

Folic Acid in Stroke Prevention in Countries without Mandatory Folic Acid Food Fortification: A Meta-Analysis of Randomized Controlled Trials

Chia-Yu Hsu,^{a,*} Shao-Wen Chiu,^{a,*} Keun-Sik Hong,^b Jeffrey L. Saver,^c Yi-Ling Wu,^d Jiann-Der Lee,^a Meng Lee,^a Bruce Ovbiagele^e

^aDepartment of Neurology, Chang Gung Memorial Hospital, Chang Gung University College of Medicine, Chiayi, Taiwan

^bDepartment of Neurology, Inje University Ilsan Paik Hospital, Inje University College of Medicine, Goyang, Korea

^cStroke Center and Department of Neurology, Geffen School of Medicine, University of California, Los Angeles, CA, USA

^dResearch Services Center for Health Information, Chang Gung University, Taoyuan, Taiwan

^eDepartment of Neurosciences, Medical University of South Carolina, Charleston, SC, USA

Background and Purpose Additional folic acid (FA) treatment appears to have a neutral effect on reducing vascular risk in countries that mandate FA fortification of food (e.g., USA and Canada). However, it is uncertain whether FA therapy reduces stroke risk in countries without FA food fortification. The purpose of this study was to comprehensively evaluate the efficacy of FA therapy on stroke prevention in countries without FA food fortification.

Methods PubMed, EMBASE, and clinicaltrials.gov from January 1966 to August 2016 were searched to identify relevant studies. Relative risk (RR) with 95% confidence interval (CI) was used as a measure of the association between FA supplementation and risk of stroke, after pooling data across trials in a random-effects model.

Results The search identified 13 randomized controlled trials (RCTs) involving treatment with FA that had enrolled 65,812 participants, all of which stroke was reported as an outcome measure. After all 13 RCTs were pooled, FA therapy versus control was associated with a lower risk of any future stroke (RR, 0.85; 95% CI, 0.77 to 0.95). FA alone or combination of FA and minimal cyanocobalamin (≤ 0.05 mg/day) was associated with a lower risk of future stroke (RR, 0.75; 95% CI, 0.66 to 0.86) whereas combination of FA and cyanocobalamin (≥ 0.4 mg/day) was not associated with a lower risk of future stroke (RR, 0.95; 95% CI, 0.86 to 1.05).

Conclusions FA supplement reduced stroke in countries without mandatory FA food fortification. The benefit was found mostly in patients receiving FA alone or combination of FA and minimal cyanocobalamin.

Keywords Folic acid; Stroke; Prevention; Food, fortified; Meta-analysis

Introduction

Homocysteine, a sulfur-containing amino acid, was thought to be an independent vascular risk factor.¹ Hyperhomocysteinemia

was related with endothelium dysfunction and procoagulation state through mechanisms of altering vascular morphology, stimulating inflammation, activating the endothelium and the blood clotting cascade, and inhibiting fibrinolysis.² A 25% low-

Correspondence: Meng Lee
Department of Neurology, Chang Gung Memorial Hospital, Chang Gung University College of Medicine, 6, West Section, Chiapu Road, Puzi City, Chiayi County 613, Taiwan
Tel: +886-5-3621000
Fax: +886-5-3623002
E-mail: menglee5126@gmail.com

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*These authors contributed equally to the manuscript as first author.

er usual total homocysteine level was associated with a 19% lower stroke risk.¹ Folic acid, as a cofactor of enzymes involved in homocysteine metabolism, could lower total homocysteine by 20% to 25%,³ and it was supposed to decrease the risk of vascular events. Many randomized controlled trials (RCTs) had been conducted to evaluate the efficacy of folic acid supplementation for the prevention of cardiovascular/cerebrovascular diseases, but their results were diverging.

To our knowledge, meta-analyses regarding this issue mostly found no benefit of folic acid for coronary artery disease prevention.^{4,5} However, there might be slight benefits in terms of stroke prevention.⁵⁻⁸ A meta-analysis found folic acid supplementation reduced the stroke risk by 11% in the 10 trials with no or partial folic acid fortification but had no benefit on stroke risk in the other five trials with folic acid mandatory fortification.⁷ Indeed, a population-based cohort study had found stroke mortality decreased in United States and Canada after mandatory folic acid fortification.⁹ Therefore, additional folic acid supplementation in these countries may have limited benefit to further reduce stroke risk. On the other hand, in the countries without mandatory folic acid fortification, whether folic acid supplementation could prevent stroke would be an important issue for public health.

Some RCTs carried out in the countries without folic acid fortification have been published in the interval since the most recent meta-analysis and offered more evidence on this issue.^{10,11} Therefore, we undertook an update meta-analysis to evaluate the efficacy of folic acid therapy on stroke prevention in countries without mandatory folic acid food fortification.

Methods

This study was performed in accordance with the recommendations of the preferred reporting items for systematic reviews and meta-analysis: the PRISMA statement.¹²

Search strategy

We searched PubMed, EMBASE, and the clinical trial registry maintained at clinicaltrials.gov from January 1966 to September 2016 using the search strategy "homocysteine" or "folate" or "folic acid" or "vitamin B12" or "cobalamin" or "vitamin B6" or "pyridoxine" or "multivitamin" and "cardiovascular disease" or "myocardial infarct" or "myocardial ischemia" or "coronary heart disease" or "angina" or "heart attack" or "stroke" or "cerebrovascular disease" or "cerebrovascular attack" or "brain attack" or "brain infarct" or "brain hemorrhage" or "intracranial hemorrhage." We restricted our search to human beings and clinical trials. There were no language restrictions. We also re-

viewed the introduction and discussion sections of retrieved trials and of prior meta-analyses to identify additional trials.

Included criteria for studies were as follows: (1) the study designed as an RCT; (2) the active treatment comprised folic acid supplementation (with or without additional vitamin B supplementation); (3) total participants and number of stroke events were reported as an outcome endpoint; (4) duration of active treatment at least 6 months; and (5) most participants (>50%) in a trial resided in countries without mandatory folic acid food fortification. Participants of any age or sex were included.

Since mandatory folic acid supplement has been applied to United States (since 1998), Canada (since 1998), Costa Rica (since 1998), Chile (since 2000), and South Africa (since 2003), trials with >50% of participants resided in these countries were excluded.

All data from eligible trials were abstracted in duplicate by two investigators independently (C.Y.H. and Y.L.W.) with a standard protocol. Discrepancies were resolved by discussion with a third investigator (M.L.) and by referencing the original report.

Study quality assessment

Since all the included studies were RCTs, the risk of bias (e.g., selection bias, performance bias, detection bias, attrition bias, and reporting bias) of the included trials was assessed by Cochrane risk-of-bias algorithm (www.cochrane.org/training/cochrane-handbook).¹³

Primary and secondary endpoints

The primary endpoint was stroke (ischemic or hemorrhagic). The secondary endpoints were ischemic stroke and hemorrhagic stroke, respectively. Subgroup analyses were conducted according to different study characteristics: treatment duration (<3 years vs. ≥3 years), sample size (<1,000 vs. ≥1,000), and mean age at entry (<65 years vs. ≥65 years).

Statistical analysis

All analyses were based on the intention-to-treat principle. Relative risk (RR) with 95% confidence interval (CI) was used as a measure of the association between folic acid supplementation and risk of stroke. We report absolute risks in terms of the difference in the number of events per 1,000 patients and the respective 95% CI. We computed a random-effects estimate based on the Mantel-Haenszel method when two or more studies provided sufficient data for a given outcome and compared the results with those obtained from a fixed-effects model. Statistical heterogeneity was assessed by the chi-square and the I^2 statistics. Heterogeneity was considered if either the chi-square test was

significant with the $P=0.10$ level, or the I^2 statistic was greater than 50%. Publication bias was estimated visually by funnel plots displaying standard error as the measure of sample size and RR as the measure of treatment effect.¹⁴ We also performed a sensitivity analysis to further explore the robustness of our results. To identify any study that might have exerted a disproportionate influence on the summary treatment effect, we removed each individual trial from the meta-analysis one at a time. Subgroup analyses were conducted based on the baseline characteristics. This meta-analysis was analyzed by Cochrane Collaboration's Review Manager Software Package (RevMan version 5.3, The Cochrane Collaboration, London, UK).

To evaluate whether the present meta-analysis had sufficient sample size to reach firm conclusions about the effect of interventions, trial sequential analysis was performed for the primary endpoint.¹⁵ Trial sequential analysis performs accumulative meta-analysis, which creates Z curve of the summarized observed effect and the monitoring boundaries for benefit, harm, and futility, and it estimates the required information size. These boundaries and analyses are adjusted to account for the amount of available evidence and to control for repeated analyses, while maintaining type I error at 5% and the power at 80%. The required information size was calculated based on event rates observed in control group and folic acid group. If the Z curve of the cumulative meta-analysis crosses one of the boundaries, no further studies are required, and there is sufficient evidence to support the conclusions.

Results

The literature review identified 54 articles for detailed assessment, among which 32 were excluded for not reporting stroke as an endpoint, nine were excluded for being conducted in countries with mandatory folic acid fortification,¹⁶⁻²⁴ and our final analysis included 13 RCTs, conducted in countries without mandatory folic acid fortification, involving treatment with folic acid (Figure 1).^{10,11,25-35} Included^{10,11,25-35} versus excluded trials¹⁶⁻²⁴ based on whether they were conducted mostly in countries without mandatory folic acid fortification were presented in Table 1. Study design characteristics and baseline characteristics of included trials were presented in Table 2.^{10,11,25-35} Overall, 65,812 participants were enrolled with 33,741 (51%) participants randomly assigned to the folic acid therapy group and 32,071 (49%) to the control group. Among 13 trials, 12 trials included individuals with preexisting conditions: stroke (one trial),³³ coronary heart disease (five trials),^{26-28,31,35} cardiovascular disease (one trial),³² end-stage renal disease or advanced chronic kidney disease (three trials),^{29,30,34} hypertension (one trial),¹⁰ and

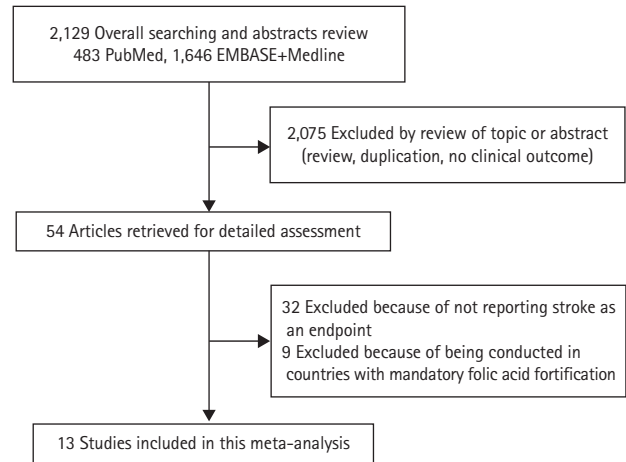


Figure 1. Flow chart of study selection.

esophageal dysplasia (one trial)²⁵ while one trial included individuals living in high altitude.¹¹ Folic acid alone or combination of folic acid and minimal cyanocobalamin (≤ 0.05 mg/day) was used in an active treatment group in eight trials^{10,25-27,29,30,32,34} whereas combination of folic acid and cyanocobalamin (≥ 0.4 mg/day) was used in an active treatment group in five trials.^{11,28,31,33,35} Three trials excluded participants with chronic kidney disease^{26,27,32} while other trials included some participants with chronic kidney disease. Cerebrovascular events analyzed were combined nonfatal and fatal strokes (ischemic and hemorrhagic) in 10 trials; for one trial each, data were available only on fatal stroke,²⁵ non-fatal and fatal ischemic stroke,¹¹ and the composite of nonfatal and fatal stroke plus transient ischemic attack.²⁷ Neuroimaging was explicitly mentioned as part of the stroke event ascertainment process in seven trials.^{10,11,28,29,31,34}

Risk-of-bias assessment of included trials was reported in Supplementary Table 1. The results from Liem et al.²⁶, Liem et al.²⁷, and Righetti et al.²⁹ had high risks of performance bias and detection bias because these were an open, non-blinded studies.

After all 13 trials were pooled, folic acid therapy versus control was associated with a lower risk of any future stroke (RR, 0.85; 95% CI, 0.77 to 0.95; $P=0.004$; 3 to 8 fewer events/1,000 patients). Folic acid alone or combination of folic acid and minimal cyanocobalamin (≤ 0.05 mg/day) was associated with a lower risk of future stroke (RR, 0.75; 95% CI, 0.66 to 0.86; $P<0.0001$) whereas combination of folic acid and cyanocobalamin (≥ 0.4 mg/day) was not associated with a lower risk of future stroke (RR, 0.95; 95% CI, 0.86 to 1.05; $P=0.30$) (Figure 2). There was slightly asymmetrical appearance on the funnel plot suggesting a small degree of publication bias, with a slight under-representation of studies showing neutral effects (Supplementary Figure 1). Sensitivity analyses excluding individual trials yielded pooled results that were not

Table 1. Included versus excluded trials based on whether they were conducted mostly in countries without mandatory folic acid fortification

Study	Preexisting condition	Control	Study period	Participants from the countries without mandatory FA fortification*	Participants from the countries with mandatory FA fortification*
Included studies					
Mark et al. (1996) ²⁵	Esophageal dysplasia	Placebo	1985–1991	100% (China)	-
Liem et al. (2004) ²⁶	Acute MI with hyperlipidemia	Usual care	1998–2003	100% (Netherlands)	-
Liem et al. (2005) ²⁷	CHD with hyperlipidemia	Usual care	1998–2003	100% (Netherlands)	-
ASFAST (2006) ³⁰	Creatinine clearance <25 mL/min	Placebo	June 1998–December 2003	100% (Australia and New Zealand)	-
NORVIT (2006) ²⁸	Acute MI	Placebo	December 1998–March 2002	100% (Norway)	-
Righetti et al. (2006) ²⁹	ESRD under hemodialysis	Usual care	January 2001–December 2005	100% (Italy)	-
WENBIT (2008) ³¹	CHD and/or aortic valve stenosis	Placebo	January 2000–April 2004	100% (Norway)	-
Heinz et al. (2010) ³⁴	ESRD under hemodialysis	Low dose vitamin B	July 2002–July 2008	100% (Germany)	-
SEARCH (2010) ³⁵	MI	Placebo	1998–2008	100% (UK)	-
SU.FOLOM3 (2010) ³²	CVD	Placebo	February 2003–June 2007	100% (France)	-
VITATOPS (2010) ³³	Stroke or TIA	Placebo	November 1998–December 2008	99.3% (Australia, Austria, Brazil, Belgium, China, India, Italy, Moldova, Malaysia, Netherlands, New Zealand, UK, Pakistan, Philippines, Portugal, Georgia, Serbia, Singapore, Sri Lanka)	0.7% (USA)
CSPT (2015) ¹⁰	Hypertension	Usual care	May 2008–August 2013	100% (China)	-
Kotwal et al. (2015) ¹¹	High-altitude	Usual care	May 2006–August 2006	100% (India)	-
Excluded studies due to more than 50% participants from countries with mandatory FA fortification					
VISP (2004) ¹⁶	Ischemic stroke	Low dose vitamin B	September 1996–May 2003	1 Center in Scotland	45 Centers in USA and 10 Centers in Canada
Wrone et al. (2004) ¹⁷	ESRD	Low dose vitamin B	March 1998–May 1999	-	100% (USA)
HOPE-2 (2006) ¹⁸	Vascular disease or diabetes	Placebo	2000–2005	27.9% (Western Europe, Brazil, Slovakia)	72.1% (USA and Canada)
Jamison et al. (2007) ²⁰	Advanced CKD and ESRD	Placebo	September 2001–May 2006	-	100% (USA)
Cole et al. (2007) ¹⁹	Colorectal adenoma	Placebo	July 1994–October 2004	-	100% (USA)
Albert (2008) ²¹	CVD	Placebo	April 1998–July 2005	-	100% (USA)
House et al. (2010) ²²	Diabetic nephropathy	Placebo	May 2001–July 2007	-	100% (Canada)
Bostom et al. (2011) ²³	Kidney transplant	Low dose vitamin B	August 2002–January 2010	14.9% (Brazil)	85.1% (Canada and USA)
Lamas et al. (2013) ²⁴	Myocardial infarction	Placebo	September 2003–October 2011	-	100% (USA and Canada)

FA, folic acid; MI, myocardial infarction; CHD, coronary heart disease; ASFAST, Atherosclerosis and Folic Acid Supplementation Trial; NORVIT, Norwegian Vitamin; ESRD, end-stage renal disease; WENBIT, Western Norway B-Vitamin Intervention Trial; SEARCH, Study of the Effectiveness of Additional Reductions in Cholesterol and Homocysteine; SU.FOLOM3, SUPplementation with FOLate, vitamin B6 and B12 and/or OMEga-3 fatty acids; CVD, cardiovascular disease; VITATOPS, VITamins TO Prevent Stroke; TIA, transient ischemic attack; CSPT, China Stroke Primary Prevention Trial; VISP, Vitamin Intervention for Stroke Prevention; HOPE-2, Heart Outcomes Prevention Evaluation 2; CKD, chronic kidney disease.

*Countries with mandatory folic acid supplement included USA (since 1998), Canada (since 1998), Costa Rica (since 1998), Chile (since 2000), and South Africa (since 2003).

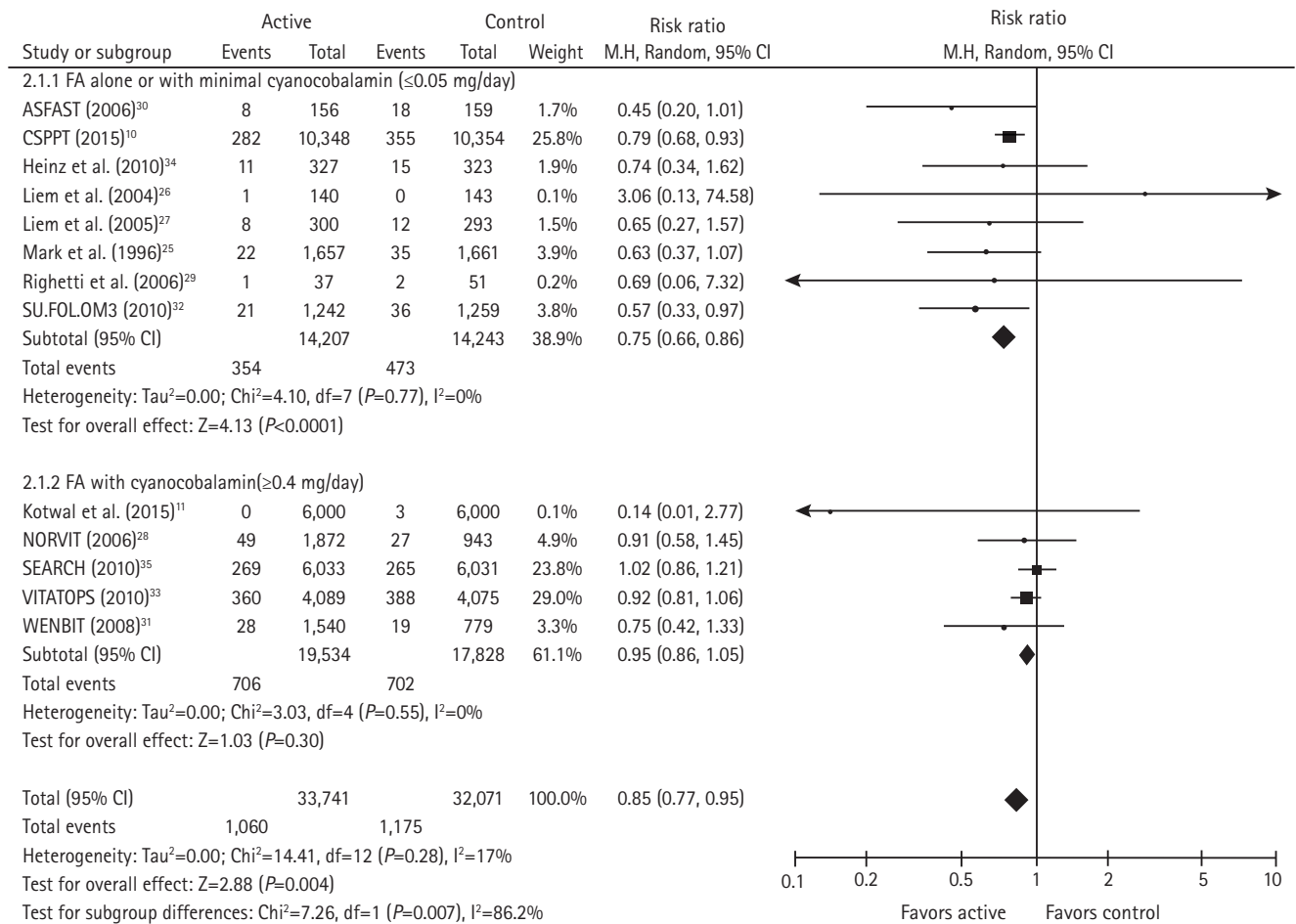


Figure 2. Risk ratio with 95% CI estimates for stroke (active treatment vs. control), by trial and pooled. M-H, Mantel-Haenszel methods; CI, confidence interval; FA, folic acid; ASFAST, Atherosclerosis and Folic Acid Supplementation Trial; CSPT, China Stroke Primary Prevention Trial; SU.FOL.OM3, SUPplementation with FOLate, vitamin B6 and B12 and/or OMega-3 fatty acids; NORVIT, Norwegian Vitamin; SEARCH, Study of the Effectiveness of Additional Reductions in Cholesterol and Homocysteine; VITATOPS, VITamins TO Prevent Stroke; WENBIT, Western Norway B-Vitamin Intervention Trial.

significantly different from the overall pooled estimates (Table 3). Trial sequential analysis was conducted and showed the number of patients evaluated (n=65,812) almost reached the required information sizes (n=66,784) and the summarized observed effect crossed benefit boundary (Figure 3).

Two trials reported data on ischemic stroke and one trial reported data on hemorrhagic stroke. There was no evidence of an effect of folic acid on ischemic (RR, 0.65; 95% CI, 0.25 to 1.71; P=0.38) and hemorrhagic stroke (RR, 0.94; 95% CI, 0.66 to 1.34; P=0.72), respectively. The overall qualities of evidence were high in stroke endpoint and low in ischemic stroke (Supplementary Table 2).

In subgroup analyses, folic acid therapy was associated with a reduction or decreasing trend in the risk of subsequent strokes when we stratified the estimates by treatment duration, sample size, and mean age at entry. There was also no obvious heterogeneity among the different study characteristics (Figure 4).

Discussion

In this meta-analysis of 13 RCTs of generally good quality, among over 65,000 people living in countries without mandatory folic acid fortification in food, we found that addition of folic acid was associated with a 15% RR reduction in future stroke risk. Folic acid alone or combination of folic acid and minimal cyanocobalamin (≤0.05 mg/day) was associated with a 25% lower risks of future stroke whereas combination of folic acid and cyanocobalamin (≥0.4 mg/day) was not associated with lower risks of future stroke. While five fewer stroke events/1,000 patients may seem modest, additional folic acid supplementation may be associated with huge benefits with inexpensive cost, since most countries have not adopted mandatory folic acid fortification. This updated meta-analysis provided robust evidence of beneficial effects of folic acid supplementation for stroke prevention in countries that do not have

Table 2. Characteristics of included trials

Study	Sample size	Age (yr)	Men (%)	FA daily dose in treatment arm (mg)	Vitamin B12 daily dose in treatment arm (mg)	Duration (mo)	Stroke definition	CKD	Prior stroke
Mark et al. (1996) ²⁵	3,318	54	44	0.8	0.018	72	Fatal stroke	NR	NR
Liem et al. (2004) ²⁶	283	59	70	5	0	12	Fatal and nonfatal stroke	0%	NR
Liem et al. (2005) ²⁷	593	65.2±9.8	78	0.5	0	42	Fatal and nonfatal stroke and TIA	0%	7%
ASFAST (2006) ³⁰	315	56±13	68	15	0	43	Fatal and nonfatal stroke	100% (ESRD)	9%
NORVIT (2006) ²⁸	2,815*	63.2±11.6	74	0.8	0.4	36	Fatal and nonfatal stroke	23.8% (Cr >1.13 mg/dL)	4%
Righetti et al. (2006) ²⁹	88	64.5±1.8	56	5	Not added in most patients	29	Fatal and nonfatal stroke	100% (ESRD)	NR
WENBIT (2008) ³¹	2,319*	61.7±10.1	79	0.8	0.4	38	Fatal and nonfatal stroke	11.9% (eGFR <60 mL/min)	6%
Heinz et al. (2010) ³⁴	650	61±13	58	5	0.05	25	Fatal and nonfatal stroke	100% (ESRD)	NR
SEARCH (2010) ³⁵	12,064	64.2±8.9	83	2	1	78	Fatal and nonfatal stroke	14% (eGFR <60 mL/min)	7%
VITATOPS (2010) ³³	8,164	62.6±12.5	64	2	0.5	41	Fatal and nonfatal stroke	11% (Cr >1.36 mg/dL)	100%
SU.FOLOM3 (2010) ³²	2,501	61±9	79	0.56	0.02	56	Fatal and nonfatal stroke	0%	26%
CSPPT (2015) ¹⁰	20,702	60±7.5	41	0.8	0	60	Fatal and nonfatal stroke	10.9%	0%
Kotwal et al. (2015) ¹¹	12,000	NR	NR	5	1	24	Ischemic stroke	NR	NR

FA, folic acid; CKD, chronic kidney disease; NR, not reported; TIA, transient ischemic attack; ASFAST, Atherosclerosis and Folic Acid Supplementation Trial; ESRD, end-stage renal disease; NORVIT, Norwegian Vitamin; WENBIT, Western Norway B-Vitamin Intervention Trial; eGFR, estimated glomerular filtration rate; SEARCH, Study of the Effectiveness of Additional Reductions in Cholesterol and Homocysteine; VITATOPS, VITamins TO Prevent Stroke; SU.FOLOM3, SUpplementation with FOlate, vitamin B6 and B12 and/or OMEga-3 fatty acids; CSPPT, China Stroke Primary Prevention Trial.

*Combined 2 factors with FA vs. placebo control: 1 factor with vitamin B6 only was not included.

Table 3. Sensitivity analyses to remove each individual trial from the meta-analysis 1 at a time

	Relative risk (95% confidence interval)
All 13 trials included	0.85 (0.77–0.95)
Removed	
ASFAST (2006) ³⁰	0.87 (0.80–0.96)
CSPPT (2015) ¹⁰	0.88 (0.77–0.99)
Heinz et al. (2010) ³⁴	0.85 (0.76–0.96)
Kotwal et al. (2015) ¹¹	0.86 (0.77–0.95)
Liem et al. (2004) ²⁶	0.85 (0.76–0.95)
Liem et al. (2005) ²⁷	0.85 (0.76–0.96)
Mark et al. (1996) ²⁵	0.87 (0.78–0.96)
NORVIT (2006) ²⁸	0.84 (0.75–0.95)
Righetti et al. (2006) ²⁹	0.85 (0.75–0.95)
SEARCH (2010) ³⁵	0.83 (0.76–0.91)
SU.FOLOM3 (2010) ³²	0.87 (0.79–0.96)
VITATOPS (2010) ³³	0.82 (0.71–0.94)
WENBIT (2008) ³¹	0.85 (0.76–0.96)

ASFAST, Atherosclerosis and Folic Acid Supplementation Trial; CSPPT, China Stroke Primary Prevention Trial; NORVIT, Norwegian Vitamin; SEARCH, Study of the Effectiveness of Additional Reductions in Cholesterol and Homocysteine; SU.FOLOM3, SUpplementation with FOlate, vitamin B6 and B12 and/or OMEga-3 fatty acids; VITATOPS, VITamins TO Prevent Stroke; WENBIT, Western Norway B-Vitamin Intervention Trial.

mandatory folic acid fortification in food.

The finding of no benefits in trials with patients receiving folic acid plus cyanocobalamin (≥0.4 mg/day) deserved further exploration.^{11,30,31,33,35} All these trials included some participants with chronic kidney disease.^{11,28,31,33,35} In the DIVINE trial,²² in patients with diabetic nephropathy, B vitamins including cyanocobalamin 1,000 mcg daily were harmful, accelerating the decline of renal function, and doubling cardiovascular events. All the events occurred among participants with estimated glomerular filtration rate <50 mL/min/1.73 m².³⁶ In the Vitamin Intervention for Stroke Prevention (VISP) subgroup analysis³⁷ that excluded participants with estimated glomerular filtration rate <48 mL/min/1.73 m² (the lowest 10%), there was a significant benefit of B vitamins including cyanocobalamin. It now appears that the reason the early studies that did not show a reduction of stroke (e.g., VISP, Norwegian Vitamin [NORVIT]) was because high cyanide levels in patients with renal failure resulted in toxicity of cyanocobalamin.^{38,39} On the other hand, the benefit of folic acid monotherapy was found in the huge Chinese trial (China Stroke Primary Prevention Trial [CSPPT]), even among participants with impaired renal function.⁴⁰ Furthermore, in countries with folate fortification the main nutritional cause of high total homocysteine is metabolic B12 deficiency, which is

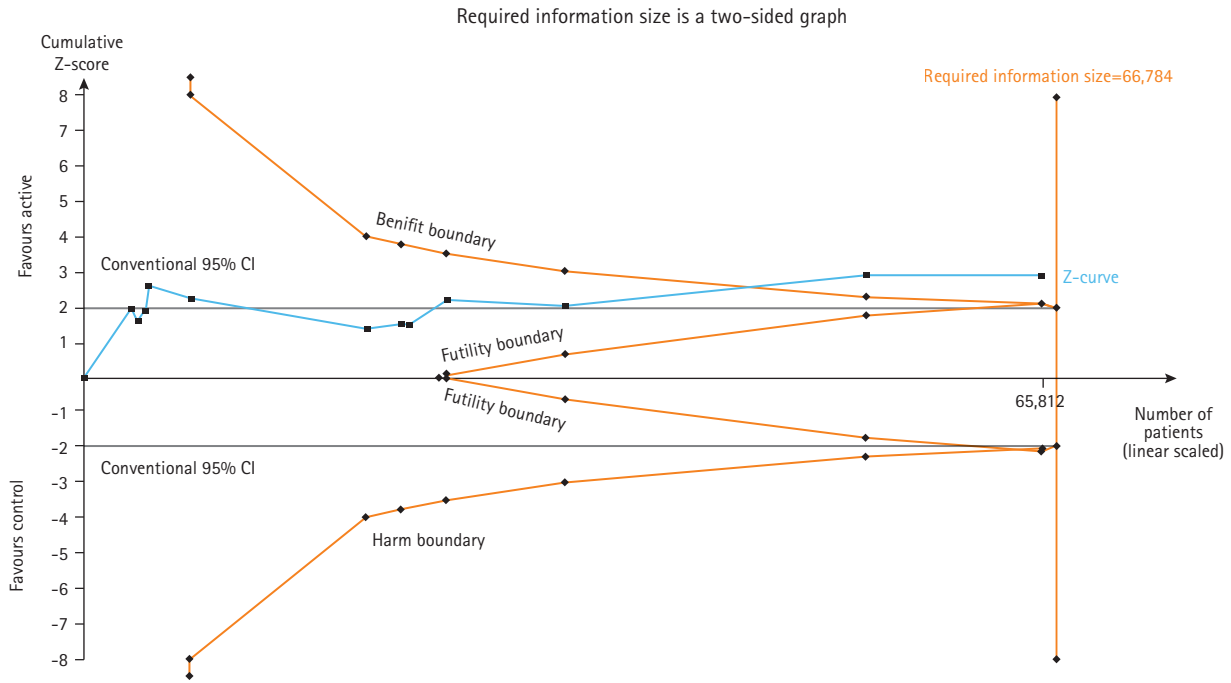


Figure 3. Trial sequential analysis of 13 trials comparing folic acid and control for stroke in countries without mandatory folic acid fortification. A cumulative Z-curve almost reached the required information size boundary and has crossed benefit boundary. CI, confidence interval.

Study or Subgroup	Active		Control		Risk ratio	Risk ratio M.H, Random, 95% CI	P value
	Events	Total	Events	Total	M.H, Random, 95% CI		
Treatment duration							
<3 years ^{11,27,30,35}	13	6,504	20	6,517	0.71 (0.36–1.41)		0.61
≥3 years ^{11,27,30,35}	1,047	27,237	1,155	25,554	0.85 (0.75–0.96)		
Sample size							
<1,000 ^{27,28,30,31}	18	633	32	646	0.57 (0.33–1.01)		0.18
≥1,000 ^{10,11,26,29,32,36}	650	27,450	704	25,768	0.85 (0.72–1.01)		
Age							
<65 years ^{10,26,27,29–32,36}	660	21,783	721	20,121	0.84 (0.72–0.99)		0.57
≥65 years ²⁸	8	300	12	293	0.65 (0.27–1.57)		

Figure 4. Effect of folic acid supplementation on the risk of stroke in prespecified subgroups. M-H, Mantel-Haenszel methods; CI, confidence interval.

very common (30% of stroke patients over age 70) and often missed.⁴¹ The reason it is missed is that a normal serum total B12 does not confirm adequate levels of active cobalamin; to do so requires measuring holotranscobalamin, methylmalonic acid or total homocysteine.⁴² It has been suggested that metabolic B12 deficiency should be investigated and treated in all stroke patients. Methylcobalamin or hydroxocobalamin should be used, particularly in patients with renal impairment.³⁸

The different effect of folic acid on myocardial infarction and stroke is worthy of comment. Myocardial infarction is mostly due to plaque rupture in the coronary artery and its mechanism is large vessel atherosclerosis. The mechanisms of stroke are more heterogeneous, including cardioembolism, large vessel atherosclerosis and small vessel disease. Within the stroke subtypes, small vessel disease was found to have strongest association with total homocysteine in many populations.^{43–46} Total homocysteine was also related to silent brain infarct and

white matter lesions on magnetic resonance imaging of brain.⁴⁷⁻⁵⁰ Beyond cerebral small vessel disease, total homocysteine has also been related to small vessel disease in other vascular beds, including in chronic kidney disease and diabetic retinopathy.^{51,52} In fact, some studies have indicated that total homocysteine-lowering therapy with vitamin B may slow the progression of white matter lesions of brain.^{53,54} Given all of the aforementioned data, we think that total homocysteine-lowering therapy might provide more benefit on small vessel disease than large vessel atherosclerosis, and would provide a larger protective effect on overall stroke versus myocardial infarction.

Folic acid food fortification program was introduced in United States and Canada since 1998 for prevention of neural tube defect in newborn.⁵⁵ The US program added 140 µg of folic acid per 100 g of enriched cereal grain product and has been estimated to provide 100 to 200 µg of folic acid per day to women of childbearing age.⁵⁵ Study found the mean folate concentrations increased from 4.6 to 10.0 ng/mL and the mean total homocysteine concentration decreased from 10.1 to 9.4 µmol/L after folic acid fortification.⁵⁶ Moreover, the prevalence of low folate concentrations (<3 ng/mL) decreased from 22% to 1.7% and the prevalence of high total homocysteine concentrations (>13 µmol/L) decreased from 18.7% to 9.8% after folic acid fortification.⁵⁶ Epidemiology study found the stroke mortality improved in United States and Canada after folic acid fortification.⁹ A dose-finding trial found folic acid dose as low as 0.2 mg/day, if given for more than 6 months, effectively lowered total homocysteine concentration by 8% to 20% as higher dose (0.4 and 0.8 mg/day).⁵⁷ The study also found folic acid 0.8 mg/day achieved maximal total homocysteine-lowering response by 6 weeks but then the total homocysteine level remained stationary by 6 months.⁵⁷ Therefore additional folic acid supplementation in the countries with folic acid food fortification may not reduce stroke risk further. A case-control study found in the era of folic acid fortification, vitamin B6 had stronger association with stroke than total homocysteine.⁵⁸ Therefore, in the countries with folic acid fortification, other risk factors other than total homocysteine may be more important for the prevention of stroke.

Although this meta-analysis included only RCTs, there were some limitations. First, meta-analysis is retrospective research that can be constrained by the comprehensiveness of searches, methodological rigor of the included studies, and publication bias. We tried to maximize study identification and minimize bias by developing the study protocol a priori, performing a thorough search of several databases, and using explicit criteria for study selection, data collection, and data analysis. Second, inevitable bias lies in the different characteristics of study par-

ticipants, treatment duration and intensity, type of cerebrovascular events identified, baseline folate and total homocysteine concentration, percentage of concomitant antiplatelet or statin use and other study design variables. Third, since only two trials reported an endpoint of ischemic stroke and one trial reported an endpoint of hemorrhagic stroke, insufficient evidence can be obtained on these endpoints.

Conclusions

In conclusion, our meta-analysis demonstrated a significant benefit of folic acid supplement in preventing stroke in countries without mandatory folic acid food fortification. The benefit was found mostly in patients receiving folic acid alone or combination of folic acid and minimal cyanocobalamin (≤ 0.05 mg/day) but not found in trials with combination of folic acid and cyanocobalamin (≥ 0.4 mg/day). Since the number of patients evaluated stroke of current RCTs almost reached the required information sizes and the summarized observed effect has crossed benefit boundary, no more relevant RCTs are needed to be conducted in countries without mandatory folic acid fortification. Because folic acid supplementation is an inexpensive, safe, and widely applicable intervention, a nutritional supplementation of folic acid by food or medication should be promoted in countries where food was not fortified with folic acid.

Supplementary materials

Supplementary materials related to this article can be found online at <https://doi.org/10.5853/jos.2017.01522>.

Disclosure

The authors have no financial conflicts of interest.

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Supplementary Table 1. Risk-of-bias assessment of included trials

Trial	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and personnel (performance bias)	Blinding of outcome assessment (detection bias)	Incomplete outcome data (attrition bias)	Selective reporting (reporting bias)	Other potential bias
ASFAST (2006) ³⁰ , Australia and New Zealand	Unclear risk Quote: patients who met the entry criteria were randomly assigned Comment: insufficient information about the sequence generation process	Unclear risk Comment: insufficient information	Low risk Quote: doubleblind randomized study Comment: probably done	Low risk Quote: doubleblind randomized study Comment: probably done	Low risk Comment: no patients lost follow-up	Low risk Comment: study protocol is available, and all of the study's prespecified outcomes of interest in the review have been reported in the prespecified way	Low risk Comment: study seems to be free of other sources of bias
CSPTT (2015) ¹⁰ , China	Low risk Quote: randomization was performed centrally by means of 4 randomization tables: 1 wasa randomization of drug code and treatment allocation, and the other 3 were MTHFR C677T genotype-specific randomized sequences with a fixed block size of 4 Comment: probably done	Low risk Quote: randomization was performed centrally by means of 4 randomization tables Comment: probably done	Low risk Quote: all study investigators and participants were blinded to the randomization procedure and the treatment assignments Comment: probably done	Low risk Quote: double blind clinical trial Comment: probably done	Low risk Comment: 0.3% vs. 0.3% patients lost follow-up	Low risk Comment: study protocol is available, and all of the study's prespecified outcomes of interest in the review have been reported in the prespecified way	Low risk Comment: study seems to be free of other sources of bias
Heinz et al. (2010) ³⁴ , Germany	Unclear risk Quote: patients were randomized to 2 treatment groups Comment: insufficient information about the sequence generation process	Low risk Quote: the code numbers were kept within the central pharmacy of the university hospital Comment: probably done	Low risk Quote: all study investigators staff, and participants were blinded to the randomization procedure Comment: probably done	Low risk Quote: doubleblind, placebocontrolled, randomized multicenter trial Comment: probably done	Low risk Comment: no patients lost follow-up except withdrew consent	Low risk Comment: study protocol is not available, but the published reports clearly include all expected outcomes, including those that were prespecified	Low risk Comment: study seems to be free of other sources of bias
Kotwal et al. (2015) ¹¹ , India	Low risk Quote: randomization was done by generating pseudo numbers by scientific calculator Comment: probably done	Unclear risk Comment: insufficient information	Low risk Quote: the participants, principal investigator as well as those involved with data analysis were unaware of the subject allocation arms Comment: probably done	Low risk Quote: coworkers were included from the local areas for appropriate randomized allocation and follow up Comment: probably done	Low risk Comment: no patients lost follow-up	Unclear risk Comment: study protocol is not available, insufficient information to permit judgement	Low risk Comment: study seems to be free of other sources of bias
Liem et al. (2004) ²⁶ , Netherlands	Unclear risk Quote: patients were randomized to treatment Comment: insufficient information about the sequence generation process	Unclear risk Comment: insufficient information	High risk Quote: open-label	High risk Quote: open-label	Low risk Comment: no patients lost follow-up	Low risk Comment: study protocol is not available, but the published reports clearly include all expected outcomes, including those that were prespecified	Unclear risk Comment: insufficient information

Supplementary Table 1. Continued

Trial	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and personnel (performance bias)	Blinding of outcome assessment (detection bias)	Incomplete outcome data (attrition bias)	Selective reporting (reporting bias)	Other potential bias
Liem et al. (2005) ²⁷ , Netherlands	Low risk Quote: a computer program randomly allocated patients Comment: probably done	Low risk Quote: a computer program randomly allocated patients Comment: probably done	High risk Quote: open-label	High risk Quote: open-label	Low risk Comment: no patients lost follow-up	Low risk Comment: study protocol is not available, but the published reports clearly include all expected outcomes, including those that were prespecified	Low risk Comment: study seems to be free of other sources of bias
Mark et al. (1996) ²⁵ , China	Low risk Quote: randomization was performed in blocks of 10 patients within strata defined by commune, gender, and age Comment: probably done	Unclear risk Comment: insufficient information	Unclear risk Comment: insufficient information	Unclear risk Comment: insufficient information	Low risk Comment: no patients lost follow-up	Low risk Comment: study protocol is not available, but the published reports clearly include all expected outcomes, including those that were prespecified	Low risk Comment: study seems to be free of other sources of bias
NORVIT (2006) ²⁸ , Norway	Low risk Quote: the randomization was performed in blocks of 20 by Alpharma Comment: probably done	Low risk Quote: the randomization was performed in blocks of 20 by Alpharma Comment: probably done	Low risk Quote: all study personnel and participants were unaware of the treatment assignments Comment: probably done	Low risk Quote: doubleblind Comment: probably done	Low risk Comment: no patients lost follow-up except withdrew consent or stop taking study medication	Low risk Comment: study protocol is not available, but the published reports clearly include all expected outcomes, including those that were prespecified	Low risk Comment: study seems to be free of other sources of bias
Righetti et al. (2006) ²⁹ , Italy	Unclear risk Quote: an independent person performed randomization for treatment using a box containing blind numbers Comment: insufficient information about the sequence generation process	Unclear risk Comment: insufficient information	High risk A single-center, open, prospective trial	High risk A single-center, open, prospective trial	Low risk Comment: no patients lost follow-up except withdrew consent or stop taking study medication	Low risk Comment: study protocol is not available, but the published reports clearly include all expected outcomes, including those that were prespecified	Low risk Comment: study seems to be free of other sources of bias
SEARCH (2010) ³⁵ , UK	Low risk Quote: the central telephone randomization system Comment: probably done	Low risk Quote: the central telephone randomization system Comment: probably done	Low risk Quote: doubleblind randomized controlled trial Comment: probably done	Low risk Quote: doubleblind randomized controlled trial Comment: probably done	Low risk Comment: no patients lost follow-up	Low risk Comment: study protocol is available, and all of the study's prespecified outcomes of interest in the review have been reported in the prespecified way	Low risk Comment: study seems to be free of other sources of bias

Supplementary Table 1. Continued

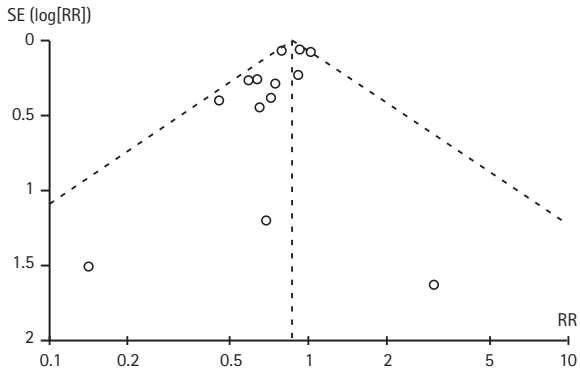
Trial	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and personnel (performance bias)	Blinding of outcome assessment (detection bias)	Incomplete outcome data (attrition bias)	Selective reporting (reporting bias)	Other potential bias
SU.FOLOM3 (2010) ³² , France	Low risk Quote: randomization was performed by means of a computerized block sequence Comment: probably done	Low risk Quote: the allocation of participants was programmed by the statistical coordinating centre Comment: probably done	Low risk Quote: patients, clinicians, trial coordinators, and outcome investigators were blinded to treatment allocation Comment: probably done	Low risk Quote: double blind, randomised, placebo controlled trial Comment: probably done	Low risk Comment: <1% participants lost to follow-up except withdrew consent	Low risk Comment: study protocol is available, and all of the study's prespecified outcomes of interest in the review have been reported in the prespecified way	Low risk Comment: study seems to be free of other sources of bias
VITATOPS (2010) ³³ , Multicountries	Low risk Quote: patients were randomly allocated by means of a central 24-h telephone service or an interactive website Comment: probably done	Low risk Quote: allocation was by use of random permuted blocks stratified by hospital Comment: probably done	Low risk Quote: patients, clinicians, trial coordinators, and outcome investigators were masked to treatment allocation Comment: probably done	Low risk Quote: a randomised, double-blind, parallel, placebocontrolled trial Comment: probably done	Low risk Comment: the rate of loss to final follow-up was 8.7% vs. 8.5%	Low risk Comment: study protocol is available, and all of the study's prespecified outcomes of interest in the review have been reported in the prespecified way	Low risk Comment: study seems to be free of other sources of bias
WENBIT (2008) ³¹ , Norway	Low risk Quote: the randomization sequence was generated in blocks of 20 by Alpha Inco Comment: probably done	Low risk Quote: the randomization code was kept at Alpha Inco until data entry was completed Comment: probably done	Low risk Quote: participants, study and laboratory personnel, and the steering and endpoints committees were unaware of the treatment allocation Comment: probably done	Low risk Quote: doubleblind Comment: probably done	Low risk Comment: no patients lost follow-up except withdrew consent	Low risk Comment: study protocol is not available, but the published reports clearly include all expected outcomes, including those that were prespecified	Low risk Comment: study seems to be free of other sources of bias

ASFAST, Atherosclerosis and Folic Acid Supplementation Trial; CSPPT, China Stroke Primary Prevention Trial; NORVIT, Norwegian Vitamin; SEARCH, Study of the Effectiveness of Additional Reductions in Cholesterol and Homocysteine; SU.FOLOM3, Supplementation with Folate, vitamin B6 and B12 and/or Omega-3 fatty acids; VITATOPS, VITamins TO Prevent Stroke; WENBIT, Western Norway B-Vitamin Intervention Trial.

Supplementary Table 2. Summary of quality assessments and findings for primary and secondary outcomes

Outcomes	Studies	Quality assessment						Summary of findings				
		Limitations	Inconsistency	Indirectness	Imprecision	Publication bias	Control	Folic acid therapy	RR (95% CI)	Control risk, events per 1,000 patients	Risk difference (95% CI)	Quality
Stroke ^{10,11,25-26}	13	No serious limitations	No serious inconsistency	No serious indirectness	No serious imprecision	Unknown due to 13 trials reported this outcome	1,175/32,071	1,060/33,741	0.85 (0.77–0.95)	37	5 fewer per 1,000 (from 2 to 8 fewer)	High
Ischemic stroke ^{10,11}	2	No serious limitations	No serious inconsistency	No serious indirectness	Serious imprecision	A small study showing harmless effects	295/16,354	223/16,348	0.65 (0.25–1.71)	21	Not significant	Low
Hemorrhagic stroke ¹⁰	1	No serious limitations	Unknown due to one trial reported this outcome	No serious indirectness	Unknown due to one trial reported this outcome	Unknown due to one trial reported this outcome	62/10,354	58/10,348	0.94 (0.66–1.34)	5	Not significant	Not applicable

RR, relative risk; CI, confidence interval.



Supplementary Figure 1. Funnel plot. SE, standard error; RR, relative risk.