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

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SUMMARY

Aims: There are growing data and a continuing controversy over the efficacy of folic acid supplementation in stroke prevention. We conducted a meta-analysis based on relevant, up-to-date published randomised trials to further examine this issue. Methods: Relative risk (RR) was used to measure the effect of folic acid supplementation on risk of stroke with a fixed-effects model. Results: Overall, folic acid supplementation reduced the risk of stroke by 8% (n = 55,764; RR: 0.92; 95% CI: 0.86–1.00, p = 0.038). In the 10 trials with no or partial folic acid fortification (n = 43,426), the risk of stroke was reduced by 11% (0.89; 0.92; 95% CI: 0.86–1.00, p = 0.038). Within these trials, a greater beneficial effect was observed among trials with a lower percent use of statins [≤ 80% (median); 0.77; 0.64–0.92, p = 0.005], and a meta-regression analysis also suggested a positive dose-response relationship between percent use of statins and log-RR for stroke associated with folic acid supplementation (p = 0.013). A daily dose of 0.4–0.8 mg folic acid appeared to be adequate for stroke prevention in comparison with larger doses. In the remaining five trials conducted in populations with folic acid fortification (n = 12,338), folic acid supplementation had no effect on stroke risk (1.03; 0.88–1.21, p = 0.69). Conclusions: Our analysis indicated that folic acid supplementation is effective in stroke prevention in populations with no or partial folic acid fortification. In addition, a greater beneficial effect was observed among trials with a lower percent use of statins. Our findings underscore the importance of identifying target populations that can particularly benefit from folic acid therapy.

Introduction

From both a clinical and public health perspective, it is critically important to determine whether folic acid supplementation can effectively prevent stroke in general or in a subset of populations. A recent meta-analysis found that the MTHFR 677C → T variant was associated with a larger effect on homocysteine concentration in regions of low folate consumption than in regions with high dietary folate intake or with established programs of folic acid fortification of flour for the prevention of neural tube defects. A similar pattern was noted for the genetic association with stroke risk (1). Similarly, our previous meta-analysis (2) reported that folic acid supplementation significantly reduced the risk of stroke by 18% [Relative risk (RR): 0.82; 95% CI: 0.68–1.00, p = 0.045] and an even greater beneficial effect was seen in those trials with no or partial folic acid fortification (0.75; 0.62–0.91, p = 0.003). In a recent meta-analysis by Lee M et al. (3), the potential mild benefits of folic acid supplementation on stroke were observed in primary stroke prevention (RR: 0.89; 95% CI: 0.79–0.99, p = 0.03), especially when folate is combined with B vitamins and in male patients. Importantly, this meta-analysis also found a trend toward treatment benefit in trials conducted only or partly in countries without a background of folic acid fortification. And folic acid supplementation was also found to significantly reduce cardiovascular disease (CVD) risk in patients with end stage renal disease (ESRD) or advanced chronic kidney disease (ACKD) by 15% (RR: 0.85; 95% CI: 0.76–0.96, p = 0.009), particularly among those trials with no or partial folic acid fortification (RR: 0.80; 95% CI: 0.65–0.99, p = 0.04) (4). Furthermore, folic acid supplementation (5) could significantly reduce the progression of carotid intima-media thickness (weighted mean differences:−0.04; 95% CI: −0.07,−0.02; p < 0.001). However, another meta-analysis on folic acid therapy
in CVD prevention (6), which included eight randomised trials \( n = 37,485 \) although only seven trials \( n = 35,603 \) had a stroke endpoint, concluded that dietary supplementation with folic acid had no significant effects within 5 years on cardiovascular events, including stroke \( (\text{RR}:0.96;95\% \text{ CI}: 0.87–1.06) \).

In light of several recently published trials (5–7), meta-analyses (1,3,6) and continuing controversy in the field, a comprehensive meta-analysis of all of the available data is warranted to address important questions before definite recommendations can be made about folic acid supplementation in stroke prevention. The questions are, first, with more published trials available, whether the effect of folic acid supplementation on stroke prevention remains significant, particularly in populations with no or partial folic acid fortification. Second, whether the use of statins, doses of folic acid or a treatment regimen (with or without other vitamins) affects the efficacy of folic acid supplementation. The present meta-analysis includes all of the pertinent published trials up to January 2012, and aims to address the aforementioned questions.

**Methods**

**Search strategy and selection criteria**

This report followed the Preferred Reporting Items for Systematic reviews and Meta-analyses (PRISMA) guidelines (10). We used a similar search strategy and selection criteria as previously reported (2). We searched the MEDLINE database (via PubMed) from January 1966 to January 2012 with the MeSH terms ‘cardiovascular disease’, ‘coronary disease’, ‘coronary thrombosis’, ‘myocardial ischemia’, ‘coronary stenosis’, ‘coronary restenosis’, ‘cerebrovascular accident’, ‘cerebrovascular disease’, ‘stroke’ and ‘folic acid’, ‘folate’ and ‘multivitamin’. Manual searches of bibliographies from all relevant trials and review articles also were conducted. The searches were restricted to human studies and clinical trials. There were no language restrictions. A team of experts in the relevant field was assembled.

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 team with relevant expertise in clinical medicine, epidemiology, clinical trials and biostatistics. Studies that met the following criteria were included: (i) a randomised controlled trial; (ii) the number of events for stroke that occurred during the study were reported by intervention and control groups, with more than ten incident cases; (iii) the intervention consisted of folic acid supplementation (with or without additional B vitamins); and (iv) the intervention duration was at least 6 months.

**Data collection and quality assessment**

All data from eligible trials were independently abstracted in duplicate by two investigators according to the standard protocol and reviewed by a third investigator. Discrepancies were resolved by discussion with the multidisciplinary research team that developed the protocol.

The following data were extracted: first author’s name, year of publication, study design, baseline characteristics (age, sex and baseline comorbidities), intervention regimen, treatment duration, dosage of folic acid, baseline homocysteine concentrations and baseline percent use of statins.

Studies were assessed for quality of randomisation, blinding, reporting of withdrawals, generation of random numbers and concealment of allocation. Trials scored one point for each area addressed, with a possible score of between 0 and 5 (highest level of quality) (11).

**Statistical analysis**

We assessed the overall effect of folic acid supplementation on the risk of stroke based on all eligible trials. We then assessed the overall effect of folic acid supplementation on the risk of stroke in two groups defined by folic acid fortification status (fortified vs. unfortified or partly fortified). We defined ‘the fortified’ in our manuscript to mean that the relevant trials were conducted in countries or regions with grain fortification of folic acid and/or the control groups were supplemented with folic acid.

Relative risk (RR) with a 95% confidence interval (95% CI) was used to measure the effect of folic acid supplementation on stroke risk. Heterogeneity between studies was assessed by Cochran’s Q test and \( I^2 \). We planned to pool data across trials according to the fixed-effects model based on Mantel-Haenszel methods if considerable heterogeneity, \( p < 0.1 \), or \( I^2 \geq 50\% \) (12) was not present. We also compared results obtained from a fixed-effects model with those obtained from a random-effects model to evaluate the influence of small-study effects on the results. Previously defined subgroup analyses and meta-regression analyses were performed to explore the influence of study characteristics in effects. The potential for publication bias was examined using a funnel plot and Egger regression test. We also conducted a sensitivity analysis by removing each individual trial from the meta-analysis. All of the analyses were done using R software, version 2.13.0 (http://www.R-project.org/).
Role of the funding source
There was no funding source for this study. All of the authors have had full access to the data used for this meta-analysis, and have assumed final responsibility for the submission of this manuscript.

Results
A total of 489 potentially relevant papers were retrieved with the systematic search. Our initial search of the literature identified 26 studies for detailed assessment. Of those studies, seven (13–19) were excluded for lack of data on stroke; three (20–22) for small number of incident cases of stroke and one (23) for the same study population as another report (24) (Figure 1). Our final analysis included 15 (7–9,24–35) randomised controlled trials that comprised a total of 55,764 individuals. The baseline characteristics of the study participants are shown in Table 1 and design characteristics are presented in Table 2.

The quality of these 15 trials ranged from 3 to 5 (maximum score), and they were all randomised, double-blind and placebo-controlled, except for one trial by Liem et al. (22), which was a randomised, open-label trial.

Among the 15 included trials, the HOPE2 (31) and SU.FOL.OM3 (8) studies represent the two positive trials in terms of stroke risk reduction. When pooling all 15 trials (n = 55,764), folic acid supplementation significantly reduced the risk of stroke by 8% (RR: 0.92; 95% CI: 0.86–1.00, p = 0.038, Figure 2) using a fixed-effects model. The estimate from a random-effects model (0.91; 0.83–0.99, p = 0.020, Figure 2) also yielded a similar result. In the subgroup analysis, a greater beneficial effect was observed among those trials with a lower percent use of statins (≤ 80% (median), 0.77; 0.64–0.92, p = 0.005); in the corresponding comparison group the estimated RRs were attenuated and insignificant. Treatment regimen (folic acid alone vs. folic acid with other B vitamins) and the dosage of folic acid (≤ 0.8 mg/d (median) vs. > 0.8 mg/d) did not significantly affect the effect of folic acid therapy (Table 3).

Furthermore, meta-regression analyses suggested a positive dose-response relationship between percent use of statins and log-RR for stroke associated with folic acid supplementation (regression coefficient = 0.0057; 95% CI: 0.0012, 0.0102; p = 0.013). However, the dosage of folic acid did not significantly correlated with the effect size.

The analysis was repeated for the five trials (7,27,29,33,34) in populations with folic acid fortification (n = 12,338). The estimated RR was 1.03 (0.88–1.21, p = 0.69) and there were no significant results in any subgroup analyses (data not shown).

Furthermore, meta-regression analyses showed that when percent use of statins and folic acid fortification status were simultaneously included in the model, both the percent use of statins and folic acid fortification status were significant effect modifiers (10 trials; fortification vs. no or partial folic acid fortification, regression coefficient = 0.4233; 95% CI: 0.0838, 0.7627; p = 0.015; percent use of statins, regression coefficient = 0.0054; 95% CI: 0.0009, 0.0098; p = 0.019) in stroke risk reduction.

Visual inspection of the funnel plot (Figure 3) did not clearly indicate the presence of publication bias, and likewise a statistical analysis of funnel plots did
not suggest any publication bias (Egger test, \( p = 0.092 \)). Heterogeneity testing for all analyses in Figure 2 and Table 3 was not significant (\( p > 0.10 \) and \( I^2 < 50\% \)). Sensitivity analyses showed that the RR and 95% CI did not alter substantially after removing any one trial (data not shown).

### Discussion

There are growing data and a continuing controversy over the efficacy of folic acid supplementation in stroke prevention. This present meta-analysis has the following unique features. We included 15 randomised trials with stroke prevention as one of the endpoints (\( n = 55,764 \)), by far the largest number of subjects included in any similar meta-analysis. We performed in-depth analysis to evaluate possible modifiers on the effect of folic acid supplementation on stroke, including folic acid fortification, use of statins, percent baseline hypertension, dosage of folic acid and the treatment regimen. As discussed below, our analysis has offered new insight and a possible explanation for the inconsistent findings between observational studies (36,37) and recent studies (6).

### Is folic acid supplementation for stroke prevention more efficacious in populations with no or partial folic acid-fortification?

Similar to our previous analysis (2), Saposnik et al. (38) further analysed stroke outcomes among the HOPE2 trial and also found that only patients from regions without folic acid fortification had a significant treatment benefit. From the present meta-analysis, with a sample size of 43,426 individuals and 1938 stroke events, we demonstrated that folic acid supplementation can significantly reduce the risk of stroke by 11% among trials conducted in no or partially folic acid fortified populations. In contrast, the RR was 1.03 (0.88–1.21) in the trials conducted in fortified populations. Folic acid fortification of North Americans \((140 \, \mu g/100 \, g \, of \, flour)\) lowered the population mean Hcy to about 8–10 \( \mu mol/l \) (39). As a consequence, the ability of folic acid supplementation to further reduce Hcy among North Americans was reduced from 25% to 15% (40). This point was not considered in the design of some clinical trials, in which folic acid fortification might contribute to their null findings. Of note, the placebo group in some trials (7,27,29) also received folic acid supple-

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**Table 1 Baseline characteristics of individual trials**

| Data source            | Total subjects | Age, year Mean (SD) | Male % | Pre-existent diseases | Use of statins % | Previous stroke % | Homocysteine Mean (SD) µmol/l |
|------------------------|----------------|---------------------|--------|-----------------------|------------------|-------------------|-------------------------------|
| **Unfortified or partly fortified** |                |                     |        |                       |                  |                   |                               |
| Mark et al., 1996 (28) | 3318           | 54 (SD)             | 44.0   | Oesophagea Dysplasia   | 0                | NR                | NR                            |
| Liem et al., 2005 (24) | 593            | 65.2 (9.8)          | 78.0   | CHD                   | 100              | 7.0                | 12.1 (4.3)                    |
| Bonata et al., 2006 (30)| 3749           | 63.0 (11.7)         | 73.7   | MI                    | 75.4             | 4.2                | 13.1 (5.2)                    |
| Lonn et al., 2006 (31) | 5522           | 68.9 (6.9)          | 71.8   | Vascular disease or diabetes | 59.0           | 8.9                | 12.2 (NR)                     |
| Zoungas et al., 2006 (32) | 315            | 56.0 (13.5)         | 68.0   | ESRD or ACKD          | 28.8             | 8.6                | 27.0 (13.0)                   |
| Ebbing et al., 2008 (35) | 3090           | 61.7 (9.9)          | 79.3   | CHD and/or aortic valve stenosis | 87.9           | 6.1                | 10.8 (4.5)                    |
| Armitage et al., 2010 (25) | 12064         | 64.0 (9.0)          | 83.0   | MI                    | 100.0            | 7.0                | 13.5 (4.8)                    |
| VITATOPS, 2010 (26)    | 8164           | 62.6 (12.5)         | 64.0   | Stroke or TIA         | NR               | 83.0              | 14.3 (8.5)                    |
| Galan P, 2010 (8)      | 2501           | 60.6                | 79.4   | CHD or stroke         | 84.5             | 25.5               | Median 12.8                   |
| Boston AG, 2011 (9)    | 4110           | 52 (9.4)            | 62.8   | Kidney transplant recipients | NR         | NR                | 16.4 (1.3)                    |
| **Fortified**          |                |                     |        |                       |                  |                   |                               |
| Tooile et al., 2004 (27) | 3680           | 66.3 (10.8)         | 63.0   | Stroke                | NR               | 100.0             | 13.4 (NR)                     |
| Wronke et al., 2004 (29) | 510            | 60.2 (15.1)         | 50.0   | ESRD                  | NR               | 32.9              | (20.0)                        |
| Jamison et al., 2007 (33) | 2056           | 65.8 (11.8)         | 98.0   | ESRD or ACKD          | 48.0             | 15.1              | 24.1 (8.8)                    |
| Albert et al., 2008 (34) | 5442           | 62.8 (8.8)          | 0      | CVD or multiple risk factors | 33.6           | NR                | 12.8 (NR)                     |
| Heinz et al., 2010 (7)  | 650            | 61.0 (13.0)         | 58.0   | ESRD                  | NR               | NR                | Median 29.0                   |

ACKD, advanced chronic kidney disease (with an estimated creatinine clearance of less than or equal to 30 mL/min); CHD, Coronary heart disease; CVD, cardiovascular disease; ESRD, end stage renal disease; MI, myocardial infarction; NR, not reported; TIA, transient ischaemic attack.
mentation, which might also contribute to the null-effect findings.

Does the use of statins affect the efficacy of folic acid supplementation?

In the analysis of the HOPE2 trial (31), Saposnik et al. (38) found that subjects not receiving lipid-lowering drugs had a larger treatment benefit. In the present meta-analysis, we also found a greater beneficial effect in those trials among subjects with a lower percent use of statins (RR: 0.77; 95% CI: 0.64–0.92, \( p = 0.005 \)); and a meta-regression analysis suggested a positive dose-response relationship between percent use of statins and RR for stroke associated with folic acid supplementation. The principal mechanisms of Hcy involve impaired endothelial function, increased oxidative stress and alterations of lipid metabolism (41). The potential confounding effect of statins may be because of the possibility that both hyperhomocysteine and hyperlipidemia are involved in atherosclerotic processes and the pathogenesis of stroke (42,43). Another possibility that remains to be tested is that folic acid supplementation may be more efficacious in populations whose atherosclerosis and stroke are because of high Hcy rather than a high calorie and/or fat diet. We speculate that this is more likely the case in developing countries with a micronutrient-poor diet such as in China. The Linxian Nutrition Intervention Trial (28) lends some support for this hypothesis.

Is there a ceiling effect of folic acid supplementation in stroke prevention?

This ceiling effect in reducing Hcy at around 0.8 mg of folic acid daily was evident in a meta-analysis of 25 randomised controlled trials (40). In our meta-analysis, five trials used a folic acid dosage of 0.8 mg daily or lower, and five used doses greater than 0.8 mg daily in no or partially fortified populations. There was no evidence of added benefit from larger

| Data Source | Blinding | Active treatment | Control | Fortification | Duration of intervention (month) | Date quality |
|-------------|----------|-----------------|---------|--------------|---------------------------------|--------------|
| Unfortified or partly fortified | | | | | | |
| Mark et al., 1996 (28) | Double | Folic acid 0.8 mg/d and vitamins B6 and B12 | Placebo | No | 72 | 3 |
| Liem et al., 2005 (24) | Open | Folic acid 0.5 mg/d | Usual care | No | 42 | 3 |
| Bonaa et al., 2006 (30) | Double | Folic acid 0.8 mg/d and vitamins B12 with or without B6 | Placebo or Vitamins B6 | No | 36 | 5 |
| Lonn et al., 2006 (31) | Double | Folic acid 2.5 mg/d and vitamins B6 and B12 | Placebo | Partly | 60 | 5 |
| Zoungas et al., 2006 (32) | Double | Folic acid 15 mg/d | Placebo | No | 43 | 3 |
| Ebbing et al., 2008 (35) | Double | Folic acid 0.8 mg/d and vitamins B12 with or without B6 | Placebo or Vitamins B6 | No | 38 | 5 |
| Armitage et al., 2010 (25) | Double | Folic acid 2 mg/d and vitamins B12 | Placebo | No | 80 | 5 |
| VITATOPS, 2010 (26) | Double | Folic acid 2 mg/d and vitamins B6 and B12 | Placebo | No | 41 | 5 |
| Galan P, 2010 (8) | Double | 5-MTHF\(\^\) 560 \(\mu g/d\) and vitamins B6 and B12 with or without omega 3 fatty acids | Placebo or omega 3 | No | 56 | 5 |
| Bostom AG, 2011 (9) | Double | Folic acid 5 mg/d and vitamins B6 and B12 | Vitamins B6 and B12 | Partly | 48 | 3 |
| Fortified | | | | | | |
| Toole et al., 2004 (27) | Double | Folic acid 2.5 mg/d and vitamins B6 and B12 | Folic acid 0.02 mg/d and vitamins B6 and B12 | Yes† | 24 | 5 |
| Wrone et al., 2004 (29) | Double | Folic acid 5 mg/d or 15 mg/d and vitamins B6 and B12 | Folic acid 1 mg/d and vitamins B6 and B12 | Yes† | 24 | 5 |
| Jamison et al., 2007 (33) | Double | Folic acid 40 mg/d and vitamins B6 and B12 | Placebo | Yes | 38 | 5 |
| Albert et al., 2008 (34) | Double | Folic acid 2.5 mg/d and vitamins B6 and B12 | Placebo | Yes | 88 | 3 |
| Heinz et al., 2010 (7) | Double | Folic acid 5 mg, 3 times/w and vitamins B6 and B12 | Folic acid 0.2 mg/d and vitamins B6 and B12 | Yes† | 24 | 4 |

†folic acid supplementation in control group. \(\^\)5-methyltetrahydrofolate.
Figure 2 Forest plot of relative risk (RR) and 95% confidence interval (95% CI) of stroke for folic acid treatment vs. control in individual trials and pooled data

Table 3 Risk of stroke for folic acid treatment vs. control in stratified analysis by pertinent factors in populations with no or partial folic acid fortification

| Stratification variables | Active group | Control group | RR (95%-CI) | p-value |
|--------------------------|--------------|--------------|-------------|---------|
| Overall (8,9,24–26,28,30–32,35) | 914/21703 | 1024/21723 | 0.89 (0.82–0.97) | 0.010 |
| ≤ 80% use of statins* | 190/6443 | 249/6461 | 0.77 (0.64–0.92) | 0.005 |
| > 80% use of statins | 326/9115 | 352/9133 | 0.93 (0.80–1.07) | 0.31 |
| Folic acid alone (24,32) | 16/456 | 30/452 | 0.53 (0.30–0.96) | 0.04 |
| Folic acid with other B vitamins (8,9,25,26,28,30,31,35) | 898/21247 | 994/21271 | 0.90 (0.83–0.99) | 0.02 |
| Daily folic acid dose (mean) | 128/6611 | 171/6640 | 0.75 (0.60–0.94) | 0.01 |
| ≤ 0.8 mg, low median (8,24,28,30,35) | 786/15092 | 853/15083 | 0.92 (0.84–1.01) | 0.08 |

*Only trials reporting the value were included in the analysis. The risk estimation is based on a fixed-effects model. RR, relative risk.
doses of folic acid supplementation in comparison with lower doses in stroke prevention (Table 3).

The most recent meta-analysis on folic acid therapy in CVD prevention (6), which included only seven trials that had a stroke endpoint and had at least 1000 subjects for a scheduled treatment duration of at least 1 year, concluded that dietary supplementation with folic acid had no significant effects on stroke \( (n = 35,603; 0.96; 0.87–1.06) \). When the three newly published trials (6,9,26) and the Linxian Nutrition Intervention Trial (28), which were eligible for this meta-analysis, were included, the estimated RR was 0.93 \( (n = 53,696; 95\% \text{ CI } 0.86–1.01; p = 0.08) \). However, in the eight trials (8,9,25,26,28,30,31,35) with no or partial folic acid fortification \( (n = 42,518) \), folic acid supplementation significantly reduced the risk of stroke by 10% \( (RR = 0.90; 0.83–0.99, p = 0.02) \). Excluding the Linxian Nutrition Intervention Trial (28) from the analyses did not materially affect the effect \( (n = 39,200; RR = 0.91; 0.84–1.00, p = 0.04) \), which was consistent with our results. Furthermore, we did not think it was necessary to exclude trials with high quality but less than 1000 subjects. Taken together, these results further underscore the importance of identifying target populations that can particularly benefit from folic acid therapy.

There are several limitations of our report. First, we are unable to evaluate the effect of folic acid supplementation on different stroke subtypes (ischaemic or haemorrhagic stroke, fatal or non-fatal stroke) because of lack of such data in the published trials. Second, in contrast to other trials of folic acid, only the SU.FOL.OM3 study (8) used 5-methyltetrahydrofolate, a more naturally occurring formulation of folic acid. However, when this trial was excluded, none of the results in Figure 2 and Table 3 were altered substantially (data not shown). Obviously, additional large, randomised studies should be performed to confirm our results and further resolve the uncertainty regarding the effect of folic acid supplementation on stroke prevention. While we are waiting for additional data from other large trials, our findings should be interpreted in the context of available evidence in the field.

In summary, given the ongoing controversy over homocysteine-lowering therapy to reduce CVD risk, clarifying the subset of populations who may benefit more from this simple intervention is very important from a population health perspective. Our analysis indicated that folic acid supplementation is effective in stroke prevention in populations with no or partial folic acid fortification. In addition, a greater beneficial effect was observed among trials with a lower percent use of statins. In general, a daily dose of 0.4–0.8 mg folic acid appeared to be adequate for stroke prevention in comparison with larger doses. Individuals with MTHFR CT or TT genotype may require at least 0.8 mg based on our data (44,45).

These results are important for those living in China, India and African countries where food is not routinely fortified with folic acid. Our findings underscore the importance of identifying target populations that can particularly benefit from folic acid therapy.

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Author contributions
XQ, XW and YH performed acquisition of data; XQ, XX, XX and YH analysed the data; All authors participated in this meta-analysis and have seen and approved the final version. They also performed the study concept and design, participated in the interpretation of data and contributed to the drafting and critical review of manuscript for important intellectual content.
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