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

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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 years 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 μg/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 intake of vitamin B-12 and the risk of stroke, with an RR of 1.01 (95% CI: 0.97–1.06) per 3 μg/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.

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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...
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 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 RR’s 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 μg/d increment in folate intake, 0.5 mg/d increment in vitamin B-6 intake, and 3 μg/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 program software 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 (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.

![Figure 1: Flow chart depicting the literature search and selection strategy.](image-url)

**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 Epidemiologic Follow-up Study (NHES) (52) | 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(47) | Finland | L, tocopherol, beta-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 | NA/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 Northern Sweden Health and Disease Cohort | M/F | 65–99 (77) | 10 | 70 stroke cases | 755 | NA/NA/0 |
| Van Guelpen et al., 2005 (46) | Sweden | | M/F | 25–74 (55.1) | 4.2 | 334 ischemic stroke cases; 62 hemorrhagic stroke cases | 1192 | 32.6/8.4/NA |
| 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 (μg/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 (μg/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) | Folate (μg/d): <272, 272–351, 352–430, 431–535, >536 | 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) | 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) | Median intake | FFQ, 131 food items | Age, BMI, physical activity, history of hypertension and hypercholesterolemia, smoking status, aspirin use, alcohol, and total calorie intakes of fiber, potassium, and vitamin E | 9 |
| Larsson et al., 2008 (47) | Foods; 0% | Median intake | 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)
| 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 (μg/d): 0–155, 156–211, 212–278, ≥279 | FFQ, 66 food items | Age, gender, current smoking status, diabetes, caloric intake, and hypertension | 7 |
| Marniemi 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 (μg/10^3 kcal/d) Vitamin B-12 (μg/10^3 kcal/d) | FFQ, 84 food items | BMI, current smoking, cholesterol, diabetes, and hypertension. Plasma homocysteine | 6 |
| Weng et al., 2008 (48) | Foods; 0% | Folate (μg/d): <297, 297–369, ≥369 | 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 |

1 Larsson 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.
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,

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 \(\mu\)g/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 \((I^2 = 46.8\%)\).

| Study                                      | RR (95% CI)   |
|--------------------------------------------|---------------|
| Overall (\(P = 11.5\%\))                 | 0.85 (0.78, 0.94) |
| Overall (\(P = 46.8\%\))                 | 0.94 (0.90, 0.98) |

![Figure 3](image-url)  
Forest plots summarizing the RR with 95% CI of stroke between the highest and lowest categories of dietary folate intake (A) and each 100 \(\mu\)g/d increase in dietary folate 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 μg/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 spine 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 $\geq 55$ y and age $< 55$ y for folate ($P = 0.98$); as for vitamin B-6, we found a significant inverse association in the $\geq 55$ 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 µg/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
| Subgroup Analysis of Dietary Intake of Homocysteine Metabolism-Related B-Vitamins and the Risk of Stroke |
|--------------------------------------------------|--------------------------------------------------|--------------------------------------------------|
| **Overall** | 10 | 0.84 (0.73, 0.97) | 48.8 | 0.041 | 16 | 0.85 (0.78, 0.94) | 11.5 | 0.322 | 10 | 1.03 (0.94, 1.13) | 0.4 | 0.434 |
| **Location** | | | | | | | | | | | | |
| USA | 2 | 1.09 (0.58, 2.05) | 56.8 | 0.128 | 6 | 0.85 (0.74, 0.97) | 0 | 0.467 | 2 | 0.84 (0.56, 1.26) | 33.1 | 0.221 |
| Europe | 4 | 0.9 (0.81, 1.01) | 0 | 0.566 | 7 | 0.88 (0.73, 1.06) | 23.3 | 0.251 | 6 | 102 (0.51, 0.69) | 0 | 0.946 |
| Asia | 4 | 0.73 (0.64, 0.83) | 0 | 0.429 | 3 | 0.86 (0.64, 1.17) | 53.6 | 0.116 | 2 | 134 (0.96, 1.88) | 5.6 | 0.303 |
| **Sex** | | | | | | | | | | | | |
| Male | 7 | 0.91 (0.78, 1.07) | 44.4 | 0.095 | 6 | 0.86 (0.74, 1.1) | 24.7 | 0.249 | 6 | 104 (0.88, 1.22) | 36.5 | 0.163 |
| Female | 2 | 0.71 (0.60, 0.84) | 0 | 0.615 | 3 | 0.96 (0.8, 1.16) | 1.5 | 0.362 | 1 | 113 (0.71, 1.79) | — | — |
| **Follow-up** | | | | | | | | | | | | |
| >10y | 9 | 0.86 (0.74, 0.99) | 50.6 | 0.04 | 12 | 0.84 (0.77, 0.92) | 2.7 | 0.418 | 7 | 104 (0.9, 1.2) | 25.6 | 0.234 |
| ≤10y | 1 | 0.54 (0.25, 1.14) | — | — | 4 | 0.96 (0.57, 1.6) | 38.2 | 0.183 | 3 | 0.98 (0.64, 1.5) | 0 | 0.705 |
| **Participants** | | | | | | | | | | | | |
| <50,000 | 6 | 0.94 (0.80, 1.11) | 26.3 | 0.237 | 13 | 0.81 (0.74, 0.89) | 0.051 | 8 | 100 (0.90, 1.10) | 0 | 0.688 |
| >50,000 | 4 | 0.73 (0.64, 0.83) | 0 | 0.429 | 3 | 0.98 (0.83, 1.15) | 0 | 0.395 | 2 | 134 (0.96, 1.88) | 5.6 | 0.303 |
| **Stroke subtype** | | | | | | | | | | | | |
| Ischemic stroke | 2 | 1.08 (0.72, 1.64) | 75.6 | 0.043 | 7 | 0.84 (0.73, 0.97) | 33.9 | 0.169 | 3 | 0.94 (0.74, 1.19) | 42.5 | 0.176 |
| Hemorrhagic stroke | 2 | 0.87 (0.65, 1.17) | 0.596 | 0 | 11 | 0.85 (0.61, 1.19) | 11.1 | 0.338 | 3 | 105 (0.81, 1.37) | 0 | 0.863 |
| **Mean age (y)** | | | | | | | | | | | | |
| ≥55 | 7 | 0.84 (0.73, 0.96) | 28.4 | 0.212 | 10 | 0.88 (0.76, 1.01) | 27.1 | 0.195 | 6 | 105 (0.95, 1.16) | 0 | 0.695 |
| <55 | 3 | 0.91 (0.51, 1.49) | 77.5 | 0.012 | 6 | 0.85 (0.74, 0.96) | 0 | 0.467 | 4 | 0.84 (0.56, 1.26) | 33.1 | 0.221 |
| **Exposure assessment** | | | | | | | | | | | | |
| FFQ | 9 | 0.86 (0.74, 0.99) | 50.6 | 0.04 | 14 | 0.87 (0.78, 0.97) | 21.1 | 0.224 | 9 | 103 (0.92, 1.15) | 5.8 | 0.387 |
| Non-FFQ | 1 | 0.54 (0.25, 1.14) | — | — | 2 | 0.80 (0.65, 0.98) | 0 | 0.844 | 1 | 0.86 (0.47, 1.57) | — | — |
| **Validation on B-vitamins** | | | | | | | | | | | | |
| Yes | 5 | 0.96 (0.83, 1.10) | 15.6 | 0.315 | 6 | 0.80 (0.71, 0.89) | 0 | 0.519 | 5 | 100 (0.90, 1.11) | 1.6 | 0.397 |
| No | 4 | 0.73 (0.64, 0.83) | 0 | 0.429 | 8 | 0.96 (0.83, 1.11) | 11.3 | 0.342 | 4 | 129 (0.96, 1.71) | 0 | 0.637 |
| **Exposure source** | | | | | | | | | | | | |
| Foods + supplements | 7 | 0.80 (0.65, 0.99) | 54.1 | 0.041 | 12 | 0.89 (0.78, 0.99) | 11.9 | 0.328 | 6 | 107 (0.81, 1.42) | 37.8 | 0.154 |
| Foods | 3 | 0.91 (0.82, 1.02) | 0 | 0.914 | 4 | 0.81 (0.71, 0.92) | 6.9 | 0.359 | 4 | 102 (0.92, 1.13) | 0 | 0.862 |
| **Quality** | | | | | | | | | | | | |
| <7 | 1 | 0.54 (0.25, 1.14) | — | — | 3 | 0.80 (0.40, 1.59) | 47.2 | 0.151 | 3 | 098 (0.64, 1.50) | 0 | 0.705 |
| ≥7 | 9 | 0.86 (0.74, 0.99) | 50.6 | 0.04 | 13 | 0.85 (0.78, 0.93) | 8.5 | 0.36 | 7 | 104 (0.90, 1.20) | 25.6 | 0.234 |
| **Adjustments** | | | | | | | | | | | | |
| Age | | | | | | | | | | | | |
| Yes | 10 | 0.84 (0.73, 0.97) | 48.8 | 0.041 | 14 | 0.85 (0.78, 0.92) | 1.9 | 0.428 | 8 | 103 (0.91, 1.16) | 16.3 | 0.302 |
| No | 0 | — | — | — | 2 | 0.56 (0.08, 3.76) | 70.1 | 0.067 | 2 | 111 (0.61, 2.04) | 0 | 0.552 |
| BMI | | | | | | | | | | | | |
| Yes | 9 | 0.86 (0.74, 0.99) | 50.6 | 0.04 | 13 | 0.86 (0.78, 0.96) | 25.1 | 0.19 | 9 | 103 (0.92, 1.15) | 5.8 | 0.387 |
| No | 1 | 0.54 (0.25, 1.14) | — | — | 3 | 0.82 (0.63, 1.07) | 0 | 0.646 | 1 | 0.86 (0.47, 1.57) | — | — |

(Continued)
|                          | Vitamin B-6 |         |         | Folate |         |         | Vitamin B-12 |         |         |         |
|--------------------------|-------------|---------|---------|--------|---------|---------|--------------|---------|---------|---------|
|                          | *N*         | RR (95% CI) | I² | *P*     | *N*         | RR (95% CI) | I² | *P*     | *N*         | RR (95% CI) | I² | *P*     |
| Physical activity        |             |         |         |        |             |         |        |         |             |         |         |        |
| 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   | 5           | 1.20 (0.92, 1.55)  | 0   | 0.545   |
| Alcohol                  |             |         |         |        |             |         |        |         |             |         |         |        |
| 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   | 3           | 0.98 (0.64, 1.50)  | 0   | 0.705   |
| 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           | —       | —       | —       | 3           | 0.72 (0.36, 1.44) | 58.9 | 0.088   | 2           | 1.11 (0.61, 2.04) | 0   | 0.552   |
| 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) |  —   | —       | 1           | 0.75 (0.38, 1.46) |  —   | —       | 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          |             |         |         |        |             |         |        |         |             |         |         |        |
| 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   | 10          | 1.03 (0.94, 1.13) | 0.4  | 0.434   |
| Adjustment model         |             |         |         |        |             |         |        |         |             |         |         |        |
| 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   | 7           | 1.05 (0.95, 1.16) | 0   | 0.635   |
| Diabetes                 |             |         |         |        |             |         |        |         |             |         |         |        |
| 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) |  —   | —       | 1           | 0.75 (0.38, 1.46) |  —   | —       | 1           | 0.86 (0.47, 1.57)  |  —   | —       |
| 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) |  —   | —       | 1           | 0.75 (0.38, 1.46) |  —   | —       | 1           | 0.86 (0.47, 1.57)  |  —   | —       |

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 μmol/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 μmol/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 ∼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 ≤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 μg/d) were all above the RDA for vitamin B-12 (2.4 μg/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 pathophysiology. 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 RR 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 ~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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References
1. Katan M, Luft A. Global burden of stroke. Semin Neurol 2018;38(2):208–11.
2. Collaborators GBD CoD. Global, regional, and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980–2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet 2018;392(10159):1736–88.
3. DALYS GBD Collaborators. H. Global, and national disability-adjusted life-years (DALYs) for 359 diseases and injuries and healthy life expectancy (HALE) for 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet 2018;392(10159):1859–922.
4. Mujumdar VS, Aru GM, Tyagi SC. Induction of oxidative stress by homocyst(e)ine impairs endothelial function. J Cell Biochem 2001;82(3):491–500.
5. Tsai JC, Perrella MA, Yoshizumi M, Heieh CM, Haber E, Schlegel RP. Dietary folate from vegetables and citrus fruit decreases plasma homocysteine concentrations in humans in a dietary controlled trial. J Nutr 1999;129(6):1135–9.
6. Homocysteine Lowering Trialists C. Dose-dependent effects of folic acid on blood concentrations of homocysteine: a meta-analysis of the randomized trials. Am J Clin Nutr 2005;82(4):806–12.
7. Wald DS, Law M, Morris JK. Homocysteine and cardiovascular disease: evidence on causality from a meta-analysis. BMJ 2002;325(7374):1202.
8. Fallon UB, Virtamo J, Young I, McMaster D, Ben-Shlomo Y, Wood PV, Willett W, Hennekens CH, Stampfer MJ. A prospective study of dietary folate and methyl group restriction. J Nutr 1994;124(7):1072–80.
9. Chasan-Taber L, Selhub J, Rosenberg IH, Malinow MR, Terry P, Tisher PV, Willett W, Hennekens CH, Stampfer MJ. A prospective study of folate and vitamin B6 and risk of myocardial infarction in US physicians. J Am Coll Nutr 1996;15(2):136–43.
comparisons from a set of estimates presented by exposure level or disease category. Stat Med 2008;27(7):954–70.

33. DerSimonian R, Laird N. Meta-analysis in clinical trials revisited. Contemporary Clinical Trials 2015;45( Pt A ):139–45.

34. Greenland S, Longnecker MP. Methods for trend estimation from summarized dose-response data, with applications to meta-analysis. Am J Epidemiol 1992;135(11):1301–9.

35. Orsini N, Bellocco R, Greenland S. Generalized least squares for trend estimation of summarized dose-response data. Stata J 2006;6(1):40–57.

36. Larsson SC, Giovannucci E, Wolk A. Folate intake and risk of breast cancer: a meta-analysis. J Natl Cancer Inst 2007;99(1):64–76.

37. Wang ZM, Zhou B, Wang YS, Gong QY, Wang QM, Yan JJ, Gao W, Wang LS. Black and green tea consumption and the risk of coronary artery disease: a meta-analysis. Am J Clin Nutr 2011;93(3):506–15.

38. Orsini N, Li R, Wolk A, Khudyakov P, Spiegelman D. Meta-analysis for linear and nonlinear dose-response relations: examples, an evaluation of approximations, and software. Am J Epidemiol 2012;175(1):66–73.

39. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ 2003;327(7414):557–60.

40. Jackson D, White IR, Riley RD. Quantifying the impact of between-study heterogeneity in multivariate meta-analyses. Stat Med 2012;31(29):3805–20.

41. Thompson SG, Higgins JPT. How should meta-regression analyses be undertaken and interpreted? Stat Med 2002;21(11):1559–73.

42. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ 1997;315(7109):629–34.

43. Begg CB, Mazumdar M. Operating characteristics of a rank correlation test for publication bias. Biometrics 1994;50(4):1088–101.

44. Duval S, Tweedie R. Trim and fill: a simple funnel-plot-based method of testing and adjusting for publication bias in meta-analysis. Biometrics 2000;56(2):453–63.

45. Marmiemi J, Alonen E, Impivaara O, Seppanen R, Hakala P, Rajala T, Ronnemaa T. Dietary and serum vitamins and minerals as predictors of myocardial infarction and stroke in elderly subjects. Nutr Metab Cardiovasc Dis 2005;15(3):188–97.

46. Van Guelpen L, Huldijn J, Johansson I, Stegmayr B, Hallmans G, Nilsson TK, Weinehall L, Wittchoff C, Palmqvist R, Winkvist A. Folate, vitamin B12, and risk of ischemic and hemorrhagic stroke: a prospective, nested case-referent study of plasma concentrations and dietary intake. Stroke 2005;36(7):1426–31.

47. Larsson SC, Mannisto S, Virtanen M, Knekt P, Albanes D, Wolk A. Folate, vitamin B6, vitamin B12, and risk of stroke subtypes in male smokers. Am J Epidemiol 2008;167(8):954–61.

48. Weng LC, Yeh WT, Bai CH, Chen HJ, Chuang SY, Chang HY, Lin BF, Chen KJ, Pan WH. Is ischemic stroke risk related to folate status or other nutrients correlated with folate intake? Stroke 2008;39(12):3152–8.

49. Cui R, Iso H, Date C, Tamakoshi A. Folate, vitamin B6 and B12 intake in relation to mortality from cardiovascular diseases: Japan collaborative cohort study. Stroke 2010;41(6):1285–9.

50. Liu HN, Kingah PL, North K, Boerwinkle E, Volcik KA. Interaction of folate intake and the paraoxonase Q192R polymorphism with risk of incident coronary heart disease and ischemic stroke: The Atherosclerosis Risk in Communities Study. Ann Epidemiol 2011;21(11):815–23.

51. Al-Delaimy WK, Rexrode KM, Hu FB, Albert CM, Stampfer MJ, Willett WC, Manson JE. Folate intake and risk of stroke among women. Stroke 2004;35(6):1259–63.

52. The alpha-tocopherol, beta-carotene lung cancer prevention study: design, methods, participant characteristics, and compliance. The ATBC Cancer Prevention Study Group. Ann Epidemiol 1994;4(1):1–10.

53. Rosenberg IH. B vitamins, homocysteine, and neurocognitive function. Nutr Rev 2001;59(8 Pt 2):569–73; discussion 573–4.

54. Selhub J. Homocysteine metabolism. Annu Rev Nutr 1999;19:217–46.

55. Lowering blood homocysteine with folic acid based supplements: meta-analysis of randomised trials. Homocysteine Lowering Trials’ Collaboration. BMJ 1998;316(7155):894–8.

56. Bonaa KH, Njoslad I, Ueland PM, Schirmer H, Tverdal A, Steigen T, Wang H, Nordrehaug JE, Arnesen E, Rasmussen K, et al. Homocysteine lowering and cardiovascular events after acute myocardial infarction. N Engl J Med 2006;354(15):1578–88.

57. Huang T, Chen Y, Yang B, Yang J, Wahlqvist ML, Li D. Meta-analysis of B vitamin supplementation on plasma homocysteine, cardiovascular and all-cause mortality. Clin Nutr 2012;31(4):448–54.

58. McCully KS. Vascular pathology of homocysteinaemia: implications for the pathogenesis of arteriosclerosis. Am J Pathol 1969;56(1):111–28.

59. Mangoni AA, Jackson SH. Homocysteine and cardiovascular disease: current evidence and future prospects. Am J Med 2002;112(7):556–65.

60. Rolland PH, Friggi A, Barlatier A, Piquet P, Latrielle V, Faye MM, Guillou J, Charpiot P, Bodard H, Ghiringhelli O, et al. Hyperhomocysteinaemia-induced vascular damage in the minipig. Captopril-hydrochlorothiazide combination prevents elastic alterations. Circulation 1995;91(4):1161–74.

61. Scott IM, Weir DG. Folic acid, homocysteine and one-carbon metabolism: a review of the essential biochemistry. J Cardiovasc Risk 1998;5(4):223–7.

62. Carnicer R, Navarro MA, Arbones-Mainar MJ, Acin S, Guzman MA, Surra JC, Arnaud C, de Las Heras M, Blanco-Vaca F, Osada J. Folic acid supplementation delays atherosclerotic lesion development in apoE-deficient mice. Life Sci 2007;80(7):638–43.

63. Villa P, Perri C, Suriano R, Cucinelli F, Panunzi S, Ranieri M, Mele C, Lanza A. L-folic acid supplementation in healthy postmenopausal women: effect on homocysteine and glycolipid metabolism. J Clin Endocrinol Metab 2005;90(8):4622–9.

64. Stroes ES, van Faassen EE, Yo M, Martasek P, Boer P, Govers R, Rabelink TJ. Folic acid reverts dysfunction of endothelial nitric oxide synthase. Circ Res 2000;86(11):1129–34.

65. Stanhewicz AE, Kenney WL. Role of folic acid in nitric oxide bioavailability and vascular endothelial function. Nutr Rev 2017;75(1):61–70.

66. Clarke R, Halsey J, Lewington S, Lonn E, Armitage J, Manson JE, Bonaa KH, Spence JD, Nygard O, Jamison R, et al. Effects of lowering homocysteine levels with B vitamins on cardiovascular disease, cancer, and cause-specific mortality: meta-analysis of 8 randomized trials involving 37 485 individuals. Arch Intern Med 2010;170(18):1622–31.

67. Hodis HN, Mack WJ, Dustin L, Mahrer PR, Azen SP, Detrano R, Selhub J, Alaupovic P, Liu CR, Liu CH, et al. High-dose B vitamin supplementation and progression of subclinical atherosclerosis: a randomized controlled trial. Stroke 2009;40(3):730–6.

68. Figueiredo JC, Grau MV, Haile RW, Sandler RS, Summers RW, Bresalier RS, Burke CA, McKeown-Eysen GE, Baron JA. Folic acid and risk of prostate cancer: results from a randomized clinical trial. J Natl Cancer Inst 2009;101(6):432–5.

69. Ebbing M, Bonaa KH, Nygard O, Arnesen E, Ueland PM, Nordrehaug JE, Rasmussen K, Njoslad I, Reismu H, Nilsen DW, et al. Cancer incidence and mortality after treatment with folic acid and vitamin B12. Jama 2009;302(19):2119–26.

70. Jayedi A, Zargar MS. Intake of vitamin B6, folate, and vitamin B12 and risk of coronary heart disease: a systematic review and dose-response meta-analysis of prospective cohort studies. Crit Rev Food Sci Nutr 2019;59(16):2697–707.

71. Kannan K, Jain SK. Effect of vitamin B6 on oxygen radicals, mitochondrial membrane potential, and lipid peroxidation in H2O2-treated U937 monocytes. Free Radic Biol Med 2004;36(4):423–8.

72. Mahfouz MM, Zhou SQ, Kummerow FA. Vitamin B6 compounds are capable of reducing the superoxide radical and lipid peroxide levels induced by H2O2 in vascular endothelial cells in culture. Int J Vitam Nutr Res 2009;79(4):218–29.

73. N Y, Oiao J, Han Y, Huang Y, Bai H, Chen Q, Fan L, Ferro A. Pyridoxine prevents dysfunction of endothelial cell nitric oxide production in response to low-density lipoprotein. Atherosclerosis 2006;188(1):84–94.

74. Lotto V, Choi SW, Friso S. Vitamin B6: a challenging link between nutrition and inflammation in CVD. Br J Nutr 2011;106(2):183–95.
75. Chamorro A. Role of inflammation in stroke and atherothrombosis. Cerebrovasc Dis 2004;17(Suppl 3):1–5.
76. Friso S, Jacques PF, Wilson PW, Rosenberg IH, Selhub J. Low circulating vitamin B(6) is associated with elevation of the inflammation marker C-reactive protein independently of plasma homocysteine levels. Circulation 2001;103(23):2788–91.
77. Friso S, Girelli D, Martinelli N, Olivieri O, Lotto V, Bozzini C, Pizzolo F, Faccini G, Beltrame F, Corrocher R. Low plasma vitamin B-6 concentrations and modulation of coronary artery disease risk. Am J Clin Nutr 2004;79(6):992–8.
78. Spinneker A, Sola R, Lemmen V, Castillo MJ, Pietrzik K, Gonzalez-Gross M. Vitamin B6 status, deficiency and its consequences – an overview. Nutr Hosp 2007;22(1):7–24.
79. Ueland PM, Ulvik A, Rios-Avila L, Midttun O, Gregory JF. Direct and functional biomarkers of vitamin B6 status. Annu Rev Nutr 2015;35:33–70.
80. Haller J, Lowik MR, Ferry M, Ferro-Luzzi A. Nutritional status: blood vitamins A, E, B6, B12, folic acid and carotene. Euronut SENECA investigators. Eur J Clin Nutr 1991;45 (Suppl 3):63–82.
81. Herrmann W, Knapp JP. Hyperhomocysteinemia: a new risk factor for degenerative diseases. Clin Lab 2002;48(9–10):471–81.
82. Bates CJ, Pentieva KD, Prentice A, Mansoor MA, Finch S. Plasma pyridoxal phosphate and pyridoxic acid and their relationship to plasma homocysteine in a representative sample of British men and women aged 65 years and over. Br J Nutr 1999;81(3):191–201.
83. Ribaya-Mercado JD, Russell RM, Sahyoun N, Morrow FD, Gershoff SN. Vitamin B-6 requirements of elderly men and women. J Nutr 1991;121(7):1062–74.
84. National Research C. Recommended dietary allowances: National Academy of Sciences, 1974.
85. Collaborators GBDLRoS, Feigin VL, Nguyen G, Cercy K, Johnson CO, Alam T, Parmar PG, Abajobir AA, Abate KH, Abd-Allah F, et al. Global, regional, and country-specific lifetime risk of stroke, 1990–2016. N Engl J Med 2018;379(25):2429–37.
86. Strong K, Mathers C, Bonita R. Preventing stroke: saving lives around the world. Lancet Neurol 2007;6(2):182–7.
87. Refsum H, Yajnik CS, Gadkari M, Schneede J, Vollset SE, Orning L, Guttormsen AB, Joglekar A, Sayyad MG, Ulvik A, et al. Hyperhomocysteinemia and elevated methylmalonic acid indicate a high prevalence of cobalamin deficiency in Asian Indians. Am J Clin Nutr 2001;74(2):233–41.
88. Antony AC. Vegetarianism and vitamin B-12 (cobalamin) deficiency. Am J Clin Nutr 2003;78(1):3–6.
89. Marti-Carvajal AJ, Sola I, Lathyris D, Dayer M. Homocysteine-lowering interventions for preventing cardiovascular events. Cochrane Database Syst Rev 2017;8:CD006612.
90. Hassan A, Hunt BJ, O’Sullivan M, Bell R, D’Souza R, Jeffery S, Bannford JM, Markus HS. Homocysteine is a risk factor for cerebral small vessel disease, acting via endothelial dysfunction, Brain 2004;127(1):212–9.
91. Sanchez-Villegas A, Doreste J, Schlatter J, Pla J, Bes-Rastrollo M, Martinez-Gonzalez MA. Association between folate, vitamin B(6) and vitamin B(12) intake and depression in the SUN cohort study. J Hum Nutr Diet 2009;22(2):122–33.
92. Sellers TA, Kushi LH, Cerhan JR, Vierkant RA, Gapstur SM, Vachon CM, Olson JE, Therneau TM, Folsom AR. Dietary folate intake, alcohol, and risk of breast cancer in a prospective study of postmenopausal women. Epidemiology 2001;12(4):420–8.
93. Larsson SC, Giovannucci E, Wolk A. Methionine and vitamin B6 intake and risk of pancreatic cancer: a prospective study of Swedish women and men. Gastroenterology 2007;132(1):113–8.
94. Rimm EB, Willett WC, Hu FB, Sampson L, Colditz GA, Manson JE, Hennekens C, Stampfer MJ. Folate and vitamin B6 from diet and supplements in relation to risk of coronary heart disease among women. JAMA 1998;279(5):535–64.
95. Crider KS, Bailey LB, Berry RJ. Folic acid food fortification – its history, effect, concerns, and future directions. Nutrients 2011;3(3):370–84.