The American Journal of Clinical Nutrition 119 (2024) 821–828

The American Journal of CLINICAL NUTRITION

journal homepage: https://ajcn.nutrition.org/

Original Research Article

Dosage exploration of combined B-vitamin supplementation in stroke prevention: a meta-analysis and systematic review

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A B S T R A C T

Background: The optimal dosage range for B-vitamin supplementation for stroke prevention has not received sufficient attention.
Objective: Our aim was to determine the optimal dosage range of a combination of folic acid, vitamin B12, and vitamin B6 supplementation in stroke prevention.
Methods: We searched PubMed, the Cochrane Central Register of Controlled Trials, and Embase database for randomized controlled trials published between January 1966 and April 2023, whose participants received B-vitamin supplementation and that reported the number of stroke cases. Relative risk (RR) was used to measure the effect of combined supplementation on risk of stroke using a fixed-effects model. Risk of bias was assessed with the Cochrane risk-of-bias algorithm.
Results: The search identified 14 randomized controlled trials of folic acid combined with vitamin B12 and vitamin B6 supplementation for stroke prevention that included 76,664 participants with 2720 stroke cases. In areas without and with partial folic acid fortification, combined B-vitamin supplementation significantly reduced the risk of stroke by 34% [RR: 0.66; 95% confidence interval (CI): 0.50, 0.86] and 11% (RR: 0.89; 95% CI: 0.79, 1.00), respectively. Further analysis showed that a dosage of folic acid $\leq$0.8 mg/d and vitamin B12 $\leq$0.4 mg/d was best for stroke prevention (RR: 0.65; 95% CI: 0.48, 0.86) in these areas. In contrast, no benefit of combined supplementation was found in fortified areas (RR: 1.04; 95% CI: 0.94, 1.16).
Conclusions: Our meta-analysis found that the folic acid combined with vitamin B12 and vitamin B6 supplementation strategy significantly reduced the risk of stroke in areas without and with partial folic acid fortification. Combined dosages not exceeding 0.8 mg/d for folic acid and 0.4 mg/d for vitamin B12 supplementation may be more effective for populations within these areas.
This trial was registered at PROSPERO asCRD42022355077.

Keywords: stroke, B-vitamin supplementation, dosages, folic acid fortification, prevention

Introduction

Stroke remains the second leading cause of death worldwide, with an ever-increasing disease burden, especially in low-income countries [1, 2]. Nutrient supplementation may be an inexpensive and effective stroke prevention strategy. The Guidelines for the Primary Prevention of Stroke issued by the American Heart Association/American Stroke Association state that B complex vitamins (folic acid, vitamin B12, and vitamin B6) may be considered for the prevention of ischemic stroke in patients with homocysteinemia [3]. However, although a number of large clinical trials of B complex vitamins supplementation have been conducted to date, the results of the trials have been inconsistent [4–6]. Some meta-analyses have even shown that folic acid, combined with other B-vitamin supplementation (vitamin B12 and/or vitamin B6), is less effective than folic acid supplementation alone for stroke prevention [7–9]. The effectiveness of B complex vitamin supplementation for

Abbreviations: CI, confidence interval; GRADE, Grading of Recommendations Assessment, Development, and Evaluation; I^2, inconsistency index; RCT, randomized clinical trials; RR, relative risk.

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https://doi.org/10.1016/j.ajcnut.2023.12.021
Received 12 October 2023; Received in revised form 22 December 2023; Accepted 26 December 2023; Available online 1 February 2024

stroke prevention still needs further confirmation [3,10]. We postulate that the reasons for these inconsistent study results are complicated and may be because of differences in national and regional nutrition strategies, population demographics, and study design characteristics.

Substantial evidence suggests that the dosage of B-vitamin supplementation may be an essential factor in stroke prevention [11]. In some studies, folic acid dosage ceilings of 0.8 mg/d [9], 1 mg/d [12], or 2.5 mg/d [8] have been reported for stroke benefit, whereas other studies concluded that higher dosages of vitamin B12 supplementation, such as >0.05 mg/d [13], 0.18 mg/d [9], or 0.4 mg/d [11] no longer decreased stroke risk, compared with lower dosages. Although these findings have clinical and public health implications, the focus of these studies remains on the supplemental dosage of an individual B vitamin. The dosage ranges of folic acid, vitamin B12, and vitamin B6, when the supplementation is a combination of all 3, that minimize the risk of stroke have not received sufficient attention.

To address the above research gaps, this meta-analysis re-evaluated clinical trials of folic acid, vitamin B12, and vitamin B6 combination supplementation in stroke prevention, utilizing evidence from the most recent trials and the largest sample size to date. Our aim was to evaluate the efficacy of concomitant supplementation with the 3 B vitamins and to identify the optimal dosage range for stroke prevention.

Methods

Search strategy and selection criteria

Our study was performed according to the recommendations of the PRISMA [14]. The protocol has been registered in the PROSPERO (registration number: CRD42022355077). We searched MEDLINE (via PubMed), the Cochrane Central Register of Controlled Trials and Embase database using the following terms: ("Folic Acid," "folic acid," "folate," "multivitamins," "5-methyltetrahydrofolate") and ("Stroke," "cerebrovascular accident," "Cardiovascular Disease," "Cardiovascular Diseases," "Coronary Disease," "Coronary Thrombosis," "Myocardial Ischemia," "Coronary Restenosis," "Coronary Stenosis"). We restricted our search to human studies and randomized clinical trials (RCTs) published from January 1966 up to 1 April 2023. There were no language restrictions.

Inclusion and exclusion criteria

Study selection and data extraction were performed by a study team according to a standard protocol. The study team consisted of experts in clinical medicine, clinical trials, epidemiology, and statistics. The selection criteria had strict inclusion criteria as follows: *1*) the study was an RCT; *2*) the intervention consisted of folic acid combined with vitamin B12 and vitamin B6 supplementation (with or without other multivitamins); *3*) the control group received either a placebo or low dosages of B vitamins (for clinical trials that did not have placebo controls, the low-dose group was selected as the control); *4*) intervention duration was ≥6 mo; *5*) the number of strokes in both the treatment and control groups was reported during the study period, with >10 incident cases in any given study; and *6*) participants were adults (aged ≥18 y) of any sex. Duplicate articles were discarded.

Data extraction

Data for this meta-analysis were extracted independently by 3 investigators (NZ, ZZ, and ZW) using a standard protocol. Any disagreements or unresolved differences were resolved by discussion with a fourth reviewer (YH) and by referencing the original report. The information extracted from each eligible trial included the following: study name or first author's name, study country, study population, folic acid fortification status, total number of participants, number of participants in each intervention group, duration of follow-up, dosage of intervention medication, total number of strokes in the treatment and control groups, concomitant diseases, mean age of the study participants, number and percentage of women, and type of control group. If appropriate, all supplementation dosages were converted to units of mg/d.

Trials conducted entirely in countries or regions with folic acid grain fortification policies were categorized as full folic acid grain fortification trials in this study. Trials conducted in multiple countries, including both folic acid fortified and nonfortified countries, were classified as partial folic acid grain fortification studies. For example, the FAVORIT study [15], conducted in the United States, Canada, and Brazil, was categorized in our study as a trial in a partially folic acid fortified area.

Statistical analysis

The effect of folic acid combined with vitamin B12 and vitamin B6 supplementation on stroke risk was evaluated based on data from the 14 eligible trials. The primary outcome was all types of stroke. Relative risk (RR) with 95% CI was used as a measure of the association between supplementation and risk of stroke among different folic acid fortification status. A stratified analysis was further conducted according to the combined dosage of folic acid and vitamin B12 (folic acid ≤0.8 and vitamin B12 ≤0.4 mg/d compared with folic acid >0.8 or vitamin B12 >0.4 mg/d) by fortification status within a region.

In this study, heterogeneity was assessed through Cochran's Q test and inconsistency index (I^2). Data were pooled across all trials according to the fixed-effects model based on Mantel–Haenszel methods, if considerable heterogeneity ($P < 0.1$, or $I^2 \geq 50\%$) was not present. Because heterogeneity was <50% in all of our analyses, fixed-effects models were adopted to estimate the treatment effect. Sensitivity analyses were performed by removing each individual trial from the meta-analysis to evaluate the stability of the pooled RR. Publication bias was also assessed by Begg's funnel plot and Peter's test, with an asymmetric funnel plot with P value of <0.05 indicating the possibility of publication bias. All statistical analyses were conducted using R software (version 4.1.2, http://www.R-project.org/), developed by the R Foundation for Statistical Computing.

Study quality and certainty assessment

Two reviewers (NZ and ZZ) independently assessed the risk of bias for all studies, including selection bias, performance bias, detection bias, attrition bias, reporting bias and other bias, using the Cochrane risk-of-bias algorithm [16,17]. Review Manager software, version 5.4.1, was used for plotting the risk-of-bias graph and the risk of bias summary. The quality and strength of the evidence was assessed using the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) tool [18]. Evidence was classified as high quality, moderate quality, low quality, or very low quality. Initially, by default, RCTs are rated as high-quality evidence.

Results

Three investigators (NZ, ZZ, and ZW) identified 2006 potential studies and after an initial review of abstracts, excluded 1940 of them including those with non-RCTs, no cardiovascular disease outcomes,

no folic acid intervention, and with only study protocols and registration information. An additional 52 studies were further excluded because of duplicate reporting, no reporting of stroke or cerebrovascular endpoints, low numbers of incident cases (defined as <10 cases), and no inclusion of vitamin B12 and vitamin B6 in the treatment (Supplemental Figure 1). Finally, 14 studies of folic acid combined with vitamin B12 and vitamin B6 supplementation for stroke prevention were included in the meta-analysis [4–6,15,19–28]. Each study's intervention group included the 3 B vitamins (folic acid, vitamin B12, and vitamin B6) with a combined total of 76,664 participants and 2720 cases of stroke. All trials reported the number of stroke events in the treatment and control groups.

Characteristics of the included studies

Table 1 shows the basic characteristics of all 14 trials that were included in this analysis, which enrolled 76,664 participants with 2720 stroke cases. Of these 14 trials, 5 were from Canada and the United States [19,20,24,27,28], 4 trials were from European countries [6,22,23,26], 1 was from China [21], and 4 were conducted in mixed regions [4,5,15,25]. Six trials were conducted in regions with folic acid grain fortification [4,19,20,24,27,28], 5 were conducted in regions without folic acid grain fortification [6,21–23,26], and 3 were conducted in regions with partial folic acid grain fortification [5,15,25]. Dosages in the intervention groups ranged from 0.4 to 40 mg/d for folic acid, from 0.018 to 2 mg/d for vitamin B12, and from 0.05 to 100 mg/d for vitamin B6. Additional baseline study characteristics in each of the RCTs are presented in Supplemental Table 1.

Efficacy of combined B-vitamin supplementation for stroke risk stratified by grain fortification status

Among populations in regions without grain fortification or with partial grain fortification, combined B-vitamin supplementation significantly reduced the risk of stroke by 34% (RR: 0.66; 95% CI: 0.50, 0.86) and 11% (RR: 0.89; 95% CI: 0.79, 1.00), respectively. In contrast, among populations in regions with grain fortification, the RR of stroke was 1.04 (95% CI: 0.94, 1.16). Furthermore, a significant interaction between combined B-vitamin supplementation and folic acid fortification status on stroke was observed (P-interaction = 0.003, Figure 1).

Dosage exploration on combined B-vitamin supplementation for stroke risk among areas of differing folic acid fortification status

After combining those trials without grain fortification and those with partial grain fortification, a significant 35% reduction in stroke risk was found in studies with folic acid ≤0.8 mg/d combined with vitamin B12 ≤0.4 mg/d (RR: 0.65; 95% CI: 0.48, 0.86) and an 11% reduction in stroke risk was found in trials with either folic acid >0.8 mg/d or/and vitamin B12 >0.4 mg/d (RR: 0.89; 95% CI: 0.79, 0.99; P-interaction = 0.045, Table 2). Detailed results of the subgroup analysis are presented in Supplemental Table 2. A scatter plot (Figure 2) that illustrates the RRs of the combined folic acid and vitamin B12 dosages shows a difference in the distribution between the 2 dosage groups among studies without and with partial grain fortification.

In addition, stratified analysis was performed on vitamin B6 dosage (≤10 mg/d compared with >10 mg/d) for studies with folic acid ≤0.8 mg/d combined with vitamin B12 ≤0.4 mg/d, and no significant difference between the 2 groups was found (Supplemental Table 3, P-interaction = 0.642). When doses of folic acid ≤0.8 mg/d combined with vitamin B12 ≤0.4 mg/d and vitamin B6 ≤10 mg/d were used concurrently, the risk of stroke was significantly reduced by 39% (RR: 0.61; 95% CI: 0.42, 0.89; P-interaction = 0.073, Supplemental Table 4).

In trials of populations from folic acid fortified areas, no significant results were found in the stratified analysis (Table 2).

Heterogeneity and sensitivity analysis

In all tests of heterogeneity, the P values were >0.10 and the I^2 statistic was <50% in all analyses. Sensitivity analyses showed that the results did not change significantly after omitting each trial in succession (Supplemental Figure 2). We also excluded studies that included other micronutrients, studies in which the intervention group was not a placebo, and studies conducted in populations with kidney disease, the results did not change (Supplemental Figure 3).

Assessment of bias and certainty of evidence

Of the 14 trials included in this analysis, there were 2 trials that did not report information on the randomization process, and 2 trials with no information on the concealment of treatment allocation. The risk of bias was assessed as unclear in 4 trials because it was unclear whether blinding was used in the assessment of outcomes (Supplemental Figures 4 and 5). The results of the GRADE assessment indicate an overall high certainty level across the 14 trials (Supplemental Table 5).

Begg's funnel plot was symmetric (Supplemental Figure 6), and Peter's test did not find that the funnel plot was asymmetric, demonstrating that the 14 RCTs included in the analysis had no obvious publication bias.

Discussion

To our knowledge, this study is the first meta-analysis to explore the effects of concomitant supplementation with folic acid, vitamin B12, and vitamin B6 on stroke, and included 14 RCTs with a total of 76,664 individuals. For this study, concomitant supplementation with all 3 B vitamins significantly reduced the risk of stroke in populations from areas of nonfortified and partially fortified grain. The results of this analysis showed that supplementation in the form of lower dosages of folic acid combined with lower dosages of vitamin B12 may be more effective in populations from these areas. No similar findings were found in trials among populations from areas of grain fortification.

Folic acid fortification status may be the most critical factor influencing the efficacy of combined B-vitamin supplementation for stroke prevention [29]. A meta-analysis by Wang et al. [30] found that among populations in grain-fortified countries and regions, folic acid supplementation did not show a stroke benefit, and a meta-analysis by Li et al. [8] confirmed that folic acid supplementation significantly reduced stroke risk in populations of unfortified regions, whereas in populations of partially fortified regions, the stroke benefit was marginally significant. In this study, we found that folic acid fortification status was also an important determinant of stroke prevention with combined B-vitamin supplementation. The combination of folic acid, vitamin B12 and vitamin B6 reduced the risk of stroke by 34% in populations from countries and regions that were not grain fortified, and by 11% in populations from countries and regions that were partially grain fortified, whereas no stroke benefit was observed in populations from fortified regions. Grain fortification may result in a higher background dietary intake of folic acid in the population, which limits the ability of B-vitamin supplementation to reduce homocysteine [12], thus,

TABLE 1
Summary of study characteristics in each of the randomized controlled trials included in this meta-analysis

Study	Region	Age (y)	Women (%)	Sample size	Concomitant diseases	Folic acid fortification	Intervention duration (y)	Dosage of intervention group (mg/d)			Control
								Folic acid	Vitamin B12	Vitamin B6	
PHS-II [19]	United States	64.3 (9.2)	0 (0)	14,641	CVD	Yes	11.2	0.4	0.025	3	Placebo
COSMOS [20]	United States	72.1 (6.6)	12,666 (59.1)	21,442	Free of CVD and cancer	Yes	3.6	0.4	0.025	3	Placebo
SU.FOL.OM3 [6]	France	60.6 (NR)	514 (20.6)	2501	CHD/stroke	No	4.7	0.5	0.02	3	Placebo
Linxian [21]	China	54 (NR)	1857 (56.0)	3318	Esophageal dysplasia	No	6	0.8	0.018	6	Placebo
NORVIT [22]	Norway	63.0 (11.7)	527 (28.0)	1880	Myocardial infarction	No	3.3	0.8	0.4	40	Placebo
WENBIT [23]	Norway	61.7 (9.9)	328 (21.2)	1550	CHD	No	3.2	0.8	0.4	40	Placebo
TACT [24]	United States, Canada	65 (NR)	299 (17.5)	1708	CHD	Yes	2.6	0.8	0.1	0.05	Placebo
VITATOPS [25]	20 countries	62.6 (12.5)	2946 (36.1)	8164	Stroke	Partial	2.8	2.0	0.5	25	Placebo
Heinz et al. [26]	Germany	61 (13.0)	271 (41.7)	650	ESRD	No	2.1	2.1	0.02	8.6	Folic acid 0.2 mg/d, vitamin B12 0.004 mg/d, and vitamin B6 1 mg/d
VISP [4]	United States, Canada, United Kingdom	66.3 (10.8)	1379 (37.5)	3680	Stroke	Yes	2	2.5	0.4	25	Folic acid 0.02 mg/d, vitamin B12 0.006 mg/d, and vitamin B6 0.2 mg/d
HOPE-2 [5]	13 countries	68.9 (6.9)	1559 (28.2)	5522	CVD/DM	Partial	5	2.5	1.0	50	Placebo
WAFACS [27]	United States	62.8 (8.8)	5442 (100)	5442	CVD	Yes	7.3	2.5	1.0	50	Placebo
FAVORIT [15]	United States, Canada, Brazil	52.0 (9.4)	1528 (37.2)	4110	Kidney transplantation	Partial	4	5.0	1.0	50	Vitamin B12 0.002 mg/d and vitamin B6 1.4 mg/d
HOST [28]	United States	65.8 (11.8)	33 (1.6)	2056	ESRD	Yes	3.2	40	2	100	Placebo

Abbreviations: CHD, coronary artery disease; COSMOS, COcoa Supplement and Multivitamin Outcomes Study; CVD, cardiovascular disease; DM, diabetes mellitus; ESRD, end-stage renal disease; FAVORIT, Folic Acid for Vascular Outcome Reduction in Transplantation; HOPE-2, Heart Outcomes Prevention Evaluation 2; HOST, Homocysteinemia in Kidney and End Stage Renal Disease; NORVIT, Norwegian Vitamin; NR, not reported; PHS-II, The Physicians' Health Study; SU.FOL.OM3, Supplementation with Folate, vitamin B6 and B12, and/or Omega-3 fatty acid; TACT, Trial to Assess Chelation Therapy; VISP, Vitamin Intervention for Stroke Prevention; VITATOPS, Vitamins to Prevent Stroke; WAFACS, Women's Antioxidant and Folic Acid Cardiovascular Study; WENBIT, Western Norway B-Vitamin Intervention Trial.

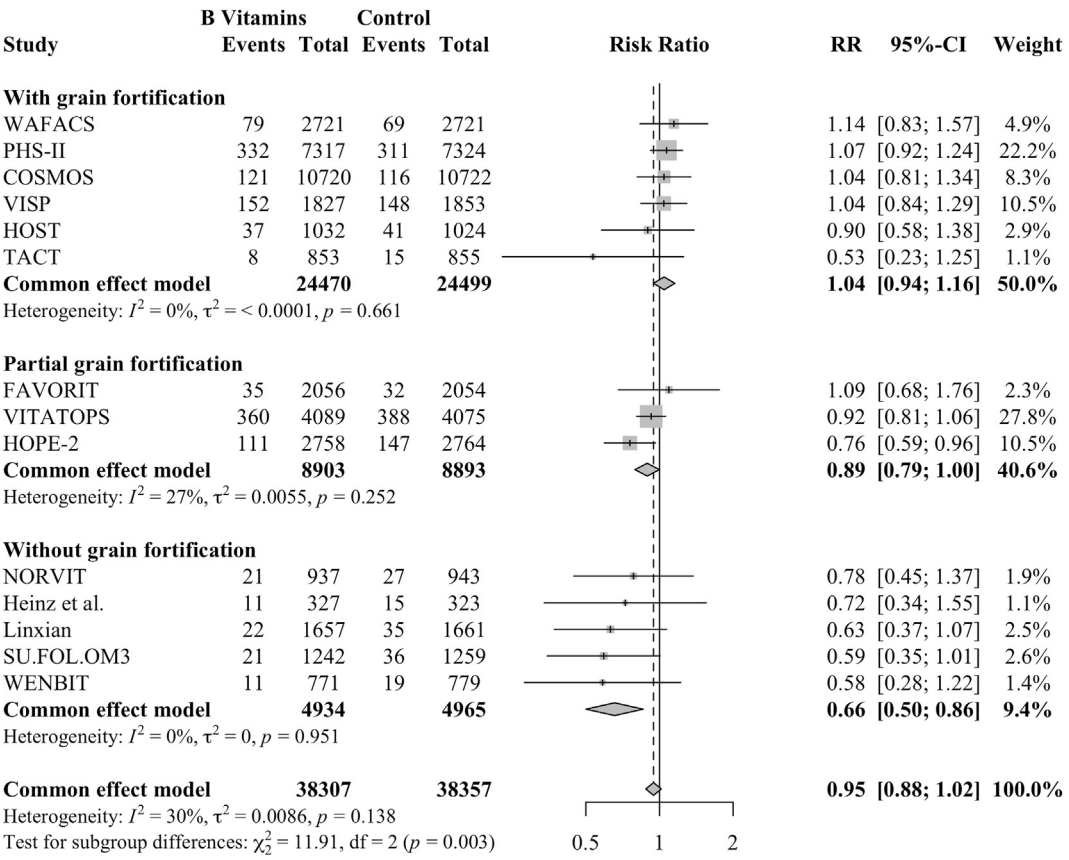

FIGURE 1. Forest plot of relative risks (RR) and 95% CI of stroke for B-vitamin treatment stratified by grain fortification status. Heterogeneity was assessed through Cochran's Q test and inconsistency index (I^2). Relative risks were calculated using a fixed-effects model based on Mantel–Haenszel methods. COSMOS, COcoa Supplement and Multivitamin Outcomes Study; FAVORIT, Folic Acid for Vascular Outcome Reduction in Transplantation; HOPE-2, Heart Outcomes Prevention Evaluation 2; HOST, Homocysteinemia in Kidney and End Stage Renal Disease; NORVIT, Norwegian Vitamin; PHS-II, The Physicians' Health Study; SU.FOL.OM3, Supplementation with Folate, vitamin B6 and B12, and/or Omega-3 fatty acid; TACT, Trial to Assess Chelation Therapy; VISP, Vitamin Intervention for Stroke Prevention; VITATOPS, Vitamins to Prevent Stroke; WAFACS, Women's Antioxidant and Folic Acid Cardiovascular Study; WENBIT, Western Norway B-Vitamin Intervention Trial.

diminishing the potential for the active treatment group to exert a therapeutic effect.

Evidence shows that since the publication of the results of the Linxian trial in 1996 [21], there have been >20 large clinical trials of B-vitamin supplementation for stroke prevention worldwide [9], with 14 trials, or more than half, of combined supplementation with the 3 B vitamins, folic acid, vitamin B12, and vitamin B6. It is important to mention that vitamin B12 and vitamin B6 are essential coenzymes for homocysteine metabolism: in nonhepatocytes, homocysteine re-methylation is dependent on vitamin B12 activity, whereas in hepatocytes, vitamin B6-dependent cystathionine β-synthase dominates the homocysteine-lowering transsulfuration pathway [31,32]. This explains why most previous trials have utilized a treatment strategy of folic acid in combination with vitamin B12 or vitamin B6, to achieve a better reduction of homocysteine. Further evidence suggests that the association between homocysteine and ischemic stroke may be stronger [33], which may explain why some clinical trials have found a significant reduction in risk of ischemic stroke, with no significant change in risk of hemorrhagic stroke [7]. Unfortunately, the primary end point of 11 of the 14 trials included in this study was set at total

TABLE 2
Relative risks (RRs) and 95% CIs of B vitamins on stroke stratified by dosage and grain fortification status

Stratification variables	Stroke events/total subjects		RR (95% CI)	*P*-interaction
	B-vitamin group	Control group		
Without and with partial folic acid fortification	592/13,837	699/13,858	0.85 (0.76, 0.94)	
Folic acid combined with vitamin B12 dosage (mg/d)				0.045
Folic acid ≤0.8 and vitamin B12 ≤0.4	75/4607	117/4642	0.65 (0.48, 0.86)	
Folic acid >0.8 or/and vitamin B12 >0.4	517/9230	582/9216	0.89 (0.79, 0.99)	
Folic acid fortification	729/24,470	700/24,499	1.04 (0.94, 1.16)	
Folic acid combined with vitamin B12 dosage (mg/d)				0.985
Folic acid ≤0.8 and vitamin B12 ≤0.4	461/18,890	442/18,901	1.04 (0.92, 1.19)	
Folic acid >0.8 or/and vitamin B12 >0.4	268/5580	258/5598	1.05 (0.89, 1.23)	

Relative risks were calculated using a fixed-effects model based on Mantel–Haenszel methods.

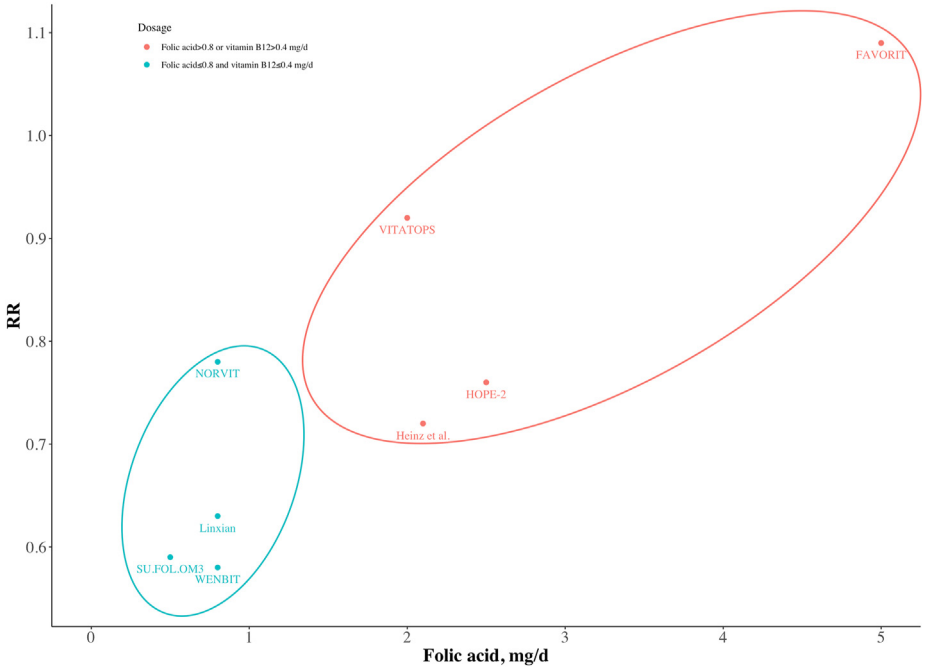

FIGURE 2. Scatterplot of relative risks stratified by folic acid and vitamin B12 supplementation dosage in studies without and with partial folic acid fortification. FAVORIT, Folic Acid for Vascular Outcome Reduction in Transplantation; HOPE-2, Heart Outcomes Prevention Evaluation 2; NORVIT, Norwegian Vitamin; RR, relative risk; SU.FOL.OM3, Supplementation with Folate, vitamin B6 and B12, and/or Omega-3 fatty acid; VITATOPS, Vitamins to Prevent Stroke; WENBIT, Western Norway B-Vitamin Intervention Trial.

stroke. Future studies may need to further consider differentiating between stroke types during the design phase to verify the effects of B-vitamin supplementation on the type of stroke.

The dosage and form of B-vitamin supplementation have been a fervid topic of debate, with previous studies mainly focusing on examining each vitamin as a single dosage. For folic acid, the meta-analyses by Huo et al. [7] and Zhao et al. [9] both showed better stroke prevention at folic acid dosages of ≤0.8 mg/d. Higher doses of folic acid supplementation may have a ceiling effect on stroke protection, leading to an accumulation of unmetabolized folic acid and placing a burden on renal function, while masking the actual stroke benefit [34]. For vitamin B12, cyanocobalamin, used in previous clinical trials, is not directly available to humans and its nephrotoxicity may obscure benefits [11]. This is illustrated in the Diabetic Intervention with Vitamins to Improve Nephropathy study [35], individuals with diabetic nephropathy who were supplemented daily with 2.5 mg folic acid, 1 mg vitamin B12, and 25 mg vitamin B6 had a significant decrease in glomerular filtration rate and an increase in stroke events. A meta-analysis by Spence et al. [11] noted no stroke benefit from B-vitamin supplementation at a vitamin B12 dosage above 0.4 mg/d. Similar results were seen in a meta-analysis by Hsu et al. [13] in populations without mandatory food fortification. Vitamin B6 is an important determinant of postprandial homocysteine levels [36]. Although there are no studies exploring vitamin B6 dosage to date, evidence from many observational studies has suggested a negative association between vitamin B6 and stroke [37]. It is worth noting, however, that in the case of combined supplementation, it is not enough to focus only on the dosage of a single nutrient, which is also bound to bring about bias in the results. No previous study has taken this into account [7–9,11].

As seen in trials among populations from areas of nonfortified and partially fortified grain, those using lower dosages of folic acid also tend to be accompanied with lower dosages of vitamin B12 [6,21–23]. This could lead to an overlap of studies when performing single nutrient stratification. For this reason, we carried out a stratified analysis of folic acid combined with vitamin B12 dosage where a 35% reduction in stroke risk was found when a dosage of folic acid ≤0.8 mg/d was combined with a dosage of vitamin B12 ≤0.4 mg/d. In contrast, a folic acid dosage that exceeded 0.8 mg/d or/and a vitamin B12 dosage that exceeded 0.4 mg/d, both diminished the efficacy of stroke prevention. These results also suggest that when using combined supplementation among populations of nonfortified and partially fortified grain areas, the dosages of folic acid and vitamin B12 need to be considered together to achieve optimal stroke prevention. For vitamin B6 dosage, although we found that a lower dosage of vitamin B6 (≤10 mg/d) combined with folic acid may also be appropriate, more studies are still needed to determine the efficacy of vitamin B6 for stroke prevention.

Our study has several limitations. First, as with any meta-analysis, inherent limitations cannot be avoided, including adjusting for demographic characteristics at baseline, despite that no significant heterogeneity between studies was found. Second, the ability to detect interactions in existing analyses is limited. Our results can be seen as hypothesis-generating and larger RCTs are needed to confirm our results. Third, we were not able to analyze the efficacy of folic acid in combination with vitamin B12 and vitamin B6 in different stroke subtypes because of a lack of data from each study. Fourth, although publication bias was assessed using a funnel plot test, this bias cannot be entirely ruled out. Our study has very important public health implications for stroke prevention in countries and regions where foods are not routinely fortified with folic acid, such as China, India, and African countries. In addition, for fortified countries where populations have a higher background dietary intake of folic acid, future studies should place more focus on the dosage of vitamin B12 and the type of

B-vitamin supplementation to identify those populations that could benefit further.

In conclusion, based on evidence from recent research, this study confirms the feasibility of supplementation of folic acid in combination with vitamin B12 and vitamin B6 for stroke prevention, especially in populations from nonfortified and partially fortified grain areas. Additionally, lower dosages of folic acid in combination with lower doses of vitamin B12 showed a surprisingly low risk. These findings emphasize that the appropriate dosage and form of supplementation need to be considered in the context of nutritional status to achieve optimal stroke prevention efficacy.

Acknowledgments

We are grateful for the contributions of all 14 studies.

Author contributions

The authors' responsibilities were as follows – ZZ, NZ: designed research; ZZ, NZ, ZW: conducted research; ZZ, NZ: analyzed data; ZZ, NZ: wrote the paper; NZ, XB, YS, PL, XL, ZZ: revised the manuscript for important intellectual content critically; ZZ: had primary responsibility for final content; and all authors: read and approved the final manuscript.

Conflict of interest

The authors report no conflicts of interest.

Funding

The authors reported no funding received for this study.

Data availability

All data in this analysis are based on published studies. The data, study materials, and analytic methods of this study will be available from the corresponding author upon reasonable request, after the request is submitted and formally reviewed and approved by the corresponding author.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ajcnut.2023.12.021.

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