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Folate, Vitamin B₆, and B₁₂ Intakes in Relation to Risk of Stroke Among Men

Ka He, MD; Anwar Merchant, DMD; Eric B. Rimm, ScD; Bernard A. Rosner, PhD;
Meir J. Stampfer, MD; Walter C. Willett, MD; Alberto Ascherio, MD

Background and Purpose—Folate, vitamin B₆, and B₁₂ deficiency are related to elevated blood homocysteine level. However, the effects of intakes of these vitamins on risk of stroke are still uncertain. This study examines intakes of folate, vitamin B₆, and B₁₂ in relation to risk of ischemic and hemorrhagic stroke.

Methods—We enrolled 43 732 men, aged 40 to 75 years, who were free of cardiovascular diseases and diabetes at baseline. Participants were followed from 1986 to 2000. Dietary information was assessed every 4 years using a detailed and validated semiquantitative food frequency questionnaire. The main outcome measures were incident ischemic and hemorrhagic strokes.

Results—A total of 725 incident strokes, including 455 ischemic, 125 hemorrhagic, and 145 unknown types of stroke, were documented during the 14-year follow-up. After adjustment for major lifestyle and dietary factors, intake of folate was associated with a significantly lower risk of ischemic but not hemorrhagic stroke. The multivariate relative risk of ischemic stroke was 0.71 (95% CI, 0.52 to 0.96; *P* for trend=0.05) for men in the highest quintile of intake compared with those who in the lowest quintile. Intake of vitamin B₁₂, but not B₆, was also inversely associated with risk of ischemic stroke.

Conclusions—Our findings suggest that increased folate intake is associated with decreased risk of ischemic stroke in men. (*Stroke*. 2004;35:169-174.)

Key Words: cerebrovascular accident ■ folic acid ■ vitamin B₆ ■ vitamin B₁₂

Abundant evidence has accumulated supporting the association between blood homocysteine level and risk of cardiovascular disease. Elevated homocysteine levels may reflect genetic defects¹; dietary factors including low intakes of folate, vitamin B₆, and B₁₂²; or renal failure. However, data directly relating intakes of these B vitamins with risk of stroke are sparse.³ In addition, most previous studies had only baseline dietary assessment and did not distinguish between hemorrhagic and ischemic strokes, which have different causes. Therefore, we examined prospectively intakes of folate and vitamins B₆ and B₁₂ in relation to incidence of ischemic and hemorrhagic stroke in a large cohort of US men with an average 14 years of follow-up.

Subjects and Methods

Study Population

The Health Professional Follow-up Study was established in 1986 when 51 529 male US health professionals, aged 40 to 75 years, answered a detailed questionnaire on medical history, lifestyle, and diet. Participants were mailed questionnaires in every other year to update information on potential risk factors and identify new cases of

diseases. At baseline, we excluded men with a history of cardiovascular diseases or diabetes mellitus. We also excluded men with inadequate dietary data (daily caloric intake <800 or >4200 kcal or ≥70 blank items out of 131 listed food items in the baseline questionnaire). These exclusions left a total of 43 732 men for the analyses. The study protocol was approved by the Harvard School of Public Health Institutional Review Board; the response to the questionnaires constituted the participants' informed consent.

Dietary Assessment

Dietary information was assessed in 1986, 1990, and 1994 through the semiquantitative food frequency questionnaires (FFQ).⁴ The FFQ contains a commonly used portion size of food such as 1 cup of cooked spinach or 1 apple. Participants were asked to record the frequency of consuming specified portions of each selected food during the previous year. There were 9 possible responses ranging from "never or <1/mo" to "≥6/d." Nutrient intakes were calculated by multiplying the average nutrient content of the specified portion size by the frequency it was consumed. Values for the amounts of nutrients in the foods were obtained from the Harvard University food composition database, which was derived from US Department of Agriculture sources, manufacturers, or published reports. Data for folate intake were all prefortification.

The FFQ's validity has been evaluated previously. The deattenuated correlation between the FFQ and diet records was 0.77 for

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From the Departments of Nutrition (K.H., A.M., E.B.R., M.J.S., W.C.W., A.A.), Epidemiology (E.B.R., M.J.S., W.C.W., A.A.), and Biostatistics (B.A.R.), Harvard School of Public Health, Boston, Mass; Channing Laboratory (E.B.R., B.A.R., M.J.S., W.C.W., A.A.), Brigham and Women's Hospital, Harvard Medical School, Boston, Mass; and Department of Preventive Medicine (K.H.), Northwestern University Feinberg School of Medicine, Chicago, Ill.

Correspondence and reprint request to Dr Ka He, Department of Preventive Medicine, Northwestern University Feinberg School of Medicine, 680 North Lake Shore Dr, Suite 1102, Chicago, IL 60611. E-mail kahe@northwestern.edu

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folate, 0.85 for vitamin B₆, and 0.56 for vitamin B₁₂.⁵ The correlation between the FFQ and serum concentration was 0.63 for folate and 0.37 for vitamin B₁₂.⁴ The FFQ has been also used in the Framingham Heart Study, in which dietary and blood levels of folate had similar relation with blood homocysteine.⁶

Outcome Assessment

End points were all incident fatal and nonfatal strokes occurring between the return of the 1986 questionnaire and the end of follow-up on January 31, 2000. A physician, blinded to risk factor status, reviewed participants' medical records if they reported incident stroke in any of the follow-up questionnaires. Most of cases were diagnosed with neuroimaging (CT/MRI). Fatal cases were identified by next of kin, colleagues, postal authorities, or by a search of the National Death Index. All deaths were confirmed by medical records, autopsy reports, or death certificates. We classified cases into ischemic (embolism or thrombosis), hemorrhagic (subarachnoid and intracerebral), or unknown type of stroke according to the criteria of the National Survey of Stroke.⁷

Statistical Analyses

Each participant contributed follow-up time from the date of return of the baseline questionnaire to the date of the first stroke, death, or end of the follow-up period, whichever came first. Participants were divided into quintiles according to their intakes of B vitamins. Incidence rates were measured as the number of cases divided by the person-time of follow-up in each quintile. Relative risks (RR) were estimated by comparing the incidence of stroke in a particular dietary intake quintile with that of the lowest one.

We used cumulative average of nutrient intakes in the analyses to best represent long-term dietary intake and reduce within-subject variation.⁴ We also used the baseline diet and the most recent diet to determine the different effects between long-term and short-term diet intake. The detail of this methodological issue has been previously described.⁸ Because changes in diet were likely to occur when participants developed some intermediate diseases, we stopped updating individual diet information if the participant had any of the following diseases: diabetes mellitus, coronary heart disease, transient ischemic attack, and peripheral arterial disease.

We estimated incidence rates and 95% CI of stroke by stratifying age (5-year categories) and cigarette smoking status using the Mantel-Haenszel method.⁹ To further adjust for other covariates, we calculated RRs and 95% CIs by using Cox proportional hazards models with age (months) as the time variable. We adjusted for cigarette smoking; body mass index (BMI); physical activity; history of hypertension and hypercholesterolemia; aspirin use; and intakes of alcohol, fiber, potassium, vitamin E, and total energy. All nutrient intakes were energy-adjusted, and total energy intake was included in all regression models.⁴ The Mantel extension test was used to test for linear trends,¹⁰ and the median values for each quintile were included in the model as a continuous variable.

Results

We documented 725 incident cases including 455 ischemic, 125 hemorrhagic, and 145 unknown types of stroke among the 43 732 men during the 14-year follow-up.

Age-adjusted baseline characteristics of the study population according to the highest and the lowest quintile of folate, vitamin B₆, and vitamin B₁₂ intakes (including supplements) are presented in Table 1. Men with higher folate intake exercised more, were less likely to be current smokers or overweight, and were more likely to use aspirin regularly. However, they drank less and had slightly higher proportions of diagnosed hypercholesterolemia. As expected, men with higher intake of vitamin B₆ showed similar characteristics as those who had higher folate intake. Also, men with higher

intakes of folate, vitamin B₆, or vitamin B₁₂ were more likely to take vitamin supplements.

In the age- and smoking-adjusted analyses, men in the highest quintile of folate intake had a significantly lower risk of ischemic stroke than those who were in the lowest quintile (RR=0.68; 95% CI, 0.50 to 0.92; *P* for trend=0.03) (Table 2). These associations were not altered appreciably after adjustment for other major lifestyle risk factors (RR=0.71; 95% CI, 0.52 to 0.96; *P* for trend=0.05). Moreover, these associations persisted after further controlling for fiber, potassium, and vitamin E intakes (RR=0.66; 95% CI, 0.45 to 0.98; *P* for trend=0.04). Furthermore, folate intake was not related to risk of hemorrhagic stroke.

Intakes of vitamin B₆ or B₁₂ were not significantly associated with risk of ischemic or hemorrhagic stroke with adjustment for age and cigarette smoking in this cohort. However, in the multivariate analysis, vitamin B₁₂ intake was inversely associated with ischemic but not hemorrhagic stroke. Comparing men in the highest quintile of vitamin B₁₂ intake with those in the lowest, the multivariate RR was 0.73 (95% CI, 0.53 to 0.99; *P* for trend=0.04), and the relation remained with additional adjustment for fiber, potassium, and vitamin E intakes (Table 2).

The repeated diet assessments enabled us to examine various temporal associations between intakes of these three B vitamins and risk of stroke. The relations between folate or vitamin B₁₂ and risk of stroke were similar for baseline and most recent diet, and as expected, cumulative average diet showed the strongest association.^{4,11} For vitamin B₆, baseline, most recent and cumulative average diet provided similar associations (Table 3).

Intakes of folate and vitamin B₆ were highly correlated (*r*=0.77), largely because the major sources of folate and vitamin B₆ were multivitamin supplements.¹² Because our ability to determine their independent effects was limited by this high correlation, we examined dietary and supplemental folate separately in relation to risk of ischemic stroke, but neither association was significant (data not shown). In addition, a previous study suggested that alcohol consumption and folate intake jointly affected blood homocysteine level and risk of coronary heart disease.¹² We therefore examined the joint relation of alcohol consumption and folate intake to risk of ischemic or hemorrhagic stroke, but no apparent interaction was observed (data not shown).

Discussion

In this large prospective follow-up study, men in the highest quintile of folate intake had an approximately 30% lower risk of ischemic stroke than those in the lowest quintile. Intake of vitamin B₁₂ but not B₆ was also inversely related to risk of ischemic stroke. No significant associations with risk of hemorrhagic stroke were observed.

We considered the possibility that the apparent beneficial effect of folate intake on ischemic stroke could be explained by other health-related factors because men with higher folate intake had an overall healthier lifestyle than those with relatively low folate intake. However, the similar results from both age- and smoking-adjusted and multivariate analyses largely reduced this likelihood. In addition, because vitamin

TABLE 1. Age-Adjusted Baseline Characteristics of Study Population (n=43 732) by Lowest and Highest Quintile of Energy-Adjusted Total Folate, Vitamin B₆, and B₁₂ Intake in 1986

	Folate		Vitamin B ₆		Vitamin B ₁₂	
	Quintile 1	Quintile 5	Quintile 1	Quintile 5	Quintile 1	Quintile 5
Mean age, y	52 (9.2)	55 (9.6)	52 (9.1)	55 (9.6)	53 (9.6)	55 (9.5)
Risk factors						
Current smoking, %	15.8	8.3	14.4	9.4	9.0	11.6
Overweight (BMI>25), %	56.6	46.5	54.7	46.5	48.1	52.5
Hypertension, %	19.9	20.0	18.8	20.2	20.3	19.5
Hypercholesterolemia, %	9.2	11.8	8.2	12.4	11.7	10.1
Aspirin use, %	24.0	30.5	22.3	31.3	23.1	28.8
Antihypertensive medication use, %						
Thiazide diuretics	8.3	10.4	7.6	10.0	8.5	10.4
β-blocker	6.8	7.7	6.1	7.7	7.2	7.8
Other	3.0	3.6	2.9	3.5	3.6	3.2
Alcohol, g/d	13.2 (17.9)	10.7 (14.2)	12.1 (15.9)	11.4 (15.5)	12.6 (17.2)	10.4 (14.3)
Physical activity, METs/wk	16.3 (24.9)	25.1 (32.8)	16.6 (23.2)	24.1 (32.1)	21.5 (30.4)	22.2 (31.9)
Dietary daily intake, mean (SD)						
Total, kcal	1898 (618)	1915 (583)	1816 (581)	1934 (600)	1919 (607)	1908 (603)
Potassium, mg*	2920 (544)	3736 (823)	2968 (536)	3683 (821)	3309 (734)	3535 (775)
Vitamin B ₆ , mg*	3.5 (11.9)	22.4 (43.4)	1.7 (0.2)	33.4 (49.2)	4.0 (14.6)	19.8 (40.8)
Vitamin B ₁₂ , μg*	8.6 (5.5)	21.9 (33.4)	8.1 (4.9)	22.8 (36.0)	5.0 (1.1)	29.2 (35.0)
Vitamin E, mg*	43.9 (136.3)	225.0 (278.8)	24.9 (96.3)	280.5 (295.0)	45.5 (135.6)	186.9 (277.1)
Vitamin C, mg*	228.6 (314.0)	816.7 (604.6)	200.1 (245.2)	958.6 (613.6)	296.7 (336.8)	704.9 (643.0)
Folic acid, μg*	237 (36.1)	936 (275.9)	282 (85.7)	745 (404.8)	345 (120.7)	725 (400.9)
Dietary fiber*	16.0 (4.4)	23.5 (8.5)	16.9 (4.5)	22.8 (8.1)	21.6 (7.6)	20.9 (7.5)
Vegetables, servings	2.0 (1.0)	3.4 (2.0)	2.2 (1.1)	3.3 (1.9)	3.1 (1.8)	3.1 (1.8)
Fruits, servings	1.4 (1.0)	2.8 (1.8)	1.6 (1.1)	2.6 (1.7)	2.5 (1.8)	2.3 (1.5)
Fish, servings/wk	1.7 (1.6)	2.6 (2.2)	1.5 (1.3)	2.7 (2.3)	1.9 (1.6)	2.6 (2.3)
Supplements, %						
Multivitamin	14.1	93.0	4.7	88.7	11.7	70.8
Folic acid	0.0	13.8	0.1	13.2	1.0	7.0
B-complex	4.1	26.3	0.0	50.5	3.5	20.9
Vitamin B ₆	2.8	25.4	0.0	42.8	3.7	19.5

METs indicates metabolic equivalent tasks.

*Adjusted for total calorie intake.

supplements were the major source of folate, residual confounding from some other constituents of the supplements could not be excluded. Presumably, the magnitude would be small since no significant inverse associations for other micronutrients in vitamin supplements such as vitamin E, vitamin C, and carotenoids were observed in this cohort,¹³ and further adjustment for fiber, potassium, and vitamin E intakes did not appreciably change our findings.

Another concern is inaccurate dietary assessment. The FFQ that we used in the dietary assessment has been evaluated and reasonably reflects long-term diet intakes.⁴ The inverse associations that we found in our cohort between intakes of folate, vitamin B₆, and vitamin B₁₂ and risk of coronary heart disease¹⁴ further support the validity of our estimates of intakes. In addition, we reduced error in dietary assessment by using repeated measurements. Participants might change their diets after they developed some intermediate diseases,

and these changes would possibly attenuate an association between intake of folate, vitamin B₆, or vitamin B₁₂ and risk of stroke. To reduce the magnitude of bias from this source, we excluded men with history of cardiovascular diseases and diabetes at baseline and stopped updating an individual's dietary information once the participant reported any of the intermediate diseases during the follow-up period. In fact, the associations with folate were just slightly weaker when using baseline or the most recent diet compared with that from cumulative average diet. Finally, our findings were unlikely to be explained by recall or selection bias because of the prospective study design and high follow-up rate.

Whereas both folate and vitamin B₁₂ intakes were inversely related to risk of ischemic stroke, vitamin B₆ intake did not show clear association with stroke in this cohort. A possible explanation is that the amount of vitamin B₆ intake in the reference group approached the maximal benefit, and higher

TABLE 2. Relative Risks of Stroke and 95% Confidence Intervals According to Quintiles of Folate, Vitamin B₆, and B₁₂ Intakes

	Quintiles of Nutrient Intake					P for Trend
	1	2	3	4	5	
Folate						
Median intake, $\mu\text{g}/\text{d}$	262	336	413	547	821	
Ischemic stroke						
No. of cases	99	98	75	104	79	
Age/smoking-adjusted	1.0	0.97 (0.73–1.28)	0.72 (0.53–0.97)	0.94 (0.71–1.25)	0.68 (0.50–0.92)	0.03
Multivariate model 1*	1.0	0.97 (0.72–1.29)	0.74 (0.54–1.01)	0.97 (0.73–1.30)	0.71 (0.52–0.96)	0.05
Multivariate model 2†	1.0	1.00 (0.74–1.36)	0.75 (0.53–1.06)	0.96 (0.68–1.35)	0.66 (0.45–0.98)	0.04
Hemorrhagic stroke						
No. of cases	22	27	27	30	19	
Age/smoking-adjusted	1.0	1.24 (0.70–2.18)	1.13 (0.63–2.03)	1.36 (0.78–2.35)	0.74 (0.39–1.41)	0.53
Multivariate model 1*	1.0	1.18 (0.67–2.11)	1.26 (0.70–2.27)	1.15 (0.65–2.05)	0.79 (0.42–1.50)	0.27
Multivariate model 2†	1.0	1.28 (0.71–2.32)	1.49 (0.79–2.83)	1.31 (0.67–2.55)	0.86 (0.40–1.88)	0.37
Vitamin B₆						
Median intake, mg/d	1.8	2.3	2.8	4.2	10.9	
Ischemic stroke						
No. of cases	89	79	83	95	109	
Age/smoking-adjusted	1.0	0.96 (0.71–1.30)	0.89 (0.66–1.21)	0.91 (0.68–1.23)	1.09 (0.82–1.46)	0.55
Multivariate model 1*	1.0	0.94 (0.68–1.28)	0.85 (0.62–1.16)	0.93 (0.69–1.26)	1.10 (0.82–1.48)	0.16
Multivariate model 2†	1.0	1.02 (0.73–1.41)	0.97 (0.68–1.38)	1.16 (0.79–1.71)	1.40 (0.94–2.08)	0.04
Hemorrhagic stroke						
No. of cases	26	26	24	26	23	
Age/smoking-adjusted	1.0	1.09 (0.64–1.86)	0.89 (0.51–1.54)	0.86 (0.50–1.49)	0.74 (0.42–1.32)	0.35
Multivariate model 1*	1.0	1.08 (0.62–1.89)	0.83 (0.46–1.49)	0.86 (0.49–1.52)	0.80 (0.45–1.43)	0.43
Multivariate model 2†	1.0	1.17 (0.66–2.09)	0.91 (0.48–1.74)	0.85 (0.42–1.74)	0.72 (0.34–1.55)	0.33
Vitamin B₁₂						
Median intake ($\mu\text{g}/\text{d}$)	5.0	7.5	10.0	13.3	21.0	
Ischemic stroke						
No. of cases	89	84	101	106	75	
Age/smoking-adjusted	1.0	0.95 (0.71–1.28)	1.05 (0.79–1.40)	1.03 (0.77–1.36)	0.74 (0.54–1.00)	0.14
Multivariate model 1*	1.0	0.97 (0.71–1.31)	1.02 (0.76–1.38)	1.03 (0.77–1.38)	0.73 (0.53–0.99)	0.04
Multivariate model 2†	1.0	0.97 (0.71–1.32)	1.04 (0.77–1.41)	1.04 (0.76–1.43)	0.73 (0.52–1.03)	0.05
Hemorrhagic stroke						
No. of cases	23	21	21	33	27	
Age/smoking-adjusted	1.0	0.94 (0.52–1.70)	0.88 (0.49–1.58)	1.28 (0.75–2.17)	1.03 (0.59–1.79)	0.50
Multivariate model 1*	1.0	0.98 (0.54–1.80)	0.88 (0.48–1.60)	1.26 (0.73–2.18)	1.07 (0.60–1.90)	0.60
Multivariate model 2†	1.0	0.99 (0.54–1.83)	0.91 (0.49–1.68)	1.34 (0.74–2.43)	1.14 (0.61–2.14)	0.53

*Adjusted for body mass index (<21, 21–22.9, 23–24.9, 25–29.9 or ≥ 30), physical activity (quintiles), history of hypertension (yes or no) and hypercholesterolemia (yes or no), smoking status (never, past, and current with 1–14, 15–24 or ≥ 25 cigarettes/d), aspirin use (yes or no), alcohol (0, 0.1–9.9, 10–19.9, 20–29.9 or ≥ 30 g/d) and total calorie (quintiles).

†Further adjusted for intakes (quintiles) of fiber, potassium, and vitamin E.

intake would thus offer no further benefit. In fact, the median intake of vitamin B₆ in the lowest quintile was 1.8 mg/d, which was close to the recommended dietary allowance (2 mg/d). In addition, vitamin B₆ is less strongly correlated with homocysteine level than are folate and vitamin B₁₂.^{15,16} Moreover, since intake of vitamin B₆ was highly correlated with folate intake, it is possible that folate intake confounded the association between vitamin B₆ and ischemic stroke. However, when we analyzed the data by excluding supple-

ment users, the association between vitamin B₆ and ischemic stroke was not materially changed.

An inverse relation between blood concentrations of folate and ischemic stroke was previously identified.¹⁷ In addition, Bazzano and colleagues³ examined the relation between dietary intake of folate and risk of stroke in the First National Health and Nutrition Examination Survey (NHANES I). They found an $\approx 20\%$ reduction of incident stroke comparing participants in the highest quartile of dietary folate intake

TABLE 3. Relative Risks* of Ischemic Stroke and 95% Confidence Intervals According to Quintiles of Folate, Vitamin B₆, and B₁₂ Intakes in Different Models

	Quintiles of Nutrient Intake					P for Trend
	1	2	3	4	5	
Folate						
Median intake, $\mu\text{g}/\text{d}$	262	336	413	547	821	
No. of cases	99	98	75	104	79	
Baseline	1.0	1.00 (0.75–1.33)	0.85 (0.62–1.16)	1.00 (0.74–1.34)	0.78 (0.58–1.06)	0.11
Most recent	1.0	0.84 (0.63–1.13)	0.78 (0.58–1.06)	0.82 (0.61–1.10)	0.80 (0.60–1.07)	0.29
Cumulative average	1.0	0.97 (0.72–1.29)	0.74 (0.54–1.01)	0.97 (0.73–1.30)	0.71 (0.52–0.96)	0.05
Vitamin B₆						
Median intake, mg/d	1.8	2.3	2.8	4.2	10.9	
No. of cases	89	79	83	95	109	
Baseline	1.0	0.90 (0.66–1.23)	0.85 (0.62–1.15)	0.94 (0.70–1.27)	1.06 (0.79–1.41)	0.26
Most recent	1.0	0.94 (0.69–1.27)	0.76 (0.55–1.04)	0.89 (0.66–1.19)	1.02 (0.77–1.36)	0.36
Cumulative average	1.0	0.94 (0.68–1.28)	0.85 (0.62–1.16)	0.93 (0.69–1.26)	1.10 (0.82–1.48)	0.16
Vitamin B₁₂						
Median intake, $\mu\text{g}/\text{d}$	5.0	7.5	10.0	13.3	21.0	
No. of cases	89	84	101	106	75	
Baseline	1.0	0.85 (0.62–1.15)	1.00 (0.75–1.32)	0.95 (0.72–1.27)	0.78 (0.58–1.05)	0.17
Most recent	1.0	0.90 (0.66–1.21)	0.87 (0.65–1.17)	0.83 (0.62–1.11)	0.82 (0.61–1.10)	0.23
Cumulative average	1.0	0.97 (0.71–1.31)	1.02 (0.76–1.38)	1.03 (0.77–1.38)	0.73 (0.53–0.99)	0.04

*Adjusted for covariates cited in Table 2 multivariate model 1.

with those in the lowest quartile (RR, 0.79; 95% CI, 0.63 to 0.99; *P* for trend=0.03). This association may have been diluted by the inclusion of cases of hemorrhagic stroke and is overall consistent with our findings.

Beneficial effects of intakes of folate, vitamin B₆, and vitamin B₁₂ with respect to risk of ischemic stroke are biologically plausible because they are inversely associated with blood homocysteine, high level of which may cause vascular damage via toxic accumulation in endothelial cells and generation of free radicals.^{2,18} A randomized, controlled trial indicated that a regular intake of 100 μg folic acid/d was sufficient to lower homocysteine level in persons at the upper end of the normal range for plasma homocysteine.¹⁹ In the Framingham Heart Study,²⁰ Selhub and colleagues found that homocysteine exhibited strong inverse association with plasma folate and weaker associations with plasma vitamin B₁₂ and pyridoxal-5'-phosphate. In addition, they found that plasma concentrations of folate and pyridoxal-5'-phosphate and folate intake were inversely associated with extracranial carotid stenosis after adjustment for age, sex, and other risk factors. Previous studies have also found that elevated homocysteine level in plasma was an independent risk factor for ischemic stroke.²¹ In the NHANES III, the multivariate odds ratio for nonfatal stroke was 2.3 (95% CI, 1.2 to 4.6), comparing participants in the highest quartile of homocysteine level in plasma with those in the lowest quartile.²² We did not observe any significant association between B vitamins and hemorrhagic stroke, but we could not exclude any important association because the number of case of hemorrhagic stroke was modest.

In conclusion, in this large cohort of US men without history of cardiovascular diseases and diabetes, we found that

increased folate and vitamin B₁₂ intakes were associated with decreased risk of ischemic stroke. These data, and those of other epidemiological and experimental studies, support a beneficial effect of folate intake on risk of ischemic stroke.

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