses, we used simple updated intake of folate (updated to the most recent questionnaire) to assess short-term exposure. Because change in diet after development of intermediate conditions such as angina, hypercholesterolemia, and diabetes may confound the exposure–disease association, we stopped updating diet and covariates at the beginning of the time interval during which individuals developed those intermediate end points. Excluding hypercholesterolemia and diabetes at baseline did not change the results.

Mantel extension tests for trend[32] were obtained by assigning the median value for each category and modeling this as a continuous variable. All *P* values are 2-sided.

Results

During 1 379 614 person-years of follow-up, we identified 1140 incident cases of stroke. Folate intake was unrelated to age, BMI, calories, or alcohol intake, but was inversely associated with intake of saturated and transfatty acids and prevalence of smoking, and positively associated with use of vitamin E supplements and the likelihood of exercising.

Table I (available online at http://stroke.ahajournals.org) shows the characteristics of our study population at baseline according to folate quintiles. The age-adjusted models for total stroke (fatal and nonfatal) suggested a significant protective trend (Table 1). However, these became close to null and the trends became nonsignificant in the multivariable-adjusted models. In multivariable analyses, folate intake was also not significantly associated with specific subtypes of stroke (Table 2). Further analyses using simple update of folate intake during follow-up did not change the overall conclusions.

Dietary folate intake (excluding supplements) and folate supplement intake were not significantly related to risk of stroke (Table II; available online at http://stroke.ahajournals.org). There was no significant interaction between folate and alcohol intake (*P* for interaction=0.1). When premenopausal women were excluded from the analyses and the analyses stratified by never-use or ever-use of hormonal replacement therapy, the association between folate intake and total or ischemic incidence of stroke remained nonsignificant.

Discussion

In this prospective cohort study, we found no significant association between intake of folate and risk of stroke after adjusting for CVD risk factors (including other nutrient variables). Total, dietary, and supplemental folate intake were not associated with total stroke or its subtypes. This finding was consistent when using different definitions of folate intake, which was updated 5 times during the follow-up. Recall bias would not have influenced our results because all the dietary data were collected prospectively. Food frequency questionnaires are subject to inaccuracies, which might account for a null finding. However, our questionnaire has been found to be of good validity.[22] Using the same dietary assessment, folate intake was associated with reduced incidence of coronary heart disease.[33] Thus, the lack of observed association cannot be attributed to our inability to measure folate intake. In addition, folate fortification cannot account for the null results observed because fortification began after the time period of the study. Although we adjusted for possible confounders in the association between folate intake and CVD, there is still the possibility of residual or unmeasured confounding.

TABLE 1. Age- and Multivariable*-Adjusted Relative Risks of Total, Fatal, and Nonfatal Stroke According to the Quintiles of Total Folate Intake

	Quintiles of Total Folate Intake and Their Range (μg/d)					*P* for trend
	1	2	3	4	5	
	30–210	211–271	272–354	355–526	>526	
Total Stroke (fatal and nonfatal)						
N of cases	235	219	238	217	231	
Age-adjusted RR	1.00	0.87 (0.73–1.05)	0.89 (0.74–1.06)	0.79 (0.66–0.96)	0.83 (0.69–0.99)	*P*=0.06
Multivariable RR	1.00	0.99 (0.82–1.20)	1.09 (0.89–1.33)	1.01 (0.81–1.25)	0.99 (0.78–1.25)	*P*=0.8
Multinutrient RR†	1.00	1.03 (0.85–1.25)	1.14 (0.92–1.40)	1.04 (0.83–1.31)	1.01 (0.79–1.29)	*P*=0.8
Fatal Stroke						
N of cases	37	39	39	30	41	
Age-adjusted RR	1.00	1.20 (0.77–1.89)	1.27 (0.81–1.99)	1.03 (0.64–1.68)	1.17 (0.75–1.83)	*P*=0.8
Multivariable RR	1.00	1.30 (0.82–2.07)	1.48 (0.91–2.40)	1.14 (0.66–1.99)	1.10 (0.61–1.97)	*P*=0.9
Multinutrient RR†	1.00	1.32 (0.82–2.11)	1.52 (0.92–2.51)	1.17 (0.66–2.08)	1.14 (0.62–2.07)	*P*=0.9
Nonfatal Stroke						
N of cases	198	180	199	187	190	
Age-adjusted RR	1.00	0.81 (0.66–1.00)	0.82 (0.67–1.00)	0.74 (0.61–0.91)	0.77 (0.63–0.94)	*P*=0.03
Multivariable RR	1.00	0.94 (0.76–1.16)	1.03 (0.83–1.28)	0.98 (0.77–1.24)	0.96 (0.74–1.25)	*P*=0.8
Multinutrient RR†	1.00	0.97 (0.79–1.21)	1.07 (0.86–1.34)	1.02 (0.79–1.30)	0.97 (0.74–1.28)	*P*=0.8

*Age (5-year categories); time period (2-year interval); smoking history (never smoker, past smoker, current smoker [1 to 14, 15 to 24, and ≥25 cigarettes/day]); body mass index (BMI): <23, 23 to 24.9, 25 to 26.9, 27 to 29.9, 30 to 32.9, ≥33 kg/m^2; hormone use and menopausal status (premenopausal, postmenopausal never user, postmenopausal current user, postmenopausal past user); currently taking aspirin (yes, no); vitamin E supplements (quintiles); physical activity (<1 h/wk, ≥1 to <2 h/wk, ≥2 to <4 h/wk, ≥4 to <7 h/wk, ≥7 h/wk of moderate to vigorous activity); alcohol use (0 g/day, 0.1 to 4.9 g/day, 5.0 to 14.9 g/day, ≥15.0 g/day); history of high blood pressure, history of diabetes; history of hypercholesterolemia; parental history of myocardial infarction at or before the age of 65 years; total caloric intake (quintiles).

†The aforementioned covariates plus nutrient variables (transfatty acid, *P* ratio, monosaturated fatty acids, total protein intake, cereal fiber, omega-3 fatty acid, α linolenic acid).

Our study did not have the power to compare extreme values of folate intake (>800 μg/d) in relation to risk of stroke, but ongoing clinical trials will be able to provide evidence on the relation of very high levels of supplemental intake and the risk of stroke. The possibility of a nonlinear relation between folate and stroke and the relatively few numbers of women with very low folate intake might explain our failure to detect such an association.

The hypothesized relation between folate and stroke is believed to be mediated by homocysteine levels,[16,34] primarily because folate supplementation lowers homocysteine levels.[14,15,35] There is evidence linking homocysteine with stroke,[2–4,7] and 2 recent meta-analyses suggested that the risk of stroke might be reduced by 19% to 24% if serum homocysteine levels were lowered by 3 μmol/L,[36,37] and a third meta-analyses found relative risk (RR) of 1.37 (95% CI: 0.99 to 1.99) of ischemic stroke among individuals with high homocysteine levels.[38] However, 4 cohort studies have failed to find a significant positive association,[10–13] and this relationship was not evident even among folate-deficient patients in 1 study of subarachnoid stroke.[39] More recently, a randomized controlled trial failed to find a difference in recurrence of stroke between low- and high-dose vitamin intake (folate, vitamin B6, and vitamin B12) through lowering of homocysteine levels.[40]

Most of the studies that have examined the direct relationship between folate and stroke were based on serum folate levels[17–19,34] and only 2 recent cohort studies assessed the intake of folate in relation to the risk of stroke.[20,41] However, serum and dietary folate are well correlated. In the first National Health and Nutrition Examination Survey (NHANES 1) cohort,[18] there was a small and nonsignificant elevation in the risk of stroke for those with folate serum levels ≤9.2 nmol/L compared with those with >9.2 nmol/L (RR=1.37; 95% CI: 0.82 to 2.29). The Bronx aging cohort study found no association between serum folate levels and incidence of stroke.[17]

In the Canadian Study of Health and Aging, Maxwell et al[19] found an increased risk of cerebrovascular events (including vascular dementia, vascular cognitive impairment, and fatal stroke) among subjects in the lowest folate quartile compared with the highest quartile (odds ratio [OR]: 2.42; 95% CI: 1.04 to 5.61). After further follow-up of NHANES I, Bazzano et al[20] used dietary intake of folate instead of serum folate levels and followed-up 9764 men and women aged 25 to 75 years for an average of 19 years, with 926 incident cases documented. They reported RR of 0.79 (95% CI: 0.63 to 0.99) comparing extreme quartiles, (*P* for trend=0.03). NHANES I used only baseline folate intake from the early 1970s using a single 24-hour recall questionnaire and did not update this intake during follow-up. In addition, the NHANES I sample size included men and women who were older in age compared with our study participants. Homocysteine may be a stronger risk factor for stroke in the elderly,[35]

TABLE 2. Multivariable*-Adjusted Relative Risks of Specific Subtypes of Stroke According to the Quintiles of Total Folate Intake

	Quintiles of Total Folate Intake and Their Range (μg/d)					*P* for trend
	1	2	3	4	5	
	30–210	211–271	272–354	355–526	>526	
Ischemic Stroke						
N of cases	122	118	129	112	120	
Multivariable RR	1.00	1.01 (0.78–1.32)	1.11 (0.84–1.46)	1.00 (0.74–1.36)	1.03 (0.74–1.43)	*P*=1.0
Thrombotic Stroke						
N of cases	59	68	74	64	58	
Multivariable RR	1.00	1.20 (0.84–1.73)	1.29 (0.89–1.89)	1.14 (0.75–1.74)	1.01 (0.64–1.61)	*P*=0.7
Embolic Stroke						
N of cases	18	24	23	18	27	
Multivariable RR	1.00	1.25 (0.66–2.36)	1.14 (0.58–2.22)	0.84 (0.39–1.81)	1.13 (0.52–2.46)	*P*=0.9
Hemorrhagic Stroke						
N of cases	24	25	21	23	21	
Multivariable RR	1.00	1.20 (0.67–2.14)	1.05 (0.56–1.98)	1.32 (0.67–2.58)	1.22 (0.56–2.66)	*P*=0.7
Subarachnoid Stroke						
N of cases	32	39	25	35	35	
Multivariable RR	1.00	1.36 (0.83–2.21)	0.92 (0.52–1.61)	1.15 (0.65–2.04)	0.95 (0.51–1.76)	*P*=0.6

*Covariates are the same as in Table 1.

which may explain their finding of a protective effect of folate intake. Another factor might be the lower median level of folate intake among the NHANES I (203 μg/d) compared with our median intake level (308 μg/d). Folate intake has increased among the US population since 1970, and a folate-deficient state associated with risk may be less common now. The other cohort study that used folate intake in relation to stroke was only among men.[41] In that study, there was an inverse relation between folate intake and the risk of ischemic stroke, especially in the highest quintile (RR: 0.66; 95% CI: 0.45 to 0.98; *P* for trend=0.04). We have no explanation why folate was inversely related to ischemic stroke in men but not in women, but exposure–disease associations can vary by gender and further studies are needed to investigate this difference for the association between folate and stroke.

In conclusion, our results suggest that folate intake does not have an important relation to the risk of stroke in women. More results from clinical trials will shed further light on this relationship.[42] Regular physical exercise, a diet rich in fruits, vegetables, and whole grains, not smoking, and other lifestyle modifications are likely to decrease the overall risk of cardiovascular diseases, including stroke; however, based on our results, there is no evidence for a benefit of folate supplementation in the context of the US diet.

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