Efficacy of folic acid supplementation in stroke prevention: a meta-analysis

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Summary

Background **The efficacy of treatments that lower homocysteine concentrations in reducing the risk of cardiovascular disease remains controversial. Our aim was to do a meta-analysis of relevant randomised trials to assess the efficacy of folic acid supplementation in the prevention of stroke.**

Methods **We collected data from eight randomised trials of folic acid that had stroke reported as one of the endpoints. Relative risk (RR) was used as a measure of the effect of folic acid supplementation on the risk of stroke with a random effect model. The analysis was further stratified by factors that could affect the treatment effects.**

Findings **Folic acid supplementation significantly reduced the risk of stroke by 18% (RR 0·82, 95% CI 0·68–1·00; p=0·045). In the stratified analyses, a greater beneficial effect was seen in those trials with a treatment duration of more than 36 months (0·71, 0·57–0·87; p=0·001), a decrease in the concentration of homocysteine of more than 20% (0·77, 0·63–0·94; p=0·012), no fortification or partly fortified grain (0·75, 0·62–0·91; p=0·003), and no history of stroke (0·75, 0·62–0·90; p=0·002). In the corresponding comparison groups, the estimated RRs were attenuated and insignificant.**

Interpretation **Our findings indicate that folic acid supplementation can effectively reduce the risk of stroke in primary prevention.**

Introduction

Cardiovascular disease is the leading cause of death in the developed world,[1] and has emerged as one of the leading causes of death in developing countries such as China.[2] As early as 1969, homocysteine, the sulphur-containing aminoacid, was postulated to affect atherosclerotic processes.[3] Since that time, substantial evidence has accumulated linking homocysteine in blood to the risk of cardiovascular disease.[4–7] Raised concentrations of homocysteine in blood have been suggested to be a modifiable, independent risk factor for coronary artery disease, stroke, and deep vein thrombosis.[8–10] The initial epidemiological evidence in support of this hypothesis came from case-control studies.[11–13] Furthermore, a meta-analysis of genetic studies and prospective studies lent further support that the association between homocysteine and cardiovascular disease is causal.[8] However, inconsistent results have been reported,[14] and the efficacy of treatments that lower homocysteine concentrations in reducing the risk of cardiovascular disease has not been confirmed by randomised trials.[15–17] There are several possible reasons for the inconsistent findings between the recent clinical trials and earlier observational studies.[18] Most trials were done among patients with pre-existing cardiovascular disease as a secondary prevention strategy; it is possible that folic acid supplementation could have a greater protective effect in primary rather than secondary prevention. Additionally, cardiovascular disease is a heterogeneous clinical entity; different cardiovascular endpoints could respond differently to folic acid supplementation.

Although results were negative for other cardiovascular endpoints, the HOPE-2 study did show that folic acid supplementation reduced the risk of stroke by 24%.[17] In the USA and Canada, folic acid fortification of grain products was fully implemented by 1998. If high homocysteine concentrations are an independent risk factor for stroke, one might expect a reduction in stroke mortality following folic acid fortification. Indeed, a recent population-based study showed that decline in stroke mortality accelerated in 1998–2002 in nearly all population strata in the USA and Canada;[19] by contrast, the rate of decline in stroke mortality in England and Wales (where fortification is not mandatory) did not change significantly between 1990 and 2002. These data raised the possibility that stroke is a disease endpoint that could particularly benefit from folic acid supplementation. Our aim was thus to do a meta-analysis focusing on stroke as the disease endpoint in relation to folic acid supplementation.

Methods

Search strategy and selection criteria

We attempted to conform to Quality of Reporting of Meta-analyses (QUOROM) guidelines in the report of this meta-analysis.[20]

To select studies, we used methods similar to those in a recent meta-analysis that assessed the effect of folic acid supplementation on the risk of cardiovascular disease.[21] We (XQ, GM, JL, XX, XW) first reviewed all relevant trials included in that meta-analysis,[21] in which a comprehensive literature search of the Medline database was done from January, 1966, to July, 2006, with the MeSH terms "cardiovascular disease", "coronary disease", "coronary thrombosis", "myocardial ischemia", "coronary stenosis", "coronary restenosis", "cerebrovascular accident", "randomized controlled trial", "clinical trials", and "folic

acid", and the text words "folic acid" and "folate". Furthermore, an independent Medline search was done (by XQ and GM) with the same methods,[21] with the addition of "stroke" and "multivitamins" as the text words and extension of the search period to April, 2007. Manual searches of bibliographies of all relevant trials and review articles (by XQ, GM, XW) were also done. The search was restricted to human studies. There were no language restrictions. Experts in the field were also consulted.

We restricted our meta-analysis to randomised clinical trials, which are less likely to be subject to confounding and biases than are observational studies. A standard protocol for study selection and data abstraction was developed by our multidisciplinary research team with relevant expertise in clinical medicine, epidemiology, clinical trials, and biostatistics. Studies were eligible for inclusion if: (1) the study was a randomised controlled trial; (2) the number of events for stroke that occurred during the study were reported by intervention and control groups, with more than ten incident cases; (3) the intervention consisted of folic acid supplementation (with or without additional B vitamin supplementation); and (4) the intervention duration was at least 6 months. The contents of 308 abstracts were reviewed independently by two investigators (XQ, GM) to determine if they met eligibility criteria for inclusion. Where discrepancies occurred, a third investigator (XW) did additional assessment.

Data collection

All data from eligible trials were independently abstracted in duplicate by two independent investigators (XQ, GM) with the standard protocol and reviewed by a third investigator (XW). Discrepancies were resolved by discussion with the multidisciplinary research team who developed the protocol (JL, YH, NS, LL, XX). Recorded data variables were as follows: first author's name, year of publication, source of publication, country origin, study design (factorial, parallel, crossover, other), type of blinding (open, double blind), number of intervention groups, intervention regimen, type of controls (placebo, usual care, untreated), total number of individuals and number of incident cases for each treatment group, mean age in each group, percentage male, baseline characteristics (pre-existing conditions, percentage with diabetes, mean concentrations of lipids), baseline concentration and changes in homocysteine concentration, duration of intervention, fortification of grains in the country of origin, stroke outcome, and funding source.

Statistical analysis

We assessed the overall effect of folic acid supplementation on the risk of stroke based on all the data from the eight trials. We then did stratified analyses by duration of folic acid supplementation (≤36 months *vs* >36 months); decrease in homocysteine concentration (<20% *vs* ≥20%), and prior folic acid grain fortification (yes or no). If the relation between folic acid supplementation and reduction in risk of stroke is causal, a greater beneficial effect should be seen in trials with longer periods of intervention, in populations with no fortification or only partly fortified grain, and with greater decreases in homocysteine concentrations. We also did a stratified analysis by history of stroke (yes or no) to assess the effect of folic acid supplementation on stroke risk in primary versus secondary prevention.

Relative risk (RR) with 95% CI was used as a measure of the effect of folic acid supplementation on risk of stroke. Unconditional maximum likelihood estimation by normal approximation[22] was used to obtain the interval estimate of the RR of stroke for folic acid supplementation compared with controls. To ensure the robustness of our estimation of RR and 95% CI, we also applied logarithmically transformed risk ratios, and calculated corresponding SE by the delta method, and then back-transformed (exponential transformation) to the original scale. The results were very similar between the two methods. We only present the results for the untransformed version. Both fixed-effects and random-effects models were used to calculate the pooled RR for folic acid supplementation compared with controls. Although both models yielded similar findings, results from the random-effects models are presented here because of the different pre-existing conditions, intervention regimens, intervention durations, and dietary intakes of folic acid that were involved in the original trials. Furthermore, many investigators consider the random effects approach to be a more natural choice than fixed effects in medical decisionmaking contexts.[23–25]

Heterogeneity between studies was assessed by Cochran's Q with a significance level set at 0·10. We also did a sensitivity analysis by removing each individual trial from the meta-analysis. All the analyses were done with R software, version 2.4.1.

Some of the studies included in our meta-analysis differed in the units used for reporting levels of lipids (mg/dL *vs* mmol/L) and homocysteine (mg/L *vs* μmol/L).

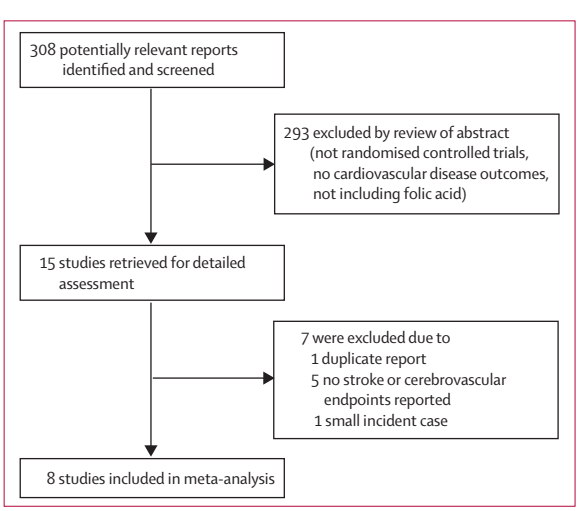

Figure 1: **Study selection**

	Number of participants	Age (years)	Sex (male)	Pre-existent diseases	Diabetes	Total cholesterol (mmol/L)	Homocysteine (μmol/L)
Toole et al[15]	3680	66·3 (10·8)	2301 (63%)	Stroke	1071 (29%)	5·2 (1·2)	13·4 (NR)
Liem et al[34]	593	65·2 (9·8)	462 (78%)	CHD	53 (9%)	4·6 (0·8)	12·1 (4·3)
Lonn et al[17]	5522	68·9 (6·9)	3963 (72%)	CHD	2209 (40%)	4·8 (0·8)	12·2 (NR)
Bonaa et al[16]	2815	63·0 (11·7)	2815 (74%)	CHD	282 (10%)	5·8 (1·2)	13·1 (5·2)
Zoungas et al[36]	315	56 (13·5)	213 (68%)	ESRD	73 (23%)	5·2 (1·2)	27 (13·0)
Wrone et al[33]	510	60·2 (15·1)	255 (50%)	ESRD	232 (46%)	4·6 (1·1)	32·9 (20)
Righetti et al[35]	88	64·5 (1·8)	49 (56%)	ESRD	17 (19%)	5·0 (0·2)	35·0 (1·4)
Mark et al[37]	3318	Median 54; mean NR	1461 (44%)	Oesophageal dysplasia	NR	NR	NR

Data are mean (SD) or n (%). CHD=coronary heart diseases. ESRD=end-stage renal disease. NR=not reported.

Table 1: **Baseline characteristics of study participants**

	Blinding	Folic acid dosage in intervention group	Control	Intervention duration (months)	Grain fortification	Funding sources
Toole et al[15]	Double	2·5 mg/d	20 μg/d folic acid	24	Yes	Public, corporate
Liem et al[34]	Open	0·5 mg/d	Usual care	42	No	Foundation
Lonn et al[17]	Double	2·5 mg/d	Placebo	60	Partly	Public, private
Bonaa et al[16]	Double	0·8 mg/d	Placebo	36	No	Public, foundation, private
Zoungas et al[36]	Double	15 mg/d	Placebo	43	Yes	Public, foundation
Wrone et al[33]	Double	5 mg/d or 15 mg/d	1 mg/d folic acid	24	Yes	Public, corporate
Righetti et al[35]	Open	5 mg/d or 5 mg/every other day	Usual care	29	No	Not listed
Mark et al[37]	Double	0·8 mg/d	Placebo	72	No	Not listed

Table 2: **Study design characteristics**

Therefore, we converted these different units to mmol/L for lipids and μmol/L for homocysteine, using the conversion factors 1 mg/dL=0·0259 mmol/L for cholesterol, and 1 mg/L=7·397 μmol/L for homocysteine.

For studies in which more than one folic acid intervention regimen existed, we reported the mean concentration of homocysteine both before and after the intervention for all the intervention groups combined. In these studies, we also combined the number of events and participants across folic acid intervention groups to obtain a single event rate for folic acid supplementation.

	Stroke events/total patients		Relative risk (95% CI)	p value
	Intervention group	Control group		
Overall	373/8949	405/7892	0·82 (0·68–1·00)	0·045
Duration of intervention				
≤36 months[15,16,34,35]	224/4078	193/3015	1·00 (0·83–1·21)	0·95
>36 months[17,34,36,37]	149/4871	212/4877	0·71 (0·57–0·87)	0·001
Homocysteine lowering				
<20%[15,33,36]	179/2325	174/2180	0·89 (0·55–1·42)	0·62
≥20%[16,17,34,35]	172/4967	196/4051	0·77 (0·63–0·94)	0·012
Grain fortification				
Yes[15,33,36]	179/2325	174/2180	0·89 (0·55–1·42)	0·62
No[16,17,34,35,37]	194/6624	231/5712	0·75 (0·62–0·91)	0·003
History of stroke				
Yes[15]	152/1827	148/1853	1·04 (0·84–1·29)	0·71
No[16,17,33-37]	221/7122	257/6039	0·75 (0·62–0·90)	0·002

Table 3: **Pooled relative risk for stroke, stratified by intervention duration, percentage change in homocysteine concentration, grain fortification, and history of stroke**

Role of the funding source

There was no funding source for this study. The corresponding author (XW) had full access to all the data used for this meta-analysis and had final responsibility to submit for publication.

Results

Of the 15 trials retrieved for detailed assessment, five were excluded for lack of data on stroke,[26–30] one[31] because it was derived from the same study population as another report,[34] and one for small number incident cases of stroke[32] (figure 1). Our final analysis included eight randomised controlled trials, consisting of 16 841 individuals.[15–17,33–37] These trials compared folic acid supplementation (with or without combination with other B vitamins, including B6 and B12) with either placebo, a lower dose of folic acid, or usual care for a minimum duration of 6 months and with stroke reported as one of the endpoints. The baseline characteristics of the study participants are shown in table 1 and design characteristics are presented in table 2. Of the eight

trials, three were done in the USA and Canada,[15,17,33] three in European countries,[16,34,35] one in Australia and New Zealand,[36] and one in China.[37] The number of participants ranged from 88[34] to 5522.[17] All trials included both men and women. The dosage of folic acid in the intervention groups ranged from 0·5 mg/day[34] to 15 mg/day.[36] All eight trials included individuals with pre-existing conditions: one trial with history of stroke,[15] and the remaining trials with coronary heart disease,[16,17,34] end-stage renal disease,[33,35,36] or oesophageal dysplasia.[37] The duration of intervention ranged from 24 months[15,33] to 72 months.[37] Three trials were done in regions with grain fortification,[15,33,36] four were in regions without grain fortification,[16,34,35,37] and one in a population from both fortified and non-fortified regions (ie, partly fortified).[17] Baseline homocysteine concentrations varied substantially among the trials, ranging from 12·1[34] to 35·0[35] µmol/L (table 1).

Pooling all eight trials, folic acid supplementation (with or without other B vitamins) significantly reduced the risk of stroke by 18% (RR 0·82, 95% CI 0·68–1·00; p=0·045; table 3). Heterogeneity testing for all analyses in table 3 showed that all p values are larger than 0·10; thus heterogeneity is not significant in the overall analysis and in stratified analyses (data not shown). Sensitivity analyses showed that the RR and 95% CI did not alter substantially by removing any one trial (data not shown).

Longer intervention duration seemed to be associated with greater reduction in the RR of stroke (figure 2). When we stratified the trials by intervention duration (≤36 months *vs* >36 months), the pooled RR for the trials with shorter duration was 1·00 (95% CI 0·83–1·21; p=0·95); by contrast the pooled RR for the trials with longer duration was 0·71 (0·57–0·87; p=0·001; table 3).

When we stratified the trials by fortification status (table 3), the RR for trials in regions with fortified grain was 0·89 (95% CI 0·55–1·42; p=0·62); that for trials in regions without fortification was 0·75 (0·62–0·91; p=0·003). When we stratified the trials by history of stroke, the RR for the trial in which there was a history of stroke was 1·04 (0·84–1·29; p=0·71); the RR for trials with no such history was 0·75 (0·62–0·94; p=0·002; table 3).

Post-intervention homocysteine reduction was measured in all but one trial[37] (table 4). There was considerable variation in the net and relative reduction of homocysteine concentration among the trials, ranging from 2·3[35] to 15·1[35] µmol/L, or from 10·9%[33] to 39·4%.[35] There seemed to be an inverse relation between degree of homocysteine lowering and RR of stroke (figure 2). When we stratified the trials by the degree of homocysteine lowering, the RR for the trials with a reduction in homocysteine concentration of less than 20% was 0·89 (95% CI 0·55–1·42; p=0·62); by contrast, the RR for the trials with a reduction in homocysteine concentration of 20% or more was 0·77 (0·63–0·94, p=0·012; table 3).

	Net decrease in homocysteine (µmol/L)	Change in homocysteine (%)	Stroke events/ total patients		RR (95% CI)
			Intervention	Control	
Toole et al[15]	–2·3	–17·2%	152/1827	148/1853	1·04 (0·84–1·29)
Liem et al[34]	–2·6	–21·5%	8/300	12/293	0·65 (0·27–1·57)
Lonn et al[17]	–3·2	–26·2%	111/2758	147/2764	0·76 (0·59–0·96)
Bonaa et al[16]	–3·8	–29·0%	49/1872	27/943	0·91 (0·58–1·45)
Zoungas et al[36]	–4·7	–17·4%	8/156	18/159	0·45 (0·20–1·01)
Wrone et al[33]	–3·6	–10·9%	19/342	8/168	1·17 (0·52–2·61)
Righetti et al[35]	–15·1	–39·4%	4/37	10/51	0·55 (0·19–1·62)
Mark et al[37]	NR	NR	22/1657	35/1661	0·63 (0·37–1·07)

NR=not reported.

Table 4: **Relative risk of stroke and change in homocysteine concentration**

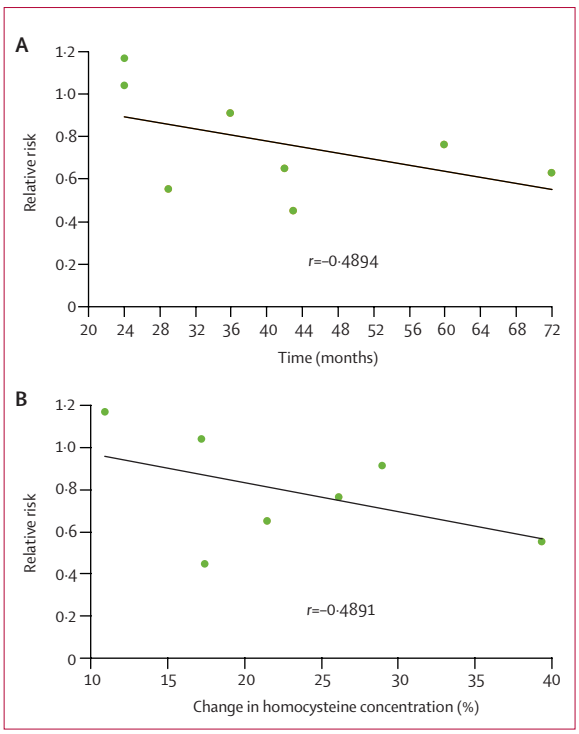

Figure 2: **Relative risk of stroke**
(A) Relative risk of stroke in relation to intervention duration. (B) Relative risk of stroke in relation to percentage change in homocysteine concentration (Mark et al[37] excluded since no baseline homocysteine concentration recorded).

Discussion

Our meta-analysis provides coherent evidence that folic acid supplementation can significantly reduce the risk of stroke in primary prevention. Although Bazzano and colleagues' meta-analysis[21] reported that the overall effect of folic acid supplementation on stroke was not significant, the association became significant after removal of the VISP trial,[15] which was done in individuals with a history of stroke.

The inverse relation between the duration of folic acid supplementation and the risk of stroke suggests that the

effect of folic acid supplementation on the risk of stroke is probably causal. This relation is consistent with observations from individual trials. In the HOPE-2 trial,[17] a beneficial treatment effect on stroke did not show until after 36 months of intervention, and the gap between treatment and control groups seemed to widen with increasing intervention duration. At 5-year follow-up, the RR of stroke was 0·75 (95% CI 0·59–0·97; p=0·03). A similar relation was seen in the Linxian Nutrition Intervention Trial, done in China,[37] which did not show significant beneficial effect on the risk of death due to cerebrovascular disease until after 24 months. At 6-year follow-up, the RR of cerebrovascular disease was 0·63 (95% CI 0·37–1·07; p=0·08). Because the pathogenesis of cardiovascular disease stems from a long and accumulative process, one would expect that averting the disease process would also need considerable time.

Additional evidence for a causal relation is provided by our observation that, in countries where grain is fortified with folic acid, further supplementation of the diet with folic acid had little effect on the risk of stroke; by contrast, folic acid supplementation significantly reduced the risk of stroke in countries where grain was not fortified. Like any essential nutrient, one would expect that in populations with adequate intake of folic acid, further reduction in the risk of stroke by supplementation would be limited.

Our data suggest that grain fortification might be an important determinant for the reduction of homocysteine concentration and thus the treatment effects noted. Folate and cyanocobalamin (vitamin B12) are important regulators of the metabolism of homocysteine in the body, and studies have shown an inverse relation between concentrations of these factors and the concentration of homocysteine in the blood.[38–40] Furthermore, folic acid and vitamins B6 and B12 supplementation can lower blood homocysteine concentrations.[41] Our meta-analysis showed that a decrease of less than 20% in the concentration of homocysteine did not significantly affect the RR of stroke, whereas a significant reduction in the RR of stroke occurred with a decrease in homocysteine concentration of 20% or more. This dose-response relation provides further evidence that homocysteine reduction could serve as a surrogate biomarker for reduction in the risk of stroke.

Evidence for the efficacy of folic acid supplementation in primary versus secondary prevention of stroke is not conclusive. Our meta-analysis indicated that folic acid supplementation can significantly reduce the risk of stroke in trials done in individuals without a history of stroke. By contrast, the RR of stroke for the only trial in which participants had a history of stroke was close to 1·0 and insignificant. As noted in the Heart Protection Study,[42] lowering of cholesterol concentrations with simvastatin led to a 25% reduction in the risk of stroke in primary prevention and the effect became evident after 2 years of intervention and greater with longer intervention; however, such an effect was not seen in individuals with pre-existing cerebrovascular disease. One would expect that secondary prevention of stroke by folic acid supplementation—even if it is efficacious—would require even longer periods of intervention, greater reduction in homocysteine concentrations, or larger sample size to detect any beneficial effect.

Although one cannot completely separate the effect of folic acid from that of other vitamins and minerals, there has been no evidence that supplements other than B vitamins could have a therapeutic effect on stroke. A randomised trial of multivitamins and minerals found no significant reduction in stroke deaths with vitamin A and zinc, riboflavin and niacin, ascorbic acid and molybdenum, or beta-carotene, selenium, and alpha-tocopherol.[43] The Linxian Nutrition Intervention Trial[37] is unique in exploring the efficacy of supplements of folic acid plus vitamins or minerals in a rural population with a micronutrient-poor diet. However, it had important limitations: cerebrovascular death was not the primary endpoint of the study; no homocysteine or folic acid concentrations were measured; and only death due to stroke was reported, and the diagnosis was made clinically without CT scan, MRI, or autopsy confirmation.

Although there is evidence that folic acid supplementation can reduce the risk of stroke, there is continued controversy with regard to whether it can lead to improved outcomes for other cardiovascular endpoints. Several randomised clinical trials assessing the effects of supplemental folic acid and B vitamins on the risk of cardiovascular disease have in general yielded negative results, as did Bazzano and colleagues' meta-analysis of randomised trials.[21] There is evidence to suggest that different endpoints could respond differently to folic acid supplementation. For example, in the HOPE-2 study,[17] although folic acid supplementation did not reduce the risk of major cardiovascular events, it did show a significant beneficial effect on the risk of stroke among patients assigned to active treatment compared with those assigned to placebo. Other epidemiological studies also support the notion that risk factors and strength of associations differ between stroke and cardiovascular disease. For example, in a nested case-control study, concentrations of plasma lipids can be an indicator of increased risk of myocardial infarction but not of stroke in patients with established cerebrovascular disease.[44] By contrast, raised concentrations of homocysteine were more strongly associated with stroke than with ischaemic heart disease:[10] for each 5 µmol/L rise in serum homocysteine, the risk was increased by 59% for stroke versus 32% for ischaemic heart disease; and for each 3 µmol/L decrease in homocysteine concentration, the risk reduction was 24% for stroke versus 16% for ischaemic heart disease. Another meta-analysis showed that, for a 25% reduction in homocysteine, the reduction in the risk of stroke was greater than that for ischaemic heart disease (19% *vs* 11%).[9] Furthermore, for every 10 mm Hg increase in

systolic blood pressure, hypertension was more strongly associated with stroke than with coronary heart disease (RR 1·26 *vs* 1·07).[45] Although the reasons for the different associations between stroke and cardiovascular disease are not completely understood, they could be due, in part, to the fact that stroke is more likely to be associated with small blood vessel pathology, whereas cardiovascular disease tends to involve larger blood vessels. Some studies suggest that homocysteine is more likely to affect small blood vessels.[46] A better understanding of the reasons for inconsistent findings across studies is important for clinical and public health practice and for future research.

One should note that meta-analyses have inherent limitations, including their retrospective and aggregate nature and the inability to adjust for individual variables. However, there was no significant heterogeneity between studies. The sample size of the trials included in this analysis varied, and the results were more likely affected by the trials with larger sample sizes. We did sensitivity testing and found that the RR and 95% CI did not alter substantially after removing any one trial. Publication bias is an important issue for meta-analysis, in which positive results are more likely to be published, and as such, meta-analyses could overestimate the true effect or association. The primary endpoints are cardiovascular disease rather than stroke in most of the published trials, of which most studies reported a non-significant association with cardiovascular disease. Therefore, the publication bias, if any, will probably underestimate the effect of folic acid supplementation. Since none of the included trials was designed exclusively for stroke, the effect of publication bias on our estimated effect of folic acid supplementation on stroke should be limited. Most previous clinical trials used folic acid in combination with other B vitamins, including B6 and B12, and the dosage also varied considerably across the trials. This meta-analysis is limited by the original study design of the trials, and cannot assess the efficacy of single versus combination regimen, nor on dosage. Most trials included in this meta-analysis were designed to assess the effects of lowering plasma homocysteine concentrations on the risk of coronary heart disease, as well as stroke. However, as long as the study has sufficient sample size and adequate methods for assessing incident cases of stroke, these trials should be informative. Our findings remain to be confirmed by data from several large trials that have yet to report results,[47–49] and should be interpreted in the context of available evidence in the field.

As the controversy over homocysteine-lowering therapy continues to evolve, clarifying whether there are groups of individuals who might benefit from this simple intervention is very important from a research and population health perspective. Our analyses showed greater beneficial effects among trials with a longer period of follow-up, greater reduction in homocysteine, without history of stroke, and in regions without grain fortification. We speculate that populations with those characteristics would be more likely to benefit from folic acid supplementation than would populations without these traits.

To efficiently assess the efficacy and causality of folic acid supplementation on stroke, future clinical trials should be done in regions without grain fortification, with a longer period of follow-up (4 years or longer), and among individuals without a history of stroke. The issue of folic acid supplementation alone versus folic acid in combination with other B vitamins, as well as optimum dosage, should also be carefully considered in future trials.

Contributors

XW, XQ, JL, GM, YH, NS, LL, and XX participated in the study conception and design. XQ, GM, and XW took part in the acquisition of data. XW, XQ, HD, GM, and XX analysed the data. XW, XQ, HD, JL, GM, YH, NS, LL, and XX interpreted the data. XW, XQ, HD, JL, GM, YH, NS, LL, and XX drafted and critically reviewed the manuscript for important intellectual content. All authors saw and approved the final version of the manuscript.

Conflict of interest statement

We declare that we have no conflict of interest.

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