

Dietary Intake of Homocysteine Metabolism-Related B-Vitamins and the Risk of Stroke: A Dose-Response Meta-Analysis of Prospective Studies

Liyun Chen,¹ Qianwen Li,² Xuexian Fang,¹ Xinhui Wang,¹ Junxia Min,¹ and Fudi Wang¹

¹The First Affiliated Hospital, School of Public Health, Institute of Translational Medicine, Zhejiang University School of Medicine, Hangzhou, China; and

²Department of Nutrition, Precision Nutrition Innovation Center, School of Public Health, Zhengzhou University, Zhengzhou, China

ABSTRACT

Observational studies regarding the putative associations between dietary intake of homocysteine metabolism-related B-vitamins (vitamin B-6, folate, and vitamin B-12) and stroke risk have yielded inconsistent results. Thus, we conducted a systematic meta-analysis of prospective studies in order to examine the relation between the dietary (from diet and supplements) intake of these B-vitamins and the risk of stroke. PubMed and Web of Science were searched for relevant articles published through to 25 February, 2020, and RR of stroke in relation to dietary intake of vitamin B-6, folate, and vitamin B-12 were pooled using a random-effects model. Eleven publications of 12 prospective studies comprising 389,938 participants and 10,749 cases were included in the final analysis. We found that dietary intake of vitamin B-6 and folate were associated with a reduced risk of stroke, and this inverse association remained significant in studies with > 10 y of follow-up periods and among participants without a pre-existing stroke event. A dose-response analysis revealed a linear inverse association between folate and vitamin B-6 intake and the risk of stroke, with a pooled RR of 0.94 (95% CI: 0.90–0.98) and 0.94 (95% CI: 0.89–0.99) for each 100 $\mu\text{g}/\text{d}$ increment in folate intake and 0.5 mg/d increment in vitamin B-6 intake, respectively. In contrast, we found no significant association between dietary vitamin B-12 intake and the risk of stroke, with an RR of 1.01 (95% CI: 0.97–1.06) per 3 $\mu\text{g}/\text{d}$ increase. In conclusion, our findings suggest that increased intake of vitamin B-6 and folate is associated with a reduced risk of stroke, supporting the notion that increasing habitual folate and vitamin B-6 intake may provide a small but beneficial effect with respect to stroke. *Adv Nutr* 2020;11:1510–1528.

Keywords: dietary intake, stroke, B-vitamins, dose-response, meta-analysis

Introduction

Stroke (also known as a cerebrovascular accident or CVA), is one of the leading causes of mortality and morbidity worldwide. In 2017, stroke accounted for 11% of all deaths and caused a global loss of 132 million disability-adjusted life years (1–3). Thus, stroke prevention is clearly an important

and urgent public health issue. Among the variety of preventive strategies studied, homocysteine-lowering therapies have attracted considerable attention, as studies suggested that homocysteine can affect atherosclerosis (4–6); moreover, a growing body of epidemiological evidence suggests an association between elevated homocysteine concentrations and an increased risk of stroke (7–10).

B-vitamins, including vitamin B-6, folate (vitamin B-9), and vitamin B-12, are important regulators of homocysteine metabolism (11). Low intake of these nutrients is associated with increased blood homocysteine concentrations, and taking B-vitamins supplements or consuming foods rich in B-vitamins significantly reduces circulating homocysteine concentrations (12–17). These findings suggest that these B-vitamins might have a beneficial effect with respect to reducing the risk of stroke. Indeed, several systematic reviews of intervention studies provided evidence that

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Supplemental Tables 1–4 and Supplemental Figures 1 and 2 are available from the “Supplementary data” link in the online posting of the article and from the same link in the online table of contents at <https://academic.oup.com/advances/>.

Address correspondence to FW (e-mail: fwang@zju.edu.cn) or JM (e-mail: junxiamin@zju.edu.cn).

Abbreviations used: CVD, cardiovascular disease; IHD, ischemic heart disease; PLP, pyridoxal 5'-phosphate; RCT, randomized controlled trial.

taking supplements containing B-vitamins, particularly folic acid, may be associated with a decreased risk of stroke (18–23). Specifically, the most recent meta-analysis of 12 randomized controlled trials (RCTs) suggested that dietary supplementation with B-vitamins significantly reduces the risk of stroke by 10%, mainly among individuals who are at high risk of developing vascular disease (23).

Despite the results obtained from intervention trials, evidence obtained from observation studies is relatively scarce. Although several epidemiological studies examined the association between dietary intake of B-vitamins and the risk of stroke, the results to date are not entirely consistent (24–27). To the best of our knowledge, no systematic analysis has been performed regarding the putative association between the dietary intake of homocysteine metabolism-related B-vitamins and the risk of stroke in the general population. Thus, we conducted a comprehensive meta-analysis of all published relevant prospective studies in order to investigate this association.

Methods

This meta-analysis was performed in accordance with the Meta-analysis of Observational Studies in Epidemiology (MOOSE) protocol (28).

Search strategy

We systematically searched the PubMed and Web of Science databases for articles published through to 25 February, 2020. The following keywords were used in our search: (vitamin B OR B-vitamins OR vitamin B-6 OR pyridoxine OR vitamin B9 OR folate OR folic acid OR vitamin B-12 OR cobalamin) AND (cardiovascular OR cardiovascular disease OR stroke OR cerebrovascular accident OR cerebrovascular disease OR ischemic stroke OR hemorrhage OR cerebral infarct OR brain ischemic) AND (prospective OR prospectively OR cohort OR longitudinal OR follow-up). No restriction was imposed with respect to the language of the publications. The references cited within the retrieved relevant articles were also reviewed in order to identify additional publications.

Study selection

Studies that satisfied the following criteria were included in our meta-analysis: 1) community-based or population-based prospective design; 2) the exposure was the intake of dietary vitamin B-6, folate, and/or vitamin B-12; 3) the outcome of interest was stroke; and 4) the study reported (or it was possible to calculate) the risk estimate of stroke using an OR, HR, or RR, with the corresponding 95% CI for the highest versus the lowest category. We excluded meta-analyses, review articles, retrospective studies, case-control studies, studies involving nonhuman subjects, nonoriginal studies, and studies lacking sufficient data. To ensure that eligible studies were identified correctly, we used a 2-step selection process (29). Two independent investigators (authors LC and QL) performed an initial screening of all titles and/or abstracts and then evaluated all potentially relevant articles based on the full-text publications.

Data extraction and quality assessment

Data were extracted using a standardized data collection form. Two investigators (authors LC and QL) independently extracted the following information from eligible studies: the first author's last name, publication year, study location, study name, duration of follow-up, participants' age and sex, sample size (i.e. the number of cases and/or participants), the percentage of participants with hypertension, diabetes, and/or pre-existing stroke, the method used to assess dietary B-vitamins intake, validation of B-vitamins in FFQs, exposure source and percentage of participants using dietary supplements, categories of intake, and the covariates adjusted for in the multivariable analysis. We extracted the risk estimates with the most adjustment.

The Newcastle–Ottawa scale was used to assess the risk of bias for each included cohort study (30). This scale assigns a maximum of 9 points to each study, with a score of 0–3, 4–6, and 7–9 indicating low, moderate, and high quality, respectively. To assess the validity and quality of the nutrition-related exposure, we modified the Newcastle–Ottawa scale “ascertainment of exposure” component (31). Specifically, we evaluated the risks of bias regarding information about the dietary assessment instrument, the use of supplements, and vitamin validation of the dietary assessment, using ratings of “A” and “B.” Detailed information regarding the modified instructions is presented in **Supplemental Table 1**. Any discrepancies were resolved through group discussion.

Statistical analysis

RR (with corresponding 95% CI) was used as the common effect size across studies; HRs and ORs were considered to approximate RR. We used the risk estimates of the original studies from multivariable models with the most complete adjustment for potential confounders. For studies that considered the highest category as the reference, we performed a transformation using the method described by Hamling et al. (32). Studies that stratified the data by sex, stroke subgroup, and/or race were treated as separate reports. We used a random-effects model to calculate the summarized RRs and their corresponding 95% CIs for the comparison between the highest and lowest categories of B-vitamins intake (33).

Given the various cut-off points among studies, we performed dose-response analyses for the increased intake of vitamin B-6, folate, and vitamin B-12 using the method recommended by Greenland and Longnecker and the publicly available Stata code written by Orsini et al. (34, 35). We extracted the mean intake (or range) of these B-vitamins in each category, the distribution of cases and participants (or person-years), and RR with 95% CI. The results of the linear dose-response shown in the forest plots are presented for each 100 $\mu\text{g}/\text{d}$ increment in folate intake, 0.5 mg/d increment in vitamin B-6 intake, and 3 $\mu\text{g}/\text{d}$ increment in vitamin B-12 intake. If the number of cases and/or person-years in each category was not reported, the variance-weighted least squares regression model was used to calculate the risk estimate (36, 37). If neither median nor mean intake per

category were provided, the midpoint of the upper and lower boundaries in each category was used as the average intake. If the highest or lowest category was open-ended, the midpoint of the category was estimated by assuming that the width of the category was the same as the next adjacent category. In addition, we evaluated possible nonlinear associations between the intake of dietary B-vitamins and the risk of stroke using restricted cubic splines, with 3 knots at the 10th, 50th, and 90th percentiles of the distribution (38). The *P* value for nonlinearity was calculated by testing the null hypothesis that the coefficient of the second spline was equal to zero.

Heterogeneity among the studies was estimated using the I^2 statistic, with values of 25%, 50%, and 75% representing low, moderate, and high degrees of heterogeneity, respectively (39). We also conducted subgroup analyses in order to explore the potential sources of heterogeneity, stratified by geographic location, sex, duration of follow-up, number of participants, stroke subtype, quality score, exclusion of pre-existing stroke, mean age, dietary assessment, the source of B-vitamins intake (foods only or foods and supplements combined), adjustment model (basic and multivariable), and adjustment for potential confounding factors (including age, BMI, physical activity, alcohol consumption, energy intake, level of education/income, hypertension/blood pressure, and diabetes). Heterogeneity between subgroups was evaluated by meta-regression analysis (40, 41). To test the robustness of each association, sensitivity analyses were conducted by omitting one study at a time.

Possible publication bias was assessed using Egger's test (42) and Begg's test (43), with the results considered to indicate publication bias at $P < 0.10$. In addition, we visually examined funnel plots for asymmetry. If possible, publication bias was indicated, we also used the trim-and-fill method to recalculate the pooled risk estimate (44). All data were analyzed using the statistical software program Stata, version 12.0 (STATA Corp.), and unless stated otherwise, a *P* value < 0.05 was considered significant.

Results

Literature search and study characteristics

The screening and selection process is shown in Figure 1. In brief, our search strategy initially identified 2233 articles in PubMed and 3054 articles in Web of Science. After we excluded duplicate publications and studies that did not meet the inclusion criteria, 11 publications (24–27, 45–51) were included in our meta-analysis. One publication (27) reported the results of 2 separate large-scale prospective cohort studies and was therefore regarded as 2 separate studies, resulting in a final analysis of 12 studies. One study (47) included male smokers who were previously enrolled in an RCT (52). These studies included a total of 389,938 participants and 10,749 stroke cases. Of the 12 included studies, 4 were conducted in the USA (24, 25, 50, 51), 3 were conducted in China (27, 48), 2 were conducted in Finland (45, 47), and 1 study each was conducted in Sweden (46), the Netherlands (26), and Japan

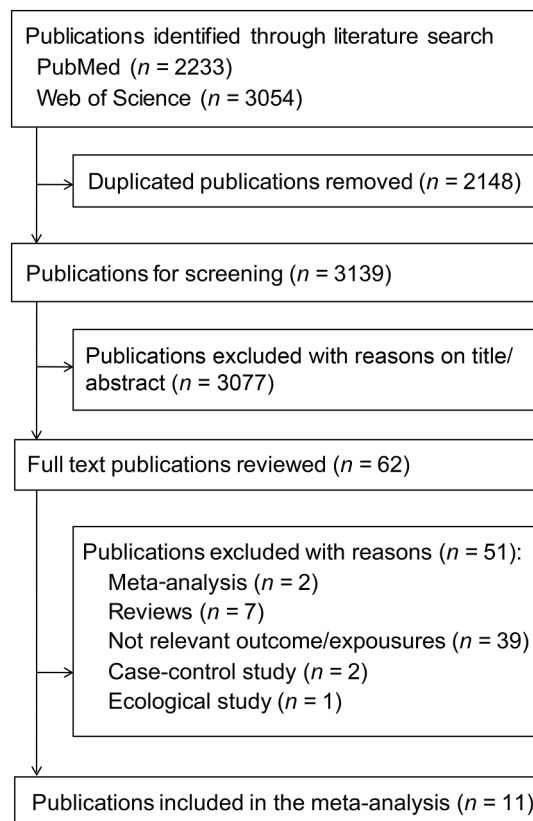


FIGURE 1 Flow chart depicting the literature search and selection strategy.

(49). One study had a nested case-control design (46), and the remaining 11 studies were prospective cohort studies. The studies were published from 2002 through to 2019, and the follow-up period ranged from 4.2 to 19 y. One study (24) assessed dietary B-vitamins intake using 24-h dietary recall, one study (45) performed dietary history interviews, and the remaining 10 studies used a validated FFQ. Three studies used FFQs that validate for B-vitamins intake, whereas others did not. Information regarding the validation of FFQs is provided in **Supplemental Table 2**. Most studies excluded pre-existing stroke at baseline. All 12 studies provided risk estimates adjusted for cigarette smoking, and most studies also adjusted for other conventional risk factors, including BMI, energy intake, alcohol consumption, hypertension, and/or physical activity. Study quality scores ranged from 5 to 9; the mean quality score was 7.4 (**Supplemental Table 3**). The detailed characteristics regarding the studies included in our analysis are summarized in **Tables 1** and **2**.

Dietary vitamin B-6 intake and the risk of stroke

Ten reports in 5 publications (25, 27, 45, 47, 49) evaluated the association between dietary vitamin B-6 intake and the risk of stroke. A significant inverse association was observed for the highest versus the lowest category of dietary vitamin B-6 intake (RR: 0.84; 95% CI: 0.73–0.97; **Figure 2A**), with moderate heterogeneity ($I^2 = 48.8\%$). The RR was 0.91 (95%

TABLE 1 Characteristics of the prospective cohort studies included in the meta-analysis

Author, year (ref)	Location	Study name	Gender	Age (mean), y	Follow-up, y	Cases, n	Participants, n	Participants with hypertension/diabetes/pre-existing stroke (%)
Al-Delaimy et al., 2004 (51)	USA	Nurses' Health Study	F	34–55 (44.5)	18	1140 stroke cases	83,272	15.4/2.0/0
Bazzano et al., 2002 (24)	USA	NHANES I Epidemiologic Follow-up Study (NHEFS)	M/F	25–74 (49)	19	926 stroke cases	9764	27/4.0/0
Cui et al., 2010 (49)	Japan	Japan Collaborative Cohort Study	M/F	40–79 (56)	14	986 stroke cases	58,730	19.6/4.5/0
Dalmeijer et al., 2008 (26)	Dutch	Dutch PROSPECT-EPIC (European Prospective study Into Cancer and nutrition) cohort	F	49–70 (57)	8.1	227 CVA cases	16,165	19.7/2.1/0
He et al., 2004 (25)	USA	Health Professional Follow-up Study	M	40–75 (52–55)	14	725 stroke cases	43,732	20.0/0/0
Larsson et al., 2008 ¹ (47)	Finland	α -Tocopherol, β -carotene Cancer Prevention Study	M	50–69 (57.1–58.1)	13.6	2702 cerebral infarction cases; 383 intracerebral hemorrhage cases; 196 subarachnoid hemorrhage cases	26,556	N.A./6.3/0
Luu et al., 2011 (50)	USA	Atherosclerosis Risk in Communities Study	M/F	45–64 (54)	16.5	594 stroke cases	13,520	33.2/10.7/0
Marniemi et al., 2005 (45)	Finland	Turku Health Survey	M/F	65–99 (77)	10	70 stroke cases	755	N.A./N.A./0
Van Guelpen et al., 2005 (46)	Sweden	Northern Sweden Health and Disease Cohort	M/F	25–74 (55.1)	4.2	334 ischemic stroke cases; 62 hemorrhagic stroke cases	1192	32.6/8.4/N.A.
Weng et al., 2008 (48)	China	Cardiovascular Disease risk Factor 2-township Study	M/F	40–70 (56.6)	10.6	132 stroke cases	1772	23/10.0/0
Zhao et al., 2019 (27)	China	Shanghai Men's Health Study, China	M	40–74 (55.39)	10.3	955 stroke cases	59,746	29.4/6.0/3.8
Zhao et al., 2019 (27)	China	Shanghai Women's Health Study, China	F	40–70 (52.36)	16.2	1317 stroke cases	74,734	24.0/4.4/1.1

¹Larsson et al. (47) included only male smokers who were previously included in an RCT (52). CVA, cerebrovascular accident; F, female; M, male; N.A., not applicable; RCT, randomized controlled trial.

TABLE 2 Characteristics of the prospective cohort studies included in the meta-analysis

Author, year (ref)	Exposure source/percentage of participants using supplements (%)	Categories of intake	Exposure assessment	Adjustment for covariates	Quality score
Al-Delaimy et al., 2004 (51)	Foods + supplements; N.A. (folate)	Folate ($\mu\text{g}/\text{d}$): 30–210, 211–271, 272–354, 355–526, > 526	FFQ, 61 food items	Age, time period, smoking history, BMI, hormone use and menopausal status, currently taking aspirin, vitamin E supplements, physical activity, alcohol use, history of high blood pressure, history of diabetes, history of hypercholesterolemia, parental history of myocardial infarction at or before the age of 65 y, total caloric intake	8
Bazzano et al., 2002 (24)	Foods + supplements; 30% (multivitamin)	Folate ($\mu\text{g}/\text{d}$): <136, 136–203.7, 203.7–300.6, >300.6	24-h dietary recall	Age, race, sex, systolic blood pressure, serum cholesterol, BMI, history of diabetes, physical activity, level of education, regular alcohol consumption, current cigarette smoking, saturated fat intake, and total energy intake	8
Cui et al., 2010 (49)	Foods + supplements; 12.5% (multivitamin)	Vitamin B-6 (mg/d) <0.79 0.79–0.96 0.97–1.11 1.12–1.32 >1.33 Folate ($\mu\text{g}/\text{d}$) 272–351 352–430 431–535 >536 Vitamin B-12 ($\mu\text{g}/\text{d}$) <4.5 4.5–5.9 6.0–7.6 7.7–9.8 >9.9	FFQ, 33 food items	Age, BMI, history of hypertension and diabetes, smoking status, ethanol and energy intakes, as well as intakes of SFAs and n-3 and n-6 PUFAs	7
Dalmeijer et al., 2008 (26)	Foods + supplements; 7.1% (multivitamin)	Folate ($\mu\text{g}/\text{d}$): <169 169–191 191–215 >215 Median intake	FFQ, 178 food items	Age, hypertension, cholesterolemia, mean systolic blood pressure, total physical activity, BMI, smoking, diabetes, intake of energy, proteins, saturated fats, monounsaturated fats, polyunsaturated fats, alcohol, vitamin B-2, vitamin B-6, vitamin B-12, betaine, and choline	8
He et al., 2004 (25)	Foods + supplements; 0%–50.5% (B-complex)	Vitamin B-6 (mg/d) 1.8 2.3 2.8 4.2 10.9 Median intake Folate ($\mu\text{g}/\text{d}$) 262 336 413 547 821	FFQ, 131 food items	Age, BMI, physical activity, history of hypertension and hypercholesterolemia, smoking status, aspirin use, alcohol, and total caloric intakes of fiber, potassium, and vitamin E	9
Larsson et al., 2008 ¹ (47)	Foods; 0%	Vitamin B-6 (mg/d) 1.9 2.2 2.5 2.7 3.0 Folate ($\mu\text{g}/\text{d}$) 262 300 330 360 410	FFQ, 276 food items	Age, supplementation group, total number of cigarettes smoked daily, BMI, systolic and diastolic blood pressure, serum total cholesterol, serum HDL cholesterol, histories of diabetes and coronary heart disease, leisure-time physical activity, and intakes of alcohol and total energy	8

(Continued)

TABLE 2 (Continued)

Author, year (ref)	Exposure source/percentage of participants using supplements (%)	Categories of intake	Exposure assessment	Adjustment for covariates	Quality score
Luu et al., 2011 (50)	Foods + supplements; N.A. (folic acid)	Folate ($\mu\text{g}/\text{d}$): 0–155 156–211 212–278 ≥ 279	FFQ, 66 food items	Age, gender, current smoking status, diabetes, caloric intake, and hypertension	7
Marmiemi et al., 2005 (45)	Foods; N.A. (nutritional)	Lowest tertile Middle tertile Highest tertile	Dietary history interview	Age, gender, smoking, functional capacity, and weight-adjusted energy intake	5
Van Guelpen et al., 2005 (46)	Foods + supplements; 17.4%–28.6% (multivitamin)	Folate ($\mu\text{g}/10^3 \text{ kcal}\cdot\text{d}$) <105 105–134, 134–173, >173(M)	FFQ, 84 food items	BMI, current smoking, cholesterol, diabetes, and hypertension. Plasma homocysteine	6
Weng et al., 2008 (48)	Foods; 0%	Folate ($\mu\text{g}/\text{d}$): <297.33 297.33–369.45 >396.45	FFQ, 49 food items	Age, sex, area, hypertension, use of antihypertensive drugs, diabetes mellitus, central obesity, alcohol consumption habits, smoking habit, sex-smoking habit interaction, BMI, self-report heart disease, hypercholesterolemia, hypertriglyceridemia, physical activity, fibrinogen, apoB, and plasminogen	9
Zhao et al., 2019 (27)	Foods + supplements; 7.73%–13.93% (B-vitamins)	Median intake vitamin B-6 (mg/d): 1.31 1.53 1.69 1.87 2.2	FFQ, 81 food items	Age at baseline, energy intake, education, income, occupation, smoke, alcohol, BMI, waist-hip ratio, physical activity, history of diabetes, hypertension, coronary heart disease, and stroke, vitamin B supplements use, menopausal status, and hormone replacement therapy	7
Zhao et al., 2019 (27)	Foods + supplements; 7.85%–12.8% (B-vitamins)	Median intake vitamin B-6 (mg/d): 1.24 1.45 1.62 1.79 2.11	FFQ, 77 food items	Age at baseline, energy intake, education, income, occupation, smoke, alcohol, BMI, waist-hip ratio, physical activity, history of diabetes, hypertension, coronary heart disease, and stroke, vitamin B supplements use, menopausal status, and hormone replacement therapy	7

¹Larsson et al. (47) included only male smokers who were previously included in an RCT (52).
F, female; M, male; N.A., not applicable; RCT, randomized controlled trial.

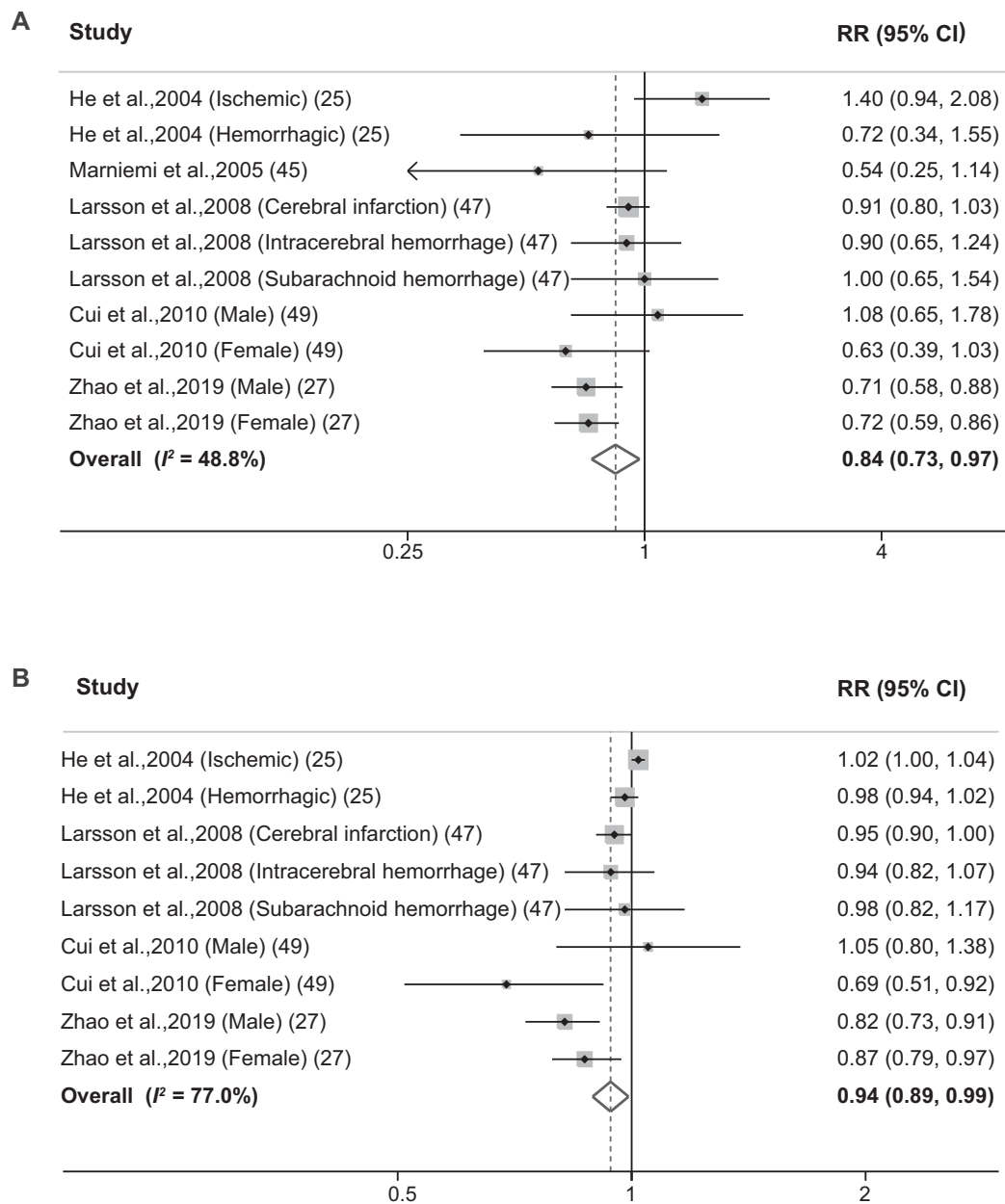


FIGURE 2 Forest plots summarizing the RR with 95% CI of stroke between the highest and lowest categories of dietary vitamin B-6 intake (A) and each 0.5 mg/d increase in dietary vitamin B-6 intake (B).

CI: 0.82–1.02; $I^2 = 0.0\%$) when intake from supplements was excluded and 0.80 (95% CI: 0.65–0.99; $I^2 = 54.1\%$) when intake from foods and supplements was taken into consideration. We found no evidence of publication bias using either Egger's test ($P = 0.966$) or Begg's test ($P = 1.00$), which is consistent with the funnel plot (Supplemental Figure 1A).

One study (45) was considered ineligible for inclusion in the dose-response analysis due to a lack of information regarding the exposure of vitamin B-6 doses in each category. Nevertheless, our analysis based on the 9 reports in the other 4 studies (25, 27, 47, 49) revealed that each 0.5 mg/d

increment in vitamin B-6 intake was associated with a 6% reduction in the risk of stroke (RR: 0.94; 95% CI: 0.89–0.99; Figure 2B), with high heterogeneity ($I^2 = 77.0\%$).

Dietary folate intake and the risk of stroke

Sixteen reports in 10 publications (24–26, 45–51) evaluated the association between dietary folate intake and the risk of stroke. We found that the highest category of dietary folate intake was associated with a 15% lower risk of stroke compared with the lowest category of dietary folate intake (RR: 0.85; 95% CI, 0.78–0.94; Figure 3A), with low heterogeneity ($I^2 = 11.5\%$). Two studies conducted in the USA (25,

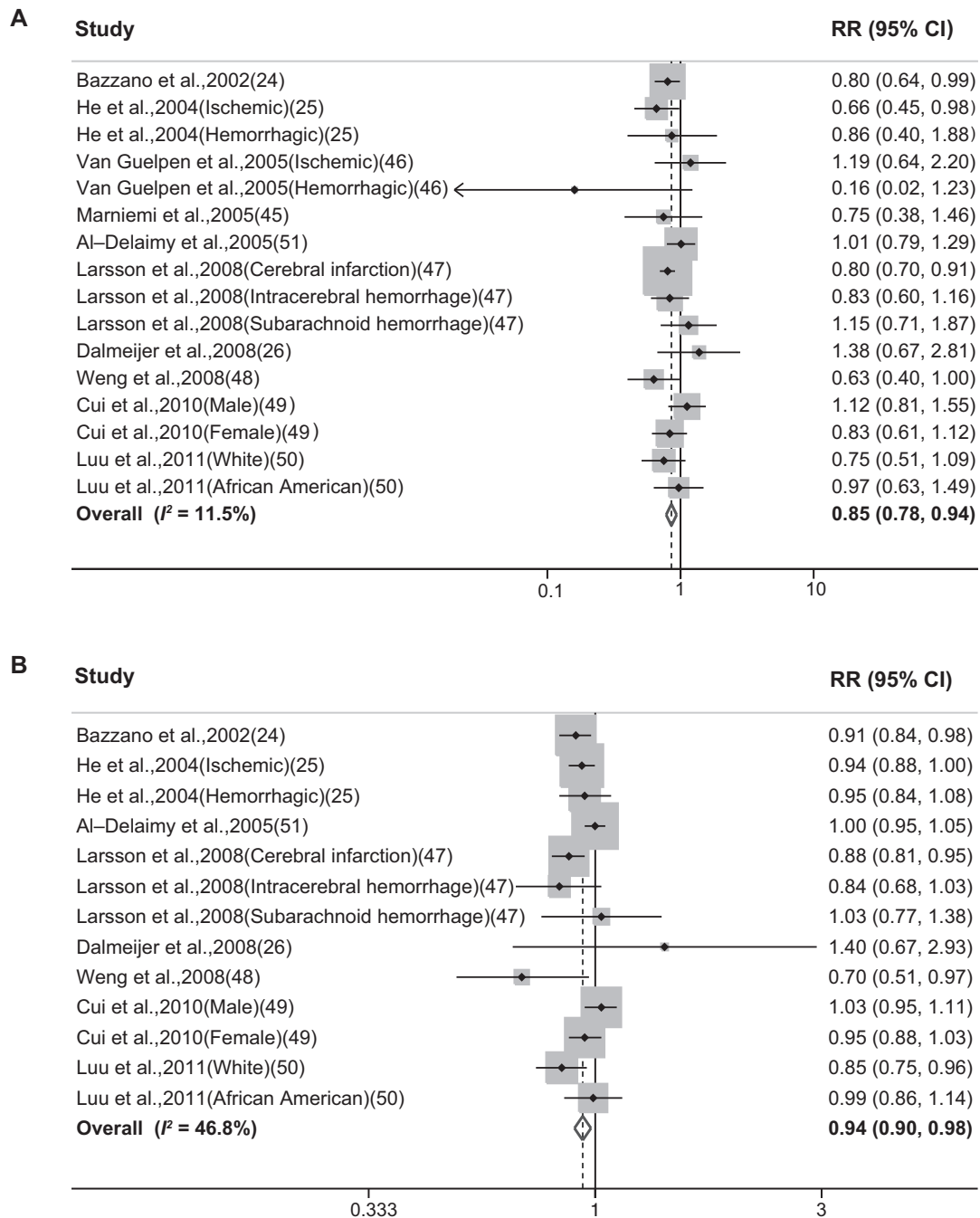


FIGURE 3 Forest plots summarizing the RR with 95% CI of stroke between the highest and lowest categories of dietary folate intake (A) and each 100 $\mu\text{g}/\text{d}$ increase in dietary folate intake (B).

50) included both the prefortification and postfortification periods based on the mandatory fortification of folic acid introduced in 1998. After these 2 studies were excluded, the inverse association remained significant (RR: 0.88; 95% CI: 0.78–0.98; $I^2 = 24.2\%$). One study (24) used 24-h dietary recall to assess diet; after omitting this study, the RR was 0.87 (95% CI: 0.78–0.96). In addition, RR was 0.81 (95% CI: 0.71–0.92; $I^2 = 6.9\%$) for folate intake from foods and 0.88 (95% CI: 0.78–0.99; $I^2 = 11.9\%$) for intake from both

foods and supplements. No evidence of publication bias was found when using either Egger's test ($P = 0.76$) or Begg's test ($P = 0.96$), and the funnel plot was basically symmetrical (**Supplemental Figure 1B**).

A total of 13 reports (24–26, 47–51) were eligible for inclusion in the dose-response analysis, which revealed that for each 100 $\mu\text{g}/\text{d}$ increase in dietary folate intake, the risk of stroke was reduced by 6% (RR: 0.94; 95% CI: 0.90–0.98; **Figure 3B**), with moderate heterogeneity ($I^2 = 46.8\%$).

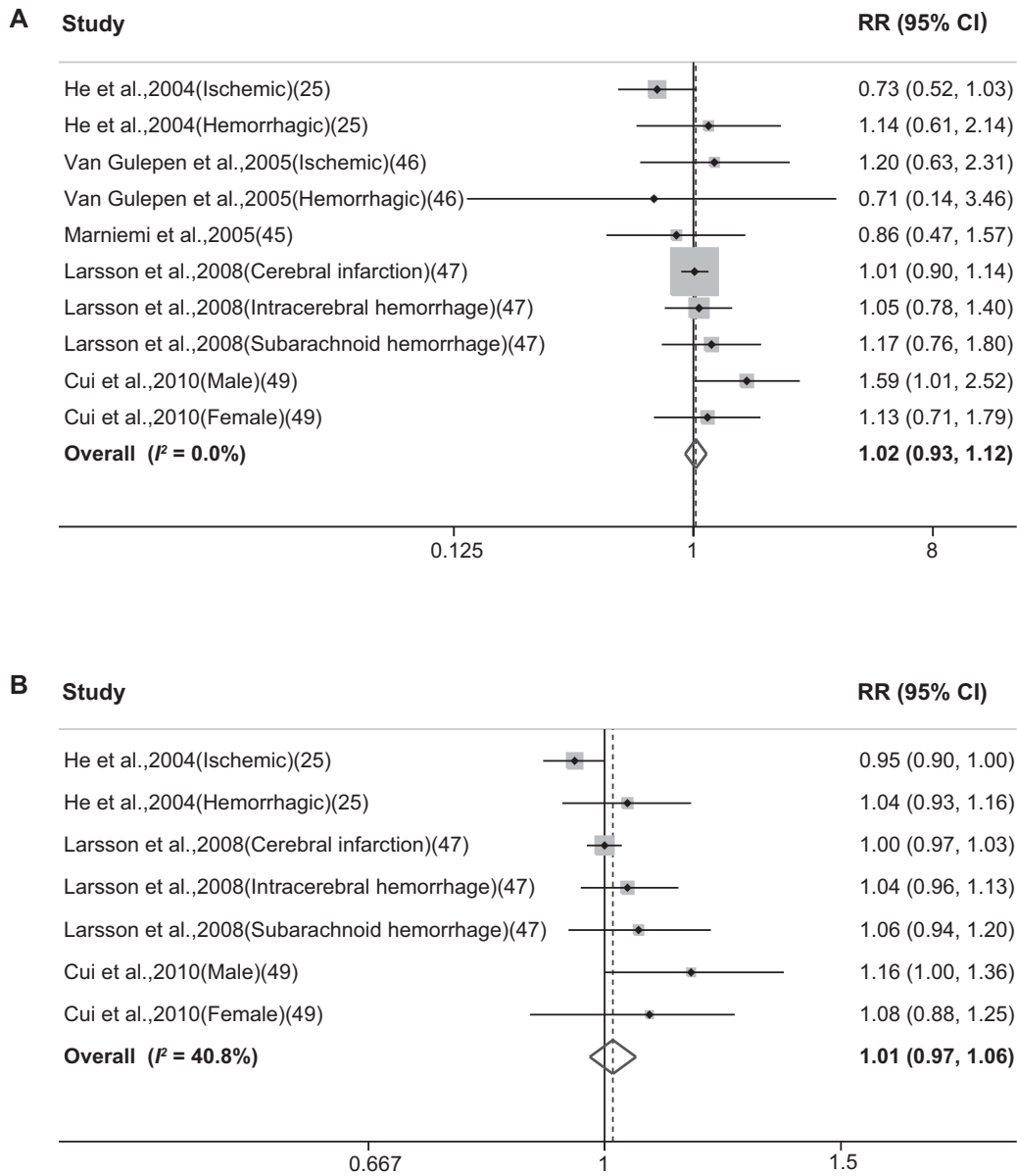


FIGURE 4 Forest plots summarizing the RR with 95% CI of stroke between the highest and lowest categories of dietary vitamin B-12 intake (A) and each 3 $\mu\text{g}/\text{d}$ increase in dietary vitamin B-12 intake (B).

Dietary vitamin B-12 intake and the risk of stroke

Ten reports in 5 publications (25, 45–47, 49) evaluated the association between dietary vitamin B-12 intake and the risk of stroke. Our analysis revealed no significant association between dietary vitamin B-12 intake and the risk of stroke, with a pooled RR for the highest versus the lowest category of vitamin B-12 intake of 1.02 (95% CI: 0.93–1.12; **Figure 4A**), with low heterogeneity ($I^2 = 8.8\%$). Similar results were obtained when we stratified the studies with various subgroups. The RR was 1.02 (95% CI: 0.92–1.13; $I^2 = 0.0\%$) for vitamin B-12 intake from foods and 1.07 (95% CI: 0.81–1.42; $I^2 = 37.8\%$) for B-12 intake from foods and supplements. We also performed a dose-response analysis and found that each 3 $\mu\text{g}/\text{d}$ increase in dietary vitamin

B-12 intake was not significantly associated with an increased risk of stroke (RR: 1.01; 95% CI: 0.97–1.06; **Figure 4B**), with moderate heterogeneity ($I^2 = 40.8\%$).

Dose-response curve between dietary B-vitamins intake and stroke risk

We next assessed the dose-response relation between the intake of dietary B-vitamins and risk of stroke using a restricted cubic spline model. We observed linear associations between the risk of stroke and dietary vitamin B-6 intake ($P = 0.18$ for nonlinearity; **Figure 5A**) or dietary folate intake ($P = 0.21$ for nonlinearity; **Figure 5B**). In addition, no evidence of a nonlinear association was found between

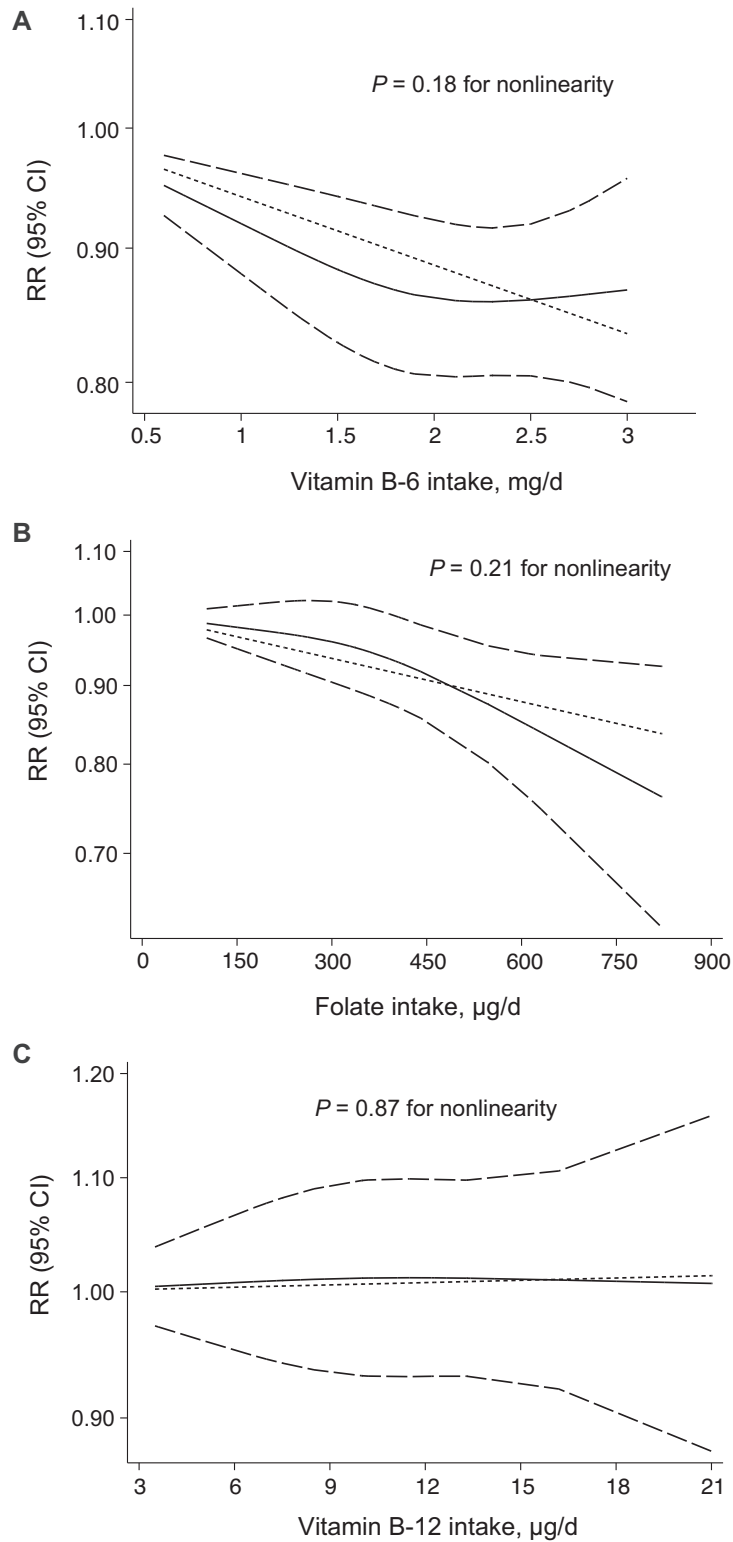


FIGURE 5 Dose-response analyses of the nonlinear association between the RR of stroke and vitamin B-6 intake (A), folate intake (B), and vitamin B-12 intake (C). Dashed lines indicate the 95% CI. In each panel, the solid line and dashed lines represent the estimated RR and 95% CI, respectively, and the dotted line represents the linear fit to the data.

dietary vitamin B-12 intake and stroke risk ($P = 0.87$ for nonlinearity; [Figure 5C](#)).

Subgroup, sensitivity, and meta-regression analyses

Next, we performed several subgroup and meta-regression analyses to examine the possible sources of heterogeneity; the results of these analyses are summarized in [Table 3](#). Overall, the inverse association between stroke risk and both folate and vitamin B-6 intake was evident in most subgroups. With respect to both folate and vitamin B-6 intake, this inverse association remained after adjusting for major confounding factors, including age, BMI, alcohol consumption, physical activity, energy intake, and diabetes. Cigarette smoking was adjusted for in all included studies; thus, no stratified results by smoking were reported. In addition, we found significant associations in studies with longer follow-up and studies without a pre-existing stroke event. When we stratified studies by mean age, we found no differences between age ≥ 55 y and age < 55 y for folate ($P = 0.98$); as for vitamin B-6, we found a significant inverse association in the ≥ 55 y subgroup. When we stratified studies based on adjustment for hypertension/blood pressure, the RR was 0.86 (95% CI: 0.78–0.95) for folate and 0.86 (95% CI: 0.74–0.99) for vitamin B-6. When using FFQs to assess diet, the RR was 0.87 (95% CI: 0.78–0.97) for folate and 0.86 (95% CI: 0.74–0.99) for vitamin B-6. When using FFQs that validate for B-vitamins intake, the RR was 0.80 (95% CI: 0.71–0.89) for folate and 0.96 (95% CI: 0.83–1.10) for vitamin B-6. With respect to folate, our analyses revealed that folate intake was inversely associated with stroke risk among males and among US-based studies, although little evidence of heterogeneity was found between these subgroups. We also found that folate intake was associated with a reduced risk of ischemic stroke (RR: 0.84; 95% CI: 0.73–0.97; 4623 cases included in the analysis), but not hemorrhagic stroke (RR: 0.85; 95% CI: 0.61–1.19; 647 cases included in the analysis). In our analysis of vitamin B-6, we found evidence of heterogeneity when studies were stratified by participant number ($P = 0.004$), with a weaker association in studies that included a relatively smaller sample size (i.e. $< 50,000$ participants). When stratified by geographic location, we found a stronger association among studies conducted in Asia compared with studies conducted in Europe and the USA. When stratified by stroke subtype, we found no significant association between vitamin B-6 intake and ischemic stroke (RR: 1.08; 95% CI: 0.72–1.64; 3157 cases included in the analysis) or hemorrhagic stroke (RR: 0.87; 95% CI: 0.65–1.17; 508 cases included in the analysis).

We also analyzed studies that provided RR values that reflect the least and greatest degree of covariate adjustments. With respect to folate, the pooled RR in the basic and multivariable adjustment model was 0.85 (95% CI: 0.76–0.95) and 0.88 (95% CI: 0.78–0.99), respectively; for vitamin B-6, the pooled RR was 0.77 (95% CI: 0.65–0.92) and 0.82 (95% CI: 0.72–0.93), respectively.

We also performed sensitivity analyses in order to further test the robustness of the associations. With respect to folate

and vitamin B-12, the overall results were not changed substantially by excluding one study at a time. With respect to vitamin B-6, when we omitted either of the 2 studies published by Zhao et al. ([27](#)), the pooled RR for each 0.5 mg/d increase in vitamin B-6 intake increased to 0.96 (95% CI: 0.92–1.01), although the heterogeneity decreased slightly to $I^2 = 69\%$. Excluding any other single study had no notable effect on overall RR.

Publication bias

We found little evidence of publication bias with respect to most of the analyses included in our study. Only for the dose-response analysis between dietary vitamin B-6 intake and stroke risk, we observed some indication of publication bias when using Egger's test ($P = 0.02$), which is consistent with the funnel plots shown in [Supplemental Figures 1 and 2](#). However, use of the trim-and-fill method did not change the pooled risk estimate, suggesting that the results were unlikely to be affected by this publication bias ([Supplemental Table 4](#)).

Discussion

This systematic meta-analysis based on 11 prospective studies involving 389,938 participants and 10,749 cases of stroke revealed a clear correlation between dietary intake of homocysteine metabolism-related B-vitamins and the risk of stroke. Specifically, we found inverse associations between stroke risk and the intake of both folate and vitamin B-6. Moreover, our dose-response analysis revealed that either a 0.5 mg/d increase in vitamin B-6 intake or a 100 $\mu\text{g}/\text{d}$ increase in folate intake reduces the risk of stroke by 6%. These inverse associations remained significant even after adjusting for conventional risk factors associated with cardiovascular disease (CVD), including BMI, smoking, physical activity, alcohol consumption, diabetes/hypertension, and energy intake. In contrast to our findings with vitamin B-6 and folate, we found no significant association between vitamin B-12 intake and stroke risk. To the best of our knowledge, our study is the first meta-analysis designed to evaluate the dose-response relation between dietary B-vitamins intake and the risk of stroke.

Vitamin B-6, folate, and vitamin B-12 are essential nutrients involved in the one-carbon metabolism pathway, which plays an important role in homocysteine metabolism in the human body ([53](#)). Specifically, vitamin B-6, folate, and vitamin B-12 act as coenzymes either to degrade homocysteine to form cysteine or to methylate homocysteine to form methionine ([54](#)). Previous RCTs showed that supplementation with folic acid, either alone or in combination with vitamin B-6 and/or vitamin B-12, significantly reduces blood homocysteine concentrations ([16, 55–57](#)). Early in 1969, McCully postulated that homocysteine affects atherosclerosis ([58](#)), and subsequent studies found that high concentrations of homocysteine can cause vascular damage, including endothelial dysfunction, increased intimal-medial thickness, and increased arterial stiffness ([5, 59, 60](#)), thus leading to atherosclerosis and vascular disease. Observational studies

TABLE 3 Subgroup analysis of the dietary intake of homocysteine metabolism-related B-vitamins and the risk of stroke¹

	Vitamin B-6			Folate			Vitamin B-12				
	N	RR (95% CI)	I ²	N	RR (95% CI)	I ²	N	RR (95% CI)	I ²	P ³	P ²
Overall	10	0.84 (0.73, 0.97)	48.8	16	0.85 (0.78, 0.94)	11.5	10	1.03 (0.94, 1.13)	0.4	0.434	0.45
Location											
USA	2	1.09 (0.58, 2.05)	56.8	6	0.85 (0.74, 0.97)	0	2	0.84 (0.56, 1.26)	33.1	0.221	0.45
Europe	4	0.9 (0.81, 1.01)	0	7	0.88 (0.73, 1.06)	23.3	6	1.02 (0.51, 0.69)	0	0.946	0.45
Asia	4	0.73 (0.64, 0.83)	0	3	0.86 (0.64, 1.17)	53.6	2	1.34 (0.96, 1.88)	5.6	0.303	0.45
Sex											
Male	7	0.91 (0.78, 1.07)	44.4	6	0.86 (0.74, 1)	24.7	6	1.04 (0.88, 1.22)	36.5	0.163	0.67
Female	2	0.71 (0.60, 0.84)	0	3	0.96 (0.8, 1.16)	1.5	1	1.13 (0.71, 1.79)	—	—	0.84
Follow-up											
>10 y	9	0.86 (0.74, 0.99)	50.6	12	0.84 (0.77, 0.92)	2.7	7	1.04 (0.9, 1.2)	25.6	0.234	0.84
≤10 y	1	0.54 (0.25, 1.14)	—	4	0.96 (0.57, 1.6)	38.2	3	0.98 (0.64, 1.5)	0	0.705	0.84
Participants											
<50,000	6	0.94 (0.80, 1.11)	26.3	13	0.81 (0.74, 0.89)	0	8	1.00 (0.90, 1.10)	0	0.688	0.10
>50,000	4	0.73 (0.64, 0.83)	0	3	0.98 (0.83, 1.15)	0	2	1.34 (0.96, 1.88)	5.6	0.303	0.10
Stroke subtype											
Ischemic stroke	2	1.08 (0.72, 1.64)	75.6	7	0.84 (0.73, 0.97)	33.9	3	0.94 (0.74, 1.19)	42.5	0.176	0.62
Hemorrhagic stroke	2	0.87 (0.65, 1.17)	0	4	0.85 (0.61, 1.19)	11.1	3	1.05 (0.81, 1.37)	0	0.863	0.62
Mean age (y)											
≥55	7	0.84 (0.73, 0.96)	28.4	10	0.88 (0.76, 1.01)	27.1	6	1.05 (0.95, 1.16)	0	0.695	0.11
<55	3	0.91 (0.51, 1.49)	77.5	6	0.85 (0.74, 0.96)	0	4	0.84 (0.56, 1.26)	33.1	0.221	0.11
Exposure assessment											
FFQ	9	0.86 (0.74, 0.99)	50.6	14	0.87 (0.78, 0.97)	21.1	9	1.03 (0.92, 1.15)	5.8	0.387	0.57
Non-FFQ	1	0.54 (0.25, 1.14)	—	2	0.80 (0.65, 0.98)	0	1	0.86 (0.47, 1.57)	—	—	0.57
Validation on B-vitamins											
Yes	5	0.96 (0.83, 1.10)	15.6	6	0.80 (0.71, 0.89)	0	5	1.00 (0.90, 1.11)	1.6	0.397	0.56
No	4	0.73 (0.64, 0.83)	0	8	0.96 (0.83, 1.11)	11.3	4	1.29 (0.96, 1.71)	0	0.637	0.56
Exposure source											
Foods + supplements	7	0.80 (0.65, 0.99)	54.1	12	0.89 (0.78, 0.99)	11.9	6	1.07 (0.81, 1.42)	37.8	0.154	0.56
Foods	3	0.91 (0.82, 1.02)	0	4	0.81 (0.71, 0.92)	6.9	4	1.02 (0.92, 1.13)	0	0.862	0.56
Quality											
<7	1	0.54 (0.25, 1.14)	—	3	0.80 (0.40, 1.59)	47.2	3	0.98 (0.64, 1.50)	0	0.705	0.84
≥7	9	0.86 (0.74, 0.99)	50.6	13	0.85 (0.78, 0.93)	8.5	7	1.04 (0.90, 1.20)	25.6	0.234	0.84
Adjustments ⁴											
Age											
Yes	10	0.84 (0.73, 0.97)	48.8	14	0.85 (0.78, 0.92)	1.9	8	1.03 (0.91, 1.16)	16.3	0.302	0.783
No	0	—	—	2	0.56 (0.08, 3.76)	70.1	2	1.11 (0.61, 2.04)	0	0.552	0.783
BMI											
Yes	9	0.86 (0.74, 0.99)	50.6	13	0.86 (0.78, 0.96)	25.1	9	1.03 (0.92, 1.15)	5.8	0.387	0.57
No	1	0.54 (0.25, 1.14)	—	3	0.82 (0.63, 1.07)	0	1	0.86 (0.47, 1.57)	—	—	0.57

(Continued)

TABLE 3 (Continued)

	Vitamin B-6				Folate				Vitamin B-12						
	N	RR (95% CI)	I ²	P ²	P ³	N	RR (95% CI)	I ²	P ²	P ³	N	RR (95% CI)	I ²	P ²	P ³
Physical activity					0.503					0.36					0.84
Yes	7	0.86 (0.74, 1.00)	56.8	0.031		9	0.84 (0.75, 0.93)	13.8	0.319		5	1.00 (0.90, 1.11)	1.6	0.397	
No	3	0.75 (0.50, 1.13)	38.4	0.197		7	0.9 (0.76, 1.08)	11.8	0.339	0.967	5	1.20 (0.92, 1.55)	0	0.545	0.21
Alcohol					0.266										
Yes	9	0.86 (0.74, 0.99)	50.6	0.04		11	0.85 (0.77, 0.95)	19	0.263		7	1.04 (0.90, 1.20)	25.6	0.234	
No	1	0.54 (0.25, 1.14)	—	—		5	0.85 (0.65, 1.11)	12.9	0.332	0.512	3	0.98 (0.64, 1.50)	0	0.705	0.78
Energy intake					—										
Yes	10	0.84 (0.73, 0.97)	48.8	0.041	—	13	0.85 (0.79, 0.92)	0	0.475		8	1.03 (0.91, 1.16)	16.3	0.302	
No	0	—	—	—	0.266	3	0.72 (0.36, 1.44)	58.9	0.088	0.705	2	1.11 (0.61, 2.04)	0	0.552	0.57
Excluding pre-existing stroke															
Yes	9	0.86 (0.74, 0.99)	50.6	0.04		15	0.86 (0.78, 0.95)	16.7	0.267		9	1.03 (0.92, 1.15)	5.8	0.387	
No	1	0.54 (0.25, 1.14)	—	—	0.004	1	0.75 (0.38, 1.46)	—	—	0.572	1	0.86 (0.47, 1.57)	—	—	—
Level of education															
Yes	2	0.72 (0.62, 0.82)	26.7	0.216		1	0.80 (0.64, 0.99)	—	—		0	—	—	—	—
No	8	0.92 (0.79, 1.08)	48.8	0.041		15	0.87 (0.78, 0.96)	15.9	0.276		10	1.03 (0.94, 1.13)	0.4	0.434	—
Level of income					0.004										
Yes	2	0.72 (0.62, 0.82)	26.7	0.216		0	—	—	—		0	—	—	—	—
No	8	0.92 (0.79, 1.08)	48.8	0.041		16	0.85 (0.78, 0.94)	11.5	0.322	0.565	10	1.03 (0.94, 1.13)	0.4	0.434	0.763
Adjustment model					0.517										
Basic	7	0.77 (0.65, 0.92)	74.3	<0.001		11	0.85 (0.76, 0.95)	32.2	0.141		7	1.10 (0.96, 1.25)	24.7	0.241	
Multivariable	7	0.82 (0.72, 0.93)	37.3	0.141		11	0.88 (0.78, 0.99)	30.2	0.158	0.705	7	1.05 (0.95, 1.16)	0	0.635	0.57
Diabetes					0.266										
Yes	9	0.86 (0.74, 0.99)	50.6	0.04		15	0.86 (0.78, 0.95)	16.7	0.267		9	1.03 (0.92, 1.15)	5.8	0.387	
No	1	0.54 (0.25, 1.14)	—	—	0.266	1	0.75 (0.38, 1.46)	—	—	0.705	1	0.86 (0.47, 1.57)	—	—	0.57
Hypertension/blood pressure															
Yes	9	0.86 (0.74, 0.99)	50.6	0.04		15	0.86 (0.78, 0.95)	16.7	0.267		9	1.03 (0.92, 1.15)	5.8	0.387	
No	1	0.54 (0.25, 1.14)	—	—	0.266	1	0.75 (0.38, 1.46)	—	—	0.705	1	0.86 (0.47, 1.57)	—	—	0.57

¹ Pooled estimates were obtained using a random-effects model by comparing the highest category and lowest category.

² P value for heterogeneity within each subgroup.

³ P value for heterogeneity between subgroups with meta-regression analysis.

⁴ Cigarette smoking was adjusted for in all studies; therefore, no stratified results by smoking are reported.

have provided evidence that a 3 $\mu\text{mol/L}$ reduction in blood homocysteine concentrations is associated with an 11% reduction in the risk of ischemic heart disease and a 19% reduction in the risk of stroke (10). On the other hand, each 5 $\mu\text{mol/L}$ increase in serum homocysteine concentration has been associated with a 32% higher risk of ischemic heart disease and a 59% higher risk of stroke (7).

The mechanism underlying the inverse association between folate intake and stroke risk remains speculative but is biologically plausible, given that folate serves as an essential methyl donor in the remethylation of homocysteine to form methionine (61). RCTs have shown that daily supplementation with 0.5–5 mg of folic acid reduces plasma homocysteine concentrations by $\sim 25\%$ (55). Moreover, as discussed above, the adverse effects of high homocysteine concentrations on stroke risk are well known and have been summarized in a number of review articles. In addition to its effects on homocysteine metabolism, some studies have also suggested that folate may play a role in other antiatherosclerotic processes, such as delaying the development of atherosclerotic lesions (62), increasing plasma HDL cholesterol concentrations (63), and reverting endothelial nitric oxide synthase (eNOS) dysfunction by increasing the production of nitric oxide (64, 65).

Since 2007, several meta-analyses of clinical trials have been conducted regarding the intervention effect of folic acid on the risk of stroke (18–21, 23, 66), and nearly all of these studies suggested that folic acid supplementation can reduce the risk of stroke, with RR values ranging from 0.82 to 0.96. Nevertheless, our findings based on observational studies are not comparable with findings based on intervention studies, as several discrepancies exist. First, the RCTs were generally performed among individuals with pre-existing vascular disease, whereas the participants in epidemiological studies were typically healthier. Based on our results, we found robust inverse associations in studies that excluded participants with a pre-existing cerebrovascular disease, which suggests that folate may play a role in the primary prevention of stroke. Indeed, a previous study conducted among participants without diabetes or CVD found that lowering homocysteine with folate-based B-vitamins significantly reduced the progression of early-stage subclinical atherosclerosis (67). In addition, folate derived from food differs from that derived from supplementation in both type and amount. Intervention studies usually use high-dose folic acid, which may be associated with an increased risk of other chronic diseases, such as cancer (68, 69). In the present meta-analysis, we found that increasing folate intake at physiologically relevant daily amounts is associated with a reduced risk of stroke. Furthermore, the power to determine associations based on long-term follow-up in RCTs may be limited by the fact that the majority of these studies were designed with a relatively short time period. In the present meta-analysis, we observed a significant association in a subgroup of studies with a long follow-up duration (i.e. >10 y), suggesting that folate intake may have long-term health benefits in terms of reducing the risk of stroke.

With respect to vitamin B-6, we found that each 0.5 mg/d increase in vitamin B-6 intake reduces the risk of stroke by 6%. A recent meta-analysis by Jayedi and Zargar revealed that a higher intake of vitamin B-6 was associated with a decreased risk of ischemic heart disease in the general population (70). Combined with our findings, it is interesting to note that consuming foods rich in vitamin B-6 may have health benefits in the context of atherosclerosis. Other than homocysteine-lowering effects, vitamin B-6 may lower the risk of stroke via other vascular-protective properties. For example, vitamin B-6 can act as an antioxidant by scavenging oxygen radicals (71), reducing the production of lipid peroxides (72), and preventing LDL-induced vascular endothelial dysfunction (73). Moreover, vitamin B-6 is also involved in the inflammatory process (74), and inflammation has been reported to play a key role in the pathogenesis of stroke (75). Several epidemiological studies have suggested that plasma concentrations of PLP (pyridoxal 5'-phosphate, the bioactive form of vitamin B-6) are negatively correlated with certain inflammatory biomarkers, including high-sensitivity C-reactive protein (hs-CRP) and fibrinogen (76, 77).

Certain factors should be taken into consideration when attempting to analyze the relation between vitamin B-6 and stroke. One important concern is age. The aging process has been reported to result in a higher requirement for vitamin B-6 intake in humans (78). Although vitamin B-6 deficiency is relatively rare in young people due to the presence of vitamin B-6 in many foods, including animal products and vegetables (79), the relatively high prevalence of vitamin B-6 deficiency among the elderly cannot be ignored (80–82). This deficiency may be attributed to increased hydrolysis of PLP with aging (83). Thus, one's intake of vitamin B-6 should increase with age, particularly in populations with poor nutritional status. When we stratified the studies by geographic location, we observed a significant inverse association between dietary vitamin B-6 intake and the risk of stroke in Asian studies, a borderline inverse association in European studies, and no significant association in US-based studies. Notably, we found that the median values of the lowest category of vitamin B-6 intake in 2 Asian studies (0.6 and 1.24 mg/d) are both lower than the lowest category in US-based studies (1.8 mg/d) (25, 27, 49). Given that the amount of vitamin B-6 intake in the reference group approached the maximal benefit in US-based studies (25), where the RDA for vitamin B-6 is 2 mg/d (84), this may have limited our ability to detect an inverse association in the highest quintile. Importantly, as the rate of stroke tends to be higher in Asia compared with most Western countries, and given that the at-risk population in Asia is extremely large, the public health benefit associated with vitamin B-6 intake cannot be overstated (85, 86). Nevertheless, because the number of studies in each subgroup is relatively low, further studies are warranted in order to investigate this relation in various regions.

Interestingly, our analysis revealed that dietary vitamin B-12 intake does not appear to be associated with the risk

of stroke. The reason for this difference between vitamin B-12 and both vitamin B-6 and folate is not clear; however, one possible explanation is that vitamin B-12 has a relatively mild effect on homocysteine concentrations. Indeed, a meta-analysis of RCTs suggested that supplementation with folic acid reduces homocysteine concentrations by $\leq 25\%$, and the addition of vitamin B-12 produced only a 7% further reduction in homocysteine concentrations (15). Another explanation is that a small percentage of study participants had vitamin B-12 deficiency; indeed, the median intake of the reference quintile in the included studies (5, 6.6, and 3.6 $\mu\text{g}/\text{d}$) were all above the RDA for vitamin B-12 (2.4 $\mu\text{g}/\text{d}$) (25, 47, 49). Vitamin B-12 deficiency is usually associated with impaired absorption (for example, due to atrophic gastritis) rather than low nutritional intake. However, in the study by Larsson et al. (47), when the authors excluded men with low serum pepsinogen I concentrations to exclude any potential bias due to impaired absorption, they still found no significant association. Thus, further studies are warranted to examine the relation between vitamin B-12 intake and the risk of stroke in vitamin B-12-deficient regions, such as India (87, 88).

Given the effect of B-vitamins on various types of vascular diseases, it is important to note that our results suggest that increasing habitual B-vitamins intake in healthy populations could be beneficial for stroke, although previous RCTs found little or no effect on the risk of IHD (23, 89). The precise mechanism underlying this apparent differential effect of B-vitamins on stroke and IHD is unknown; however, several factors may play a role in this difference. First, stroke and IHD have different etiologies and pathophysiologies. For example, IHD primarily affects large coronary vessels, whereas stroke often involves both large and small cerebral vessels. A previous study has reported that elevated homocysteine is a risk factor for cerebral small vessel disease (90); thus, the putative beneficial effect of B-vitamins in lowering homocysteine on stroke but not IHD may be attributed to reduced atherosclerosis primarily in small cerebral vessels.

Hypertension has been reported to be an important risk factor for stroke. However, due to limited information, we were unable to conduct subgroup analysis among participants with hypertension and normotensive participants. Thus, we performed analyses based on adjustments for hypertension/blood pressure. Omitting the single study that did not make this adjustment (45) yielded generally similar results (the RRs changed from 0.85 to 0.86 for folate and from 0.84 to 0.86 for vitamin B-6), suggesting that hypertension may not significantly affect the relation between folate or vitamin B-6 intake and stroke risk.

Age is another major risk factor for stroke. To examine the effect of age on our overall results, we performed a sensitivity analysis by excluding one study that did not adjust for age (46) and found no significant change in the inverse relation between folate or vitamin B-6 and stroke risk. As none of the included studies provided risk estimates for different age intervals, we performed analysis using the mean/median age in each cohort and found that increasing vitamin B-6 intake

may have an additional beneficial effect on stroke among older individuals (i.e. aged ≥ 55 y). Nevertheless, further studies are needed in order to determine the association in various age groups.

Our meta-analysis has several key strengths. First, because we based our analysis on prospective studies, our findings are not likely due to recall bias or selection bias. Moreover, most of the studies included in our analysis had a large sample size and long follow-up duration, which provided high statistical power to our assessment of the correlation between dietary vitamin-B intake and the risk of stroke. In addition, we also conducted subgroup analyses and found that the inverse associations persisted even after adjusting for major risk factors for CVD. Finally, in addition to comparing the highest and lowest categories of B-vitamins intake, we also generated dose-response curves for vitamin B-6, folate, and vitamin B-12 intake.

Despite these strengths, our analysis has several limitations that should be acknowledged when interpreting our findings. First, due to the observational nature of the included studies, we cannot exclude the potential bias effect of unmeasured or residual confounders, as subjects with a higher B-vitamins intake tend to have a healthier lifestyle, such as less smoking, a reduced incidence of overweight, higher physical activity, and lower consumption of alcohol (91–94), and they are also more likely to have a higher social-economic status. In order to estimate the potential impact of these factors on our results, we calculated the effect size using the RR values based on basic and multivariable adjustment models and found that these adjustments may contribute to $\sim 3\%$ and 5% reduction in stroke risk for folate and vitamin B-6, respectively. Nevertheless, when we stratified the studies by adjustment for major lifestyle factors (e.g. smoking, alcohol consumption, physical activity, and BMI), these inverse associations remained in the majority of subgroups. With respect to social-economic status, we found that the inverse associations persist in studies adjusted for level of income/education. Another possible limitation with respect to evaluating the association between B-vitamins intake and stroke risk is that dietary factors may not have been considered to a sufficient degree. Among the studies included in our analysis, only a few (24–26, 49) adjusted for several dietary factors, including the intake of saturated fats/polyunsaturated fats, vitamin E, and/or dietary fiber; thus, we cannot exclude the possibility that other nutrients and/or dietary components correlated with dietary B-vitamins (e.g. fruits, vegetables, and/or whole grains) may have been responsible, at least in part, for the associations identified in our study. Further studies should therefore attempt to control for adequate dietary variables to minimize any bias associated with the conclusions.

Secondly, meta-analyses can result in significant heterogeneity due to differences in the characteristics of studies. In our study, we found either low evidence or no evidence of heterogeneity in the analysis of folate and vitamin B-12, and moderate heterogeneity in the analysis of vitamin B-6. We conducted several subgroup and meta-regression

analyses to explore the potential source of heterogeneity. For both folate and vitamin B-6, we found that the significant inverse associations persisted in the subgroup of studies with relatively long follow-up (i.e. >10 y), which indicates that dietary intake of folate and vitamin B-6 may have beneficial effects on stroke in the long term, but not necessarily the short term, in the general population. In our analysis of vitamin B-6, we found significant between-subgroup heterogeneity when we stratified studies by participant number, with a stronger inverse association among studies with a relatively large number of participants (i.e. >50,000), as well as reduced heterogeneity within subgroups. We also found evidence of heterogeneity when we stratified studies by geographic location, with stronger inverse associations among Asian studies and no significant association among US studies. With respect to stroke subtypes, we found an inverse association between folate intake and the risk of ischemic stroke. In addition, we observed no evidence of heterogeneity between subgroups when we stratified the analyses by quality score or adjustment for confounding factors. Nevertheless, the overall heterogeneity appeared to be driven more by differences in the size of the association rather than by differences in the direction of the association, as the majority of subgroup analyses yielded an inverse association.

Thirdly, the potential impacts of measurement error need to be addressed. For example, the intake of B-vitamins may have changed during long follow-up periods, possibly without timely updates. Most of the studies included in our analysis assessed dietary B-vitamins intake at baseline, and only one study used repeated measurements in an attempt to reduce error (25). We found that when using baseline diet compared with that from cumulative average diet, the associations with stroke risk were only slightly weakened. In addition, the different methods used to assess diet will inevitably lead to some degree of heterogeneity. For example, Bazzano et al. (24) used a single 24-h recall, which may not be representative of the participants' long-term intake. The exclusion of this study yielded a pooled RR of 0.87. Another study performed dietary history interviews (45); after omitting these 2 studies, the results were not materially changed, suggesting that our findings are generally robust with respect to measurements of assessing diet. In addition, we conducted analysis in studies with validation for B-vitamins intake, finding that folate intake was inversely associated with stroke risk, whereas vitamin B-6 was not. Notably, as only a few studies used FFQs that provide correlation for the B-vitamins under study, more studies need to be done on this issue.

Concern also arises from different ranges of folate and vitamin B-6 intake, which might be attributed to the differences in dietary habits, dietary assessments, and geographic locations across studies. The differences of intake categories could also result in heterogeneity and lead to some degree of insecurity on the risk estimates (36). Therefore, it is important to deal with the potential bias introduced by methodological differences that seem to be inherent in

nutritional studies. Future studies would be better conducted with consistent methods estimating the intake and similar exposure levels to minimize the bias and enhance the accuracy of the overall results.

Another potential factor is the different sources of B-vitamins in the included studies. Some studies measured B-vitamins intake from foods, whereas other studies included participants who consumed B-vitamins from both food and supplements. To explore the potential impact of supplements on the results, we performed subgroup analysis stratified by the source of B-vitamins. For folate, similar inverse associations were observed across studies with or without supplements; as for vitamin B-6, a stronger association was observed in studies with supplements, suggesting that vitamin B-6 intake from supplements may have been a potential source of bias. Thus, future studies should attempt to evaluate the association among participants with or without vitamin supplements.

Another limitation that warrants discussion is the fact that in 1998, the US FDA required that flour and uncooked cereal-grain products be fortified with folic acid, which could have led to a misclassification of long-term folate intake (95). However, after we excluded 2 US studies that included both prefortification and postfortification periods (25, 50), the association between folate intake and stroke risk remained statistically significant, suggesting that the mandatory fortification of folate may not have affected the results.

In addition, our sensitivity analysis with respect to vitamin B-6 intake suggests that the result may not have been sufficiently stable. When we excluded the study by Zhao et al. (27), the pooled risk estimates remained inverse but were only marginally significant. As noted by the authors of that study, vitamin B-6 intake was lower than the reference nutrient intake in the cohorts for 36.5% and 40.3% of men and women, respectively; thus, the relatively low dose in the reference quintile may have contributed, at least in part, to the strong inverse association reported in their study. Thus, more studies are warranted in order to further elucidate the inverse relation between vitamin B-6 intake and stroke risk. Lastly, the number of studies in several of the subgroups is relatively small, which limits our analysis (when the studies were stratified by sex, geographic location, or stroke subtype). Thus, future studies should attempt to clarify the association between dietary B-vitamins intake and the risk of specific stroke subtypes in various regions and in each sex.

Conclusions

In conclusion, the results of our meta-analysis indicate that both vitamin B-6 and folate intake are inversely correlated with the risk of stroke; in contrast, vitamin B-12 intake does not appear to be associated with the risk of stroke. Our findings support the notion that increasing habitual vitamin B-6 and folate intake have a small, but beneficial effect on the risk of stroke. In the future, more prospective studies are needed to investigate the associations using consistent

dietary assessment methods and comparable intake levels to reduce the impact of methodological issues. Further studies are also warranted to report results by different stroke subtypes and regions. Moreover, well-designed RCTs should help identify the putative causal role that B-vitamins play in reducing the risk of stroke.

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