Folic Acid Therapy Reduces the First Stroke Risk Associated With Hypercholesterolemia Among Hypertensive Patients

Xianhui Qin, MD*; Jianping Li, MD*; J. David Spence, MD; Yan Zhang, MD; Youbao Li, MD; Xiaobin Wang, MD, ScD; Binyin Wang, MD, PhD; Ningling Sun, MD; Fang Chen, MD; Jingxuan Guo, MD; Delu Yin, MD; Liming Sun, MD; Genfu Tang, MD; Mingli He, MD; Jia Fu, MD; Yefeng Cai, MD; Xiuli Shi, MD; Ping Ye, MD; Hong Chen, MD; Shuiping Zhao, MD; Mao Chen, MD; Chuanyu Gao, MD; Xiangqing Kong, MD; Fan Fan Hou, MD, PhD; Yining Huang, MD; Yong Huo, MD

**Background and Purpose**—We sought to determine whether folic acid supplementation can independently reduce the risk of first stroke associated with elevated total cholesterol levels in a subanalysis using data from the CSPPT (China Stroke Primary Prevention Trial), a double-blind, randomized controlled trial.

**Methods**—A total of 20702 hypertensive adults without a history of major cardiovascular disease were randomly assigned to a double-blind daily treatment of an enalapril 10-mg and a folic acid 0.8-mg tablet or an enalapril 10-mg tablet alone. The primary outcome was first stroke.

**Results**—The median treatment duration was 4.5 years. For participants not receiving folic acid treatment (enalapril-only group), high total cholesterol (>200 mg/dL) was an independent predictor of first stroke when compared with low total cholesterol (<200 mg/dL; 4.0% versus 2.6%; hazard ratio, 1.52; 95% confidence interval, 1.18–1.97; P=0.001). Folic acid supplementation significantly reduced the risk of first stroke among participants with high total cholesterol (4.0% in the enalapril-only group versus 2.7% in the enalapril–folic acid group; hazard ratio, 0.69; 95% confidence interval, 0.56–0.84; P<0.001; number needed to treat, 78; 95% confidence interval, 52–158), independent of baseline folate levels and other important covariates. By contrast, among participants with low total cholesterol, the risk of stroke was 2.6% in the enalapril-only group versus 2.5% in the enalapril–folic acid group (hazard ratio, 1.00; 95% confidence interval, 0.75–1.30; P=0.982). The effect was greater among participants with elevated total cholesterol (P for interaction=0.024).

**Conclusions**—Elevated total cholesterol levels may modify the benefits of folic acid therapy on first stroke. Folic acid supplementation reduced the risk of first stroke associated with elevated total cholesterol by 31% among hypertensive adults without a history of major cardiovascular diseases.

**Clinical Trial Registration**—URL: http://www.clinicaltrials.gov. Unique identifier: NCT00794885.

(Stroke. 2016;47:2805-2812. DOI: 10.1161/STROKEAHA.116.014578.)

**Key Words:** cholesterol ■ folic acid ■ hypercholesterolemia ■ risk factors ■ stroke
Hypercholesterolemia is a recognized risk factor for stroke. There is a considerable interest in the efficacy of folic acid therapy in lowering the risk of stroke associated with hypercholesterolemia. In the HOPE-2 trial (Heart Outcomes Prevention Evaluation 2),¹ high total cholesterol (TC) levels were associated with an increased risk of stroke in the placebo group (6.6% versus 3.9%; \( P<0.01 \)). Consistently, folic acid therapy showed a trend toward a greater benefit in reducing stroke among patients with higher baseline TC levels than among patients with lower baseline TC levels (\( P \) for interaction=0.34). A meta-analysis² of 15 randomized controlled trials found that folic acid supplementation reduced the risk of stroke by 8% on average (relative risk [RR], 0.92; 95% confidence interval [CI], 0.86–1.00), but the effect was greater among those trials with a lower percent use of statins (RR, 0.77; 95% CI, 0.64–0.92), suggesting that the benefits of folic acid supplementation in the prevention of stroke might be hindered by concomitant use of statins because of the possibility of overlapping biological mechanisms.

Although it is plausible that folic acid therapy may lower the risk of stroke associated with hypercholesterolemia, particularly in patients not taking statins, to date, this hypothesis has not been tested in randomized trials. Indeed, hypertension is recognized as a major and modifiable risk factor of stroke. Furthermore, hypertension and elevated homocysteine concentrations have shown a multiplicative effect on cardiovascular disease risk.³ ⁴ We chose to carry out the trial in Chinese hypertensive patients because we speculated that hypertensive adults in regions without mandatory folic acid fortification may particularly benefit from homocysteine-lowering therapy along with antihypertension therapy. Therefore, the CSPPT (China Stroke Primary Prevention Trial), a multicommunity, double-blind, randomized controlled trial, compared the efficacy of a combination of the angiotensin-converting enzyme inhibitor enalapril and folic acid with enalapril alone in reducing the risk of first stroke in Chinese adults with hypertension.⁵ This report, a subanalysis using data from the CSPPT (see the Statistical Analysis Plan of the CSPPT),³ sought to determine whether folic acid supplementation can independently reduce the risk of first stroke associated with elevated TC levels. A unique aspect of the CSPPT is the low percentage of concomitant use of lipid-lowering drugs (0.8%) at baseline among the study participants, offering an opportunity to determine the independent and interactive effects of folic acid supplementation with elevated TC on first stroke without confounding by statins.

**Methods**

The methods and primary results of the CSPPT trial have been reported elsewhere.⁶ Briefly, a total of 20702 hypertensive adults without a history of major cardiovascular disease were randomly assigned to a double-blind daily treatment of an enalapril 10-mg and a folic acid 0.8-mg tablet or an enalapril 10-mg tablet alone. Participants were scheduled for follow-up every 3 months. This study was approved by the Ethics Committee of the Institute of Biomedicine, Anhui Medical University, Hefei, China (FWA assurance number: FWA00001263). All participants gave written informed consent.

**Outcomes**

The primary outcome was first stroke. The secondary outcomes included first ischemic stroke (fatal or nonfatal), first hemorrhagic stroke (fatal or nonfatal), and a composite of cardiovascular events consisting of cardiovascular death, myocardial infarction, and stroke.

**Statistical Analysis**

Means (SD) or medians (25th percentile, 75th percentile) and proportions were calculated for population characteristics by the treatment groups in accordance with baseline TC strata (≥200 versus <200 [desirable levels] mg/dL). Low-density lipoprotein cholesterol (LDL-C) was estimated by the Friedewald formula⁶ as follows: LDL-C, mg/dL=TC−(high-density lipoprotein cholesterol [HDL-C])−(triglycerides/5).

The hazard ratios (HRs) and 95% confidence intervals (CIs) for the risk of the primary outcome associated with elevated TC levels (≥200 versus <200 mg/dL) were estimated using Cox proportional hazards models stratified by treatment groups with adjustment for major covariates.

We further assessed the effect of folic acid supplementation on the prevention of study outcomes according to different TC strata, as well as assessed the interaction between baseline TC strata and folic acid therapy on the study outcomes by means of Cox proportional hazards regression both before and after adjustment for the major covariates. Interactions were examined by including interaction terms in the Cox models.

A 2-tailed \( P<0.05 \) was considered to be statistically significant in all analyses. R software, version 3.2.0 (http://www.R-project.org/) was used for all statistical analyses.

**Results**

**Study Participants and Baseline Characteristics**

The total sample of the CSPPT was 20702. Of these, 166 participants who were taking lipid-lowering medications and 370 participants who were missing TC data at baseline were excluded from the analysis. A total of 20166 participants were included in the final analysis (Figure I in the online-only Data Supplement).

At baseline, 11862 participants (58.8%) had high TC levels (≥200 mg/dL), 8304 participants (41.2%) had low TC levels (<200 mg/dL). Although there were significant differences in baseline characteristics between participants with low TC levels (<200) and with high TC levels (≥200 mg/dL; Table I in the online-only Data Supplement); all of the baseline characteristics were comparable between the 2 treatment groups within each baseline TC strata, with the exception of a higher use of antiplatelet drugs in the enalapril-only group among participants with high TC levels (Tables 1 and 2).

**Effects of Folic Acid Therapy on Serum Folate, Blood Pressure, and TC Levels**

Table 2 shows that compared with the enalapril-only group, the enalapril–folic acid group showed significantly increased serum folate concentrations. However, there were no significant group differences in TC, systolic blood pressure, or diastolic blood pressure levels both at baseline and after treatment within each baseline TC strata. We found that mean folate levels in the enalapril-only group also increased substantially ≈54% during the course of the trial. The cause of this increase is unclear. During the course of the study, subjects received nutritional health education,
which may have led to improved dietary choices. Whatever the cause, this change likely attenuated the beneficial effect.

**Interactive Effect of Folic Acid Therapy and TC on First Stroke**

Over the median treatment duration of 4.5 years, for participants not receiving folic acid (the enalapril-only group), high baseline TC levels (≥200 versus <200 mg/dL; 4.0% versus 2.6%; HR=1.52; 95% CI, 1.18–1.97; P=0.001) were significantly associated with increased risk of first stroke, after adjustment for major covariates (age, sex, MTHFR C677T genotypes, systolic blood pressure and diastolic blood pressure at baseline, mean systolic blood pressure and diastolic blood pressure over the treatment period, body mass index, baseline TC levels (≥200 versus <200 mg/dL; 4.0% versus 2.6%; HR=1.52; 95% CI, 1.18–1.97; P=0.001) were significantly associated with increased risk of first stroke, after adjustment for major covariates (age, sex, MTHFR C677T genotypes, systolic blood pressure and diastolic blood pressure at baseline, mean systolic blood pressure and diastolic blood pressure over the treatment period, body mass index,

**Table 1. Baseline Characteristics of Participants by Treatment Groups for Baseline Total Cholesterol Strata***

<table>
<thead>
<tr>
<th>Total Cholesterol&lt;200 mg/dL</th>
<th>Total Cholesterol≥200 mg/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Enalapril</td>
</tr>
<tr>
<td>n</td>
<td>4187</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>1933 (46.2)</td>
</tr>
<tr>
<td>Age, y</td>
<td>60.1±7.7</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>24.3±3.7</td>
</tr>
<tr>
<td>Methylene tetrahydrofolate reductase C677T polymorphisms, n (%)</td>
<td>0.899</td>
</tr>
<tr>
<td>CC</td>
<td>1225 (29.2)</td>
</tr>
<tr>
<td>CT</td>
<td>2046 (48.9)</td>
</tr>
<tr>
<td>TT</td>
<td>916 (21.9)</td>
</tr>
<tr>
<td>Current smoking</td>
<td>1032 (24.6)</td>
</tr>
<tr>
<td>Current alcohol drinking</td>
<td>960 (22.9)</td>
</tr>
<tr>
<td>Physical activity</td>
<td>0.621</td>
</tr>
<tr>
<td>Low</td>
<td>1323 (31.6)</td>
</tr>
<tr>
<td>Medium</td>
<td>1730 (41.4)</td>
</tr>
<tr>
<td>High</td>
<td>1131 (27.0)</td>
</tr>
<tr>
<td>Self-reported hyperlipidemia</td>
<td>86 (2.1)</td>
</tr>
<tr>
<td>Self-reported diabetes mellitus</td>
<td>105 (2.5)</td>
</tr>
<tr>
<td>Laboratory results</td>
<td></td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>133.3±79.1</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>47.9±12.3</td>
</tr>
<tr>
<td>Fasting glucose, mmol/L</td>
<td>5.5±1.4</td>
</tr>
<tr>
<td>Homocystine, μmol/L†</td>
<td>12.4 (10.4, 15.4)</td>
</tr>
<tr>
<td>Vitamin B12, pg/mL†</td>
<td>367.0 (303.6, 463.5)</td>
</tr>
<tr>
<td>Medication use, n (%)</td>
<td></td>
</tr>
<tr>
<td>Antihypertensive drugs (all types)</td>
<td>1915 (45.7)</td>
</tr>
<tr>
<td>Antihypertensive drugs (subtypes)</td>
<td></td>
</tr>
<tr>
<td>Angiotensin–converting enzyme inhibitors</td>
<td>399 (9.5)</td>
</tr>
<tr>
<td>Angiotensin II–receptor blockers</td>
<td>2 (0.1)</td>
</tr>
<tr>
<td>Calcium channel blockers</td>
<td>490 (11.7)</td>
</tr>
<tr>
<td>Diuretics</td>
<td>68 (1.6)</td>
</tr>
<tr>
<td>β-Blockers</td>
<td>41 (1.0)</td>
</tr>
<tr>
<td>Glucose-lowering drugs</td>
<td>44 (1.1)</td>
</tr>
<tr>
<td>Antiplatelet drugs</td>
<td>94 (2.2)</td>
</tr>
</tbody>
</table>

HDL indicates high-density lipoprotein.

*For continuous variables, values are presented as mean±SD.
†Values are medians (25th, 75th percentile), Wilcoxon signed-rank test was used.
study centers, vitamin B12, folate, homocysteine, TG, HDLC, creatinine, glucose levels, and smoking status). However, the increased risk associated with high TC levels (2.7% versus 2.5%; HR, 1.05; 95% CI, 0.80–1.39; P = 0.727) was no longer significant in the enalapril–folic acid group (Figure 1).

Similar results were found for ischemic stroke (Figure 1).

Kaplan–Meier curves of the cumulative event rate of first stroke and ischemic stroke for the 2 treatment groups within each baseline TC strata are shown in Figure 2. Folic acid therapy did not have a significant effect on risk of first stroke (2.6% in the enalapril-only group versus 2.5% in the enalapril–folic acid group; HR, 1.00; 95% CI, 0.75–1.30; P = 0.982) in participants with low baseline TC levels (<200 mg/dL). In contrast, among participants with high baseline TC levels (≥200 mg/dL), folic acid therapy significantly reduced the risk of first stroke to a level that was on par with participants with low baseline TC levels (4.0% in the enalapril-only group versus 2.7% in the enalapril–folic acid group; HR, 0.69; 95% CI, 0.56–0.84; P < 0.001; number needed to treat [4.5 years], 78; 95% CI, 52–158), independent of baseline folate levels and other important covariates listed above. The interaction between baseline TC levels and folic acid therapy on risk of first stroke was significant (P for interaction = 0.024). The overall results were consistent for ischemic stroke (P for interaction = 0.035), the composite of stroke, myocardial infarction, or death from cardiovascular causes (P for interaction = 0.026), and composite of stroke or all-cause death (P for interaction = 0.038), but not for hemorrhagic stroke (P for interaction = 0.485; Table 3).

Table 2. Serum Folate, Total Cholesterol Levels, and Blood Pressure at Baseline and After Treatment-by-Treatment Groups for Baseline Total Cholesterol Strata*

<table>
<thead>
<tr>
<th>Variables</th>
<th>Total Cholesterol&lt;200 mg/dL</th>
<th></th>
<th>Total Cholesterol≥200 mg/dL</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Enalapril</td>
<td>Enalapril–Folic Acid</td>
<td>P Value</td>
<td>Enalapril</td>
</tr>
<tr>
<td>Folate, ng/mL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At baseline</td>
<td>8.2 (5.6, 10.9) [4139]</td>
<td>8.3 (5.6, 10.9) [4081]</td>
<td>0.697</td>
<td>8.0 (5.6, 10.3) [5862]</td>
</tr>
<tr>
<td>At exit visit</td>
<td>13.1 (9.8, 16.1) [3380]</td>
<td>20.2 (15.2, 23.6) [3350]</td>
<td>&lt;0.001</td>
<td>12.9 (9.6, 15.9) [4806]</td>
</tr>
<tr>
<td>Change†</td>
<td>4.3 (1.6, 7.3) [3340]</td>
<td>11.5 (5.9, 17.2) [3319]</td>
<td>&lt;0.001</td>
<td>4.4 (1.7, 7.3) [4777]</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At baseline</td>
<td>177.2 (161.0, 190.0) [4187]</td>
<td>176.8 (159.8, 189.6) [4117]</td>
<td>0.133</td>
<td>233.6 (215.8, 258.3) [5899]</td>
</tr>
<tr>
<td>At exit visit</td>
<td>183.4 (163.3, 204.2) [3393]</td>
<td>181.9 (162.9, 203.9) [3347]</td>
<td>0.489</td>
<td>218.1 (195.4, 244.0) [4905]</td>
</tr>
<tr>
<td>Change†</td>
<td>8.1 (–9.3, 29.3) [3393]</td>
<td>8.1 (–10.0, 29.3) [3347]</td>
<td>0.974</td>
<td>–19.3 (–42.1, 3.1) [4905]</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>161.3 (151.3, 176.7) [4187]</td>
<td>161.3 (151.3, 177.3) [4117]</td>
<td>0.586</td>
<td>166.0 (154.7, 180.0) [5899]</td>
</tr>
<tr>
<td>Over treatment period</td>
<td>138.0 (131.7, 145.4) [4186]</td>
<td>138.2 (131.6, 145.9) [4117]</td>
<td>0.472</td>
<td>138.9 (132.2, 146.4) [5899]</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>92.0 (84.7, 100.0) [4187]</td>
<td>92.7 (85.3,100.0) [4117]</td>
<td>0.053</td>
<td>94.7 (87.3,101.3) [5899]</td>
</tr>
<tr>
<td>Over treatment period</td>
<td>82.4 (77.4, 87.3) [4186]</td>
<td>82.4 (77.5, 87.5) [4117]</td>
<td>0.996</td>
<td>82.9 (78.4, 87.6) [5899]</td>
</tr>
</tbody>
</table>

*Values are medians (25th, 75th) (number of participants with available data).
†Change=exit level (folate or total cholesterol)–baseline level.

Figure 1. Rates of first stroke (A) and ischemic stroke (B) by treatment groups and baseline total cholesterol (TC) strata.
Similar results were observed between baseline LDL-C levels (≥100 versus <100 [optimal levels] mg/dL) and folic acid therapy on incident stroke (Table II in the online-only Data Supplement). Exclusion of participants with LDL-C ≥190 mg/dL or diabetes mellitus or fasting glucose ≥7.0 mmol/L at baseline did not change the benefits for participants with high LDL-C levels (LDL-C ≥100 mg/dL: HR, 0.76; 95% CI, 0.61–0.93 versus LDL-C <100 mg/dL: HR, 1.32; 95% CI, 0.82–2.12; P for interaction=0.032).

The 20th percentiles of LDL-C and TC levels were ≈100 and 177 mg/dL, respectively. Greater beneficial results were also observed in participants with TC ≥177 mg/dL (HR, 0.72; 95% CI, 0.61–0.86) versus TC <177 mg/dL (HR, 1.28; 95% CI, 0.83–1.98; P for interaction=0.017).

**Exploratory Stratified Analyses by Important Covariables**

The Table III in the online-only Data Supplement showed that of all the listed subgroups, there was a greater beneficial effect for the enalapril–folic acid group than for the enalapril-only group on risk of first stroke for participants with high TC levels at baseline than those with low TC levels at baseline although many of the comparisons were not statistically significant. Similar results were also observed between baseline LDL-C levels and folic acid therapy on incident stroke.
Table 3. Interaction Between Folic Acid Therapy and Total Cholesterol Levels at Baseline on First Stroke

<table>
<thead>
<tr>
<th>Total Cholesterol, mg/dL</th>
<th>Enalapril</th>
<th>Enalapril–Folic Acid</th>
<th>HR (95% CI)</th>
<th>P Value</th>
<th>P for Interaction</th>
<th>Adjusted HR* (95% CI)</th>
<th>P Value*</th>
<th>P for Interaction*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary outcome</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First stroke</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;200</td>
<td>4187</td>
<td>109 (2.6)</td>
<td>4117</td>
<td>102 (2.5)</td>
<td>0.95 (0.73–1.25)</td>
<td>0.727</td>
<td>0.044</td>
<td>1.00 (0.76–1.32)</td>
</tr>
<tr>
<td>≥200</td>
<td>5899</td>
<td>237 (4.0)</td>
<td>5963</td>
<td>163 (2.7)</td>
<td>0.67 (0.55–0.82)</td>
<td>&lt;0.001</td>
<td>0.69</td>
<td>(0.56–0.84)</td>
</tr>
<tr>
<td>&lt;200</td>
<td>4187</td>
<td>80 (1.9)</td>
<td>4117</td>
<td>74 (1.8)</td>
<td>0.94 (0.69–1.29)</td>
<td>0.720</td>
<td>0.049</td>
<td>0.99 (0.72–1.37)</td>
</tr>
<tr>
<td>≥200</td>
<td>5899</td>
<td>205 (3.5)</td>
<td>5963</td>
<td>134 (2.3)</td>
<td>0.64 (0.52–0.80)</td>
<td>&lt;0.001</td>
<td>0.66</td>
<td>(0.53–0.82)</td>
</tr>
<tr>
<td>Hemorrhagic stroke</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;200</td>
<td>4187</td>
<td>28 (0.67)</td>
<td>4117</td>
<td>28 (0.68)</td>
<td>1.02 (0.60–1.72)</td>
<td>0.947</td>
<td>0.656</td>
<td>1.08 (0.63–1.83)</td>
</tr>
<tr>
<td>≥200</td>
<td>5899</td>
<td>32 (0.54)</td>
<td>5963</td>
<td>28 (0.47)</td>
<td>0.86 (0.52–1.43)</td>
<td>0.569</td>
<td>0.46</td>
<td>0.82 (0.49–1.37)</td>
</tr>
<tr>
<td>Composite of stroke, myocardial infarction, or death from cardiovascular causes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;200</td>
<td>4187</td>
<td>126 (3.0)</td>
<td>4117</td>
<td>118 (2.9)</td>
<td>0.95 (0.74–1.23)</td>
<td>0.715</td>
<td>0.035</td>
<td>1.00 (0.77–1.29)</td>
</tr>
<tr>
<td>≥200</td>
<td>5899</td>
<td>269 (4.6)</td>
<td>5963</td>
<td>187 (3.1)</td>
<td>0.68 (0.57–0.82)</td>
<td>&lt;0.001</td>
<td>0.70</td>
<td>(0.58–0.85)</td>
</tr>
<tr>
<td>Sensitivity analyses</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Composite of first stroke or all-cause death</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;200</td>
<td>4187</td>
<td>240 (5.7)</td>
<td>4117</td>
<td>228 (5.5)</td>
<td>0.97 (0.81–1.16)</td>
<td>0.722</td>
<td>0.075</td>
<td>1.02 (0.85–1.23)</td>
</tr>
<tr>
<td>≥200</td>
<td>5899</td>
<td>385 (6.5)</td>
<td>5963</td>
<td>307 (5.2)</td>
<td>0.78 (0.67–0.91)</td>
<td>0.001</td>
<td>0.79</td>
<td>(0.68–0.92)</td>
</tr>
</tbody>
</table>

*Adjusted for age, sex, MTHFR C677T genotypes, study centers, systolic blood pressure, and diastolic blood pressure at baseline, mean systolic blood pressure and diastolic blood pressure over the treatment period, body mass index, baseline laboratory measurements for vitamin B12, folate, homocysteine, triglycerides, high-density lipoproteins cholesterol, creatinine and glucose levels, and smoking status.

LDL-C and folic acid therapy on first stroke in different subgroups (Table IV in the online-only Data Supplement).

Discussion

This study was the first to demonstrate that elevated TC levels modify the benefits of folic acid supplementation on first stroke. Folic acid therapy significantly reduced the risk of first stroke associated with elevated TC levels by 31%, independent of baseline folate levels and other important covariates. Our results have important clinical and public health implications.

Elevated level of TC is a recognized risk factor for stroke and has become a major public health concern throughout the world.7 From 2011 to 2012, 38.9% of US adults aged 20 to 49 years and 75.8% of US adults aged ≥50 years had TC levels ≥200 mg/dL.8 In China, from 2000 to 2001, the age-standardized prevalence of high TC levels (≥200 mg/dL) was 32.8% in a nationally representative sample of 15,540 Chinese adults aged 35 to 74 years.8 In the recently published results of the HOPE-3 study (Heart Outcomes Prevention Evaluation 3), treatment with rosuvastatin10 resulted in a significantly lower risk of cardiovascular events than placebo in an intermediate-risk, ethnically diverse population without cardiovascular disease. However, although statins are the major class of drug most commonly used to lower cholesterol to prevent cardiovascular diseases including stroke, there are several issues associated with statin use. First, statin intolerance occurs in 10% to 15% of patients.11 Patients who take statins can experience a series of adverse effects, including myopathy and new-onset diabetes mellitus, which is particularly common in Asian populations.12,13 The value of other lipid-lowering therapies in patients who cannot tolerate statins in the primary prevention of stroke is uncertain.14 Second, even among individuals with cardiovascular disease, the usage of statin medication is much lower among middle- to low-income countries: 17.5% in upper middle-income countries, 4.3% in lower middle-income countries, 3.3% in low-income countries, and 1.7% in China, compared with 66.5% in high-income countries.15 There are >1 billion hypertensive patients worldwide16 (≈300 million in China17). From a public health perspective, in China alone, a 1.3% decrease in absolute stroke risk (number needed to treat=78) associated with folic acid supplementation in patients with hypercholesterolemia could translate into sparing ≈2 million people from stroke >4.5 years. Furthermore, we have shown that folic acid had no adverse effect on tolerability of an enalapril-based antihypertensive treatment.5

The recommendations of statin medications for the primary prevention of cardiovascular diseases vary substantially among several recently published guidelines. According to 2013 guidelines produced the American College of Cardiology/American Heart Association,18 for primary prevention, statin medications should be initiated for individuals with primary elevations of LDL-C≥190 mg/dL, diabetes mellitus, or an estimated 10-year cardiovascular events risk of ≥7.5%. Our results showed that after excluding participants...
with LDL-C ≥ 190 mg/dL or diabetes mellitus at baseline, we still found significantly beneficial results from folic acid supplementation for participants with LDL-C ≥ 100 mg/dL but < 190 mg/dL. Furthermore, the beneficial effects were consistent in participants with relatively lower cardiovascular risk, such as younger patients, women, or those with lower glucose levels or lower blood pressure at both baseline and during the treatment period. These results suggest that folic acid supplementation may possibly have a role in the primary prevention of stroke among individuals with relatively low estimated risk for cardiovascular events.

Hypercholesterolemia may influence 3 aspects important to atherothrombosis: endothelial function, platelet aggregation (primary coagulation), and secondary coagulation.\(^1\) Consistently, several studies have demonstrated that folic acid ameliorates endothelial dysfunction and nitrate tolerance and can improve pathologic features of atherosclerosis.\(^2\) Folic acid also has a potent homocysteine lowering effect, as well as direct antioxidant and antiinflammatory effects.\(^3\) In fact, the potential mechanisms underlying the benefits of statins are similar and also include improved endothelial dysfunction and a positive effect on the fibrinolytic system and platelet function.\(^4\) Our findings should stimulate further investigation into the mechanisms underlying the interactive effect of folic acid and high TC on stroke. This line of further research may provide new insights into the pathogenesis of stroke and inform novel preventive and treatment strategies for stroke.

Inadequate folate intake is prevalent in most countries lacking mandatory folic acid fortification of foods, including Asia and other continents. In the CSPPT, a folic acid dose of 0.8 mg/d, the ceiling dose\(^5\) of folic acid supplementation in reducing stroke, was used. In the United States, after the introduction of folic acid fortification of food, total folic acid intake was only \(\approx 250 - 400 \mu g/d\) in women and \(300 - 420 \mu g/d\) in men.\(^6\) From 2003 to 2004, the median serum folate concentration in the United States was \(11.9 \text{ ng/mL}\) (versus \(19.9 \text{ ng/mL}\) in the CSPPT after treatment). Therefore, we speculate that even in countries such as the United States and Canada, where folic acid fortification and use of folic acid supplements are widespread, there may still be room to further reduce stroke risk by introducing a target-specific folic acid therapy for those individuals with hypercholesterolemia. Folic acid is attractive because it is safe and inexpensive. We think that our findings on the effects of folic acid supplementation on the risk of first stroke associated with hypercholesterolemia have implications for hypertensive adults across the globe.

This is a subanalysis of the CSPPT for the primary outcome. The systematic bias in treatment allocation was minimized by the randomized process. The observer bias in the assessment of first stroke was minimized by masking the treatment allocation from investigators, participants, and the independent end point adjudication committee. Random error was reduced by the reasonably large number of outcome events.

Several potential concerns or limitations are worth mentioning. First, one should note that subanalyses of randomized trials have inherent limitations, such as the possibility of residual imbalance in some unmeasured predictive factors at baseline. However, the distribution of important covariates was comparable between treatment groups within each baseline TC strata. Second, this study focused on the primary prevention of stroke in hypertensive adults; the generalizability of our findings to the secondary prevention of stroke or in populations with a high percent use of statins remains to be determined. Third, previous studies have reported that antiplatelet therapy possibly modifies the potential benefits of folic acid supplementation in the secondary prevention of vascular events.\(^7\) In the CSPPT study, only \(\approx 3\%\) of the participants were exposed to antiplatelet drugs; this number is too small for any meaningful analysis of the possible interaction between antiplatelet drugs and the effect of folic acid therapy on the primary prevention of stroke. Fourth, in the CSPPT, the stroke was not quantified using National Institute of Health Stroke Scale. Therefore, we could not evaluate the association between folic acid supplementation and risk of moderate-to-severe stroke. Another limitation of the CSPPT is the lack of classification of subtypes of ischemic stroke based on mechanisms such as the Trial of ORG 10172 in Acute Stroke Treatment classification. In addition, C-reactive protein was not measured at baseline and could not be included in the multivariable models as a potential confounder. Therefore, we cannot rule out the possibility of residual confounders. More importantly, statistical power was calculated for the main effect (the primary objective) of the CSPPT. All the subgroup analyses were exploratory without taking multiple testing into consideration. Therefore, our results are hypothesis generating. Confirmation of our findings in an independent population is essential.

Conclusions

Among hypertensive patients without a history of major cardiovascular diseases in China, elevated TC levels modified the benefits of folic acid supplementation on risk of first stroke. Folic acid supplementation reduced the risk of first stroke associated with elevated TC levels by 31%, independent of baseline folate levels and other important covariates. If confirmed by further studies, high TC may serve as an indicator for folic acid supplementation or higher folate intake in the primary prevention of stroke in hypertensive patients, particularly those who do not have access to statins (populations from low-income countries).

Sources of Funding

The trial was jointly supported by Shenzhen Ausa Pharmed Co Ltd and national, provincial, and private funding, including funding from the following: The Major State Basic Research Development Program of China (program 973; grant No. 2012 CB517703); the National Science and Technology Major Projects Specialized for Major New Drugs Innovation and Development during the 12th Five-Year Plan Period: China Stroke Primary Prevention Trial, grant No. zx090110105, Clinical Center Grant, grant No. zx09401013; the projects of National Natural Science Foundation of China (grant No. 81473052, 81441091, and 81402735); the National Clinical Research Center for Kidney Disease, Nanfang Hospital, Nanfang Medical University, Guangzhou, China; and research grants from the Department of Development and Reform, Shenzhen Municipal Government (grant No. SFG 20201744).
Disclosures

All authors have completed the ICMJE Form for Disclosure of Potential Conflicts of Interest. Dr. Huo reports grants from the National Major Scientific and Technological Special Project and non-financial support from Shenzhen Ausa. Dr. Qin reports grants from the National Science Foundation and consulting fees from Ausa Research Institute, Shenzhen Ausa. Dr. Spence reports personal fees from Bayer, Boehringer-Ingelheim, Pfizer. Dr. B. Wang reports grants from the Ministry of Science and Technology of the People’s Republic of China, and State Key Laboratory for Organ Failure Research, Guangzhou, China. The other authors report no conflicts.

References


Folic Acid Therapy Reduces the First Stroke Risk Associated With Hypercholesterolemia Among Hypertensive Patients

Xianhui Qin, Jianping Li, J. David Spence, Yan Zhang, Youbao Li, Xiaobin Wang, Binyan Wang, Ningling Sun, Fang Chen, Jingxuan Guo, Delu Yin, Liming Sun, Genfu Tang, Mingli He, Jia Fu, Yefeng Cai, Xiuli Shi, Ping Ye, Hong Chen, Shuiping Zhao, Mao Chen, Chuanyu Gao, Xiangqing Kong, Fan Fan Hou, Yining Huang and Yong Huo

Stroke. 2016;47:2805-2812; originally published online October 11, 2016;
doi: 10.1161/STROKEAHA.116.014578

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2016 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/47/11/2805

Data Supplement (unedited) at:
http://stroke.ahajournals.org/content/suppl/2016/10/11/STROKEAHA.116.014578.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org//subscriptions/
## Table I. Baseline characteristics of participants by baseline total cholesterol strata

<table>
<thead>
<tr>
<th></th>
<th>Total cholesterol &lt;200mg/dL</th>
<th>Total cholesterol ≥200mg/dL</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>8304</td>
<td>11862</td>
<td></td>
</tr>
<tr>
<td>Male, no. (%)</td>
<td>3879(46.7)</td>
<td>4360(36.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age—yr</td>
<td>60.1±7.7</td>
<td>59.9±7.4</td>
<td>0.029</td>
</tr>
<tr>
<td>Body-mass index—kg/m²</td>
<td>24.3±3.7</td>
<td>25.3±3.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Systolic blood pressure—mmHg</td>
<td>164.7±19.7</td>
<td>168.5±20.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic blood pressure—mmHg</td>
<td>92.8±11.9</td>
<td>94.9±11.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MTHFR C677T polymorphisms—no. (%)</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CC</td>
<td>2447(29.5)</td>
<td>3078(25.9)</td>
<td></td>
</tr>
<tr>
<td>CT</td>
<td>4052(48.8)</td>
<td>5847(49.3)</td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>1805(21.7)</td>
<td>2937(24.8)</td>
<td></td>
</tr>
<tr>
<td>Current Smoking</td>
<td>2115(25.5)</td>
<td>2620(22.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Current alcohol drinking</td>
<td>1920(23.1)</td>
<td>2904(24.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Physical activity</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>low</td>
<td>2665(32.1)</td>
<td>4662(39.4)</td>
<td></td>
</tr>
<tr>
<td>medium</td>
<td>3405(41.0)</td>
<td>4692(39.1)</td>
<td></td>
</tr>
<tr>
<td>high</td>
<td>2228(26.9)</td>
<td>2554(21.6)</td>
<td></td>
</tr>
<tr>
<td>Self-reported hyperlipidemia</td>
<td>166(2.0)</td>
<td>336(2.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Self-reported diabetes</td>
<td>217(2.6)</td>
<td>402(3.4)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

### Laboratory results

<table>
<thead>
<tr>
<th></th>
<th>Total cholesterol &lt;200mg/dL</th>
<th>Total cholesterol ≥200mg/dL</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglycerides—mg/dL</td>
<td>133.6±83.6</td>
<td>156.2±113.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL cholesterol—mg/dL</td>
<td>47.9±12.4</td>
<td>54.8±14.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fasting glucose—mmol/L</td>
<td>5.5±1.4</td>
<td>6.0±1.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Homocysteine—μmol/L†</td>
<td>(10.4, 15.5)</td>
<td>(10.5, 15.5)</td>
<td></td>
</tr>
<tr>
<td>Vitamin B12—pg/mL†</td>
<td>367.0</td>
<td>388.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Folate—ng/mL</td>
<td>8.3</td>
<td>8.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>(5.6, 10.9)</td>
<td>(5.6, 10.3)</td>
<td></td>
</tr>
</tbody>
</table>

### Medication use—no. (%)

<table>
<thead>
<tr>
<th></th>
<th>Total cholesterol &lt;200mg/dL</th>
<th>Total cholesterol ≥200mg/dL</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-hypertensive drugs</td>
<td>3753(45.2)</td>
<td>5469(46.1)</td>
<td>0.202</td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>791(9.5)</td>
<td>1037(8.7)</td>
<td>0.057</td>
</tr>
<tr>
<td>Angiotensin II–receptor blockers</td>
<td>6(0.1)</td>
<td>10(0.1)</td>
<td>0.765</td>
</tr>
<tr>
<td>Calcium channel blockers</td>
<td>975(11.7)</td>
<td>1039(8.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diuretics</td>
<td>131(1.6)</td>
<td>283(2.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>β-Blockers</td>
<td>69(0.8)</td>
<td>98(0.8)</td>
<td>0.971</td>
</tr>
<tr>
<td>Glucose-lowering drugs</td>
<td>99(1.2)</td>
<td>197(1.7)</td>
<td>0.007</td>
</tr>
<tr>
<td>Antiplatelet drugs</td>
<td>186(2.2)</td>
<td>369(3.1)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*For continuous variables, values are presented as mean ±SD; †Values are medians (25th, 75th percentile), Wilcoxon signed rank test was used. **Abbreviations**: ACE, angiotensin-converting enzyme; HDL, high density lipoproteins; MTHFR, methylenetetrahydrofolate reductase.
Table II. Interaction between folic acid therapy and total cholesterol or LDL cholesterol levels at baseline on first stroke and ischemic stroke

<table>
<thead>
<tr>
<th></th>
<th>Enalapril</th>
<th>Enalapril-folic acid</th>
<th>Absolute risk reduction (%)</th>
<th>HR (95%CI)</th>
<th>Adjusted* HR (95%CI)</th>
<th>Adjusted* P for interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Stroke</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;200</td>
<td>4187</td>
<td>109(2.6)</td>
<td>4117</td>
<td>102(2.5)</td>
<td>0.1</td>
<td>0.95(0.73-1.25)</td>
</tr>
<tr>
<td>&lt;177</td>
<td>2106</td>
<td>42(2.0)</td>
<td>2096</td>
<td>47(2.2)</td>
<td>-0.2</td>
<td>1.13(0.74-1.71)</td>
</tr>
<tr>
<td>177-&lt;200</td>
<td>2081</td>
<td>67(3.2)</td>
<td>2012</td>
<td>55(2.7)</td>
<td>0.5</td>
<td>0.84(0.59-1.20)</td>
</tr>
<tr>
<td>≥200</td>
<td>5899</td>
<td>237(4.0)</td>
<td>5963</td>
<td>163(2.7)</td>
<td>1.3</td>
<td>0.67(0.55-0.82)</td>
</tr>
<tr>
<td>200-&lt;240</td>
<td>3343</td>
<td>120(3.6)</td>
<td>3354</td>
<td>83(2.5)</td>
<td>1.1</td>
<td>0.68(0.52-0.90)</td>
</tr>
<tr>
<td>≥240</td>
<td>2556</td>
<td>117(4.6)</td>
<td>2609</td>
<td>80(3.1)</td>
<td>1.5</td>
<td>0.66(0.50-0.88)</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;100</td>
<td>1981</td>
<td>39(2.0)</td>
<td>2016</td>
<td>44(2.2)</td>
<td>-0.2</td>
<td>1.12(0.72-1.72)</td>
</tr>
<tr>
<td>≥100</td>
<td>8079</td>
<td>305(3.8)</td>
<td>8034</td>
<td>220(2.7)</td>
<td>1.1</td>
<td>0.72(0.61-0.86)</td>
</tr>
<tr>
<td>100-&lt;130</td>
<td>3212</td>
<td>99(3.1)</td>
<td>3099</td>
<td>72(2.3)</td>
<td>0.8</td>
<td>0.75(0.55-1.01)</td>
</tr>
<tr>
<td>130-&lt;160</td>
<td>2490</td>
<td>94(3.8)</td>
<td>2483</td>
<td>75(3.0)</td>
<td>0.8</td>
<td>0.79(0.59-1.08)</td>
</tr>
<tr>
<td>≥160</td>
<td>2377</td>
<td>112(4.7)</td>
<td>2452</td>
<td>73(3.0)</td>
<td>1.7</td>
<td>0.63(0.47-0.84)</td>
</tr>
<tr>
<td><strong>Ischemic stroke</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;200</td>
<td>4187</td>
<td>80(1.9)</td>
<td>4117</td>
<td>74(1.8)</td>
<td>0.1</td>
<td>0.94(0.69-1.29)</td>
</tr>
<tr>
<td>&lt;177</td>
<td>2106</td>
<td>29(1.4)</td>
<td>2096</td>
<td>34(1.6)</td>
<td>-0.2</td>
<td>1.19(0.72-1.95)</td>
</tr>
<tr>
<td>177-&lt;200</td>
<td>2081</td>
<td>51(2.5)</td>
<td>2021</td>
<td>40(2.0)</td>
<td>0.5</td>
<td>0.81(0.53-1.22)</td>
</tr>
<tr>
<td>≥200</td>
<td>5899</td>
<td>205(3.5)</td>
<td>5963</td>
<td>134(2.3)</td>
<td>1.2</td>
<td>0.64(0.52-0.80)</td>
</tr>
<tr>
<td>200-&lt;240</td>
<td>3343</td>
<td>103(3.1)</td>
<td>3354</td>
<td>65(1.9)</td>
<td>1.2</td>
<td>0.62(0.46-0.85)</td>
</tr>
<tr>
<td>≥240</td>
<td>2556</td>
<td>102(4.0)</td>
<td>2609</td>
<td>69(2.6)</td>
<td>1.4</td>
<td>0.66(0.48-0.89)</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;100</td>
<td>1981</td>
<td>25(1.3)</td>
<td>2016</td>
<td>30(1.5)</td>
<td>-0.2</td>
<td>1.19(0.70-2.02)</td>
</tr>
<tr>
<td>≥100</td>
<td>8079</td>
<td>259(3.2)</td>
<td>8034</td>
<td>177(2.2)</td>
<td>1.0</td>
<td>0.68(0.56-0.83)</td>
</tr>
<tr>
<td>100-&lt;130</td>
<td>3212</td>
<td>80(2.5)</td>
<td>3099</td>
<td>53(1.7)</td>
<td>0.8</td>
<td>0.68(0.48-0.97)</td>
</tr>
<tr>
<td>130-&lt;160</td>
<td>2490</td>
<td>81(3.3)</td>
<td>2483</td>
<td>63(2.5)</td>
<td>0.8</td>
<td>0.77(0.56-1.08)</td>
</tr>
<tr>
<td>≥160</td>
<td>2377</td>
<td>98(4.1)</td>
<td>2452</td>
<td>61(2.5)</td>
<td>1.6</td>
<td>0.60(0.43-0.82)</td>
</tr>
</tbody>
</table>

Adjusted for age, sex, MTHFR C677T genotypes, study centers, systolic blood pressure, and diastolic blood pressure at baseline, mean systolic blood pressure and diastolic blood pressure over the treatment period, body-mass index, baseline laboratory measurements for vitamin B12, folate, homocysteine, triglycerides, high density lipoproteins cholesterol, creatinine and glucose levels, and smoking status.

Abbreviation: LDL, low-density lipoprotein.
Table III. Interactions between folic acid therapy and total cholesterol levels at baseline on first stroke stratified by baseline characteristics

<table>
<thead>
<tr>
<th>Total cholesterol, mg/dL</th>
<th>Enalapril</th>
<th>Enalapril-folic acid</th>
<th>HR (95% CI)</th>
<th>Adjusted HR* (95% CI)</th>
<th>P* for interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age&lt;60 years</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;200</td>
<td>2084</td>
<td>43(2.1)</td>
<td>32(1.6)</td>
<td>0.77(0.49-1.22)</td>
<td>0.85(0.53-1.35)</td>
</tr>
<tr>
<td>≥200</td>
<td>3046</td>
<td>92(3.0)</td>
<td>62(2.0)</td>
<td>0.67(0.48-0.92)</td>
<td>0.75(0.54-1.05)</td>
</tr>
<tr>
<td>Age≥60 years</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;200</td>
<td>2103</td>
<td>66(3.1)</td>
<td>70(3.3)</td>
<td>1.06(0.76-1.49)</td>
<td>1.09(0.77-1.53)</td>
</tr>
<tr>
<td>≥200</td>
<td>2853</td>
<td>145(5.1)</td>
<td>101(3.5)</td>
<td>0.68(0.52-0.87)</td>
<td>0.66(0.51-0.86)</td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;200</td>
<td>1933</td>
<td>58(3.0)</td>
<td>52(2.7)</td>
<td>0.89(0.61-1.29)</td>
<td>1.00(0.68-1.47)</td>
</tr>
<tr>
<td>≥200</td>
<td>2198</td>
<td>117(5.3)</td>
<td>61(2.8)</td>
<td>0.52(0.38-0.71)</td>
<td>0.57(0.41-0.78)</td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;200</td>
<td>2254</td>
<td>51(2.3)</td>
<td>50(2.3)</td>
<td>1.02(0.69-1.51)</td>
<td>1.00(0.67-1.48)</td>
</tr>
<tr>
<td>≥200</td>
<td>3701</td>
<td>120(3.2)</td>
<td>102(2.7)</td>
<td>0.82(0.63-1.07)</td>
<td>0.76(0.60-1.02)</td>
</tr>
<tr>
<td>MTHFR 677 CC genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;200</td>
<td>1225</td>
<td>34(2.8)</td>
<td>31(2.5)</td>
<td>0.92(0.56-1.49)</td>
<td>0.84(0.51-1.38)</td>
</tr>
<tr>
<td>≥200</td>
<td>1535</td>
<td>64(4.2)</td>
<td>30(1.9)</td>
<td>0.46(0.30-0.71)</td>
<td>0.49(0.32-0.76)</td>
</tr>
<tr>
<td>MTHFR 677 CT genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;200</td>
<td>2046</td>
<td>48(2.4)</td>
<td>43(2.1)</td>
<td>0.92(0.61-1.38)</td>
<td>1.07(0.69-1.65)</td>
</tr>
<tr>
<td>≥200</td>
<td>2905</td>
<td>103(3.6)</td>
<td>92(3.1)</td>
<td>0.88(0.66-1.16)</td>
<td>0.92(0.69-1.22)</td>
</tr>
<tr>
<td>MTHFR 677 TT genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;200</td>
<td>916</td>
<td>27(3.0)</td>
<td>28(3.2)</td>
<td>1.07(0.63-1.82)</td>
<td>1.03(0.61-1.77)</td>
</tr>
<tr>
<td>≥200</td>
<td>1459</td>
<td>70(4.8)</td>
<td>41(2.8)</td>
<td>0.57(0.39-0.84)</td>
<td>0.55(0.37-0.81)</td>
</tr>
<tr>
<td>Folate &lt;8.1 ng/mL(median)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;200</td>
<td>2009</td>
<td>57(2.8)</td>
<td>48(2.5)</td>
<td>0.87(0.59-1.27)</td>
<td>0.87(0.59-1.29)</td>
</tr>
<tr>
<td>≥200</td>
<td>3002</td>
<td>143(4.8)</td>
<td>99(3.2)</td>
<td>0.67(0.52-0.87)</td>
<td>0.68(0.53-0.88)</td>
</tr>
<tr>
<td>Folate ≥8.1 ng/mL(median)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;200</td>
<td>2130</td>
<td>47(2.2)</td>
<td>53(2.5)</td>
<td>1.13(0.77-1.68)</td>
<td>1.14(0.77-1.70)</td>
</tr>
<tr>
<td>≥200</td>
<td>2860</td>
<td>93(3.3)</td>
<td>62(2.2)</td>
<td>0.66(0.48-0.91)</td>
<td>0.70(0.51-0.97)</td>
</tr>
<tr>
<td>Folate ≥9.6 ng/mL(highest tertile)</td>
<td>1487</td>
<td>34(2.3)</td>
<td>477</td>
<td>34(2.3)</td>
<td>1.00(0.62-1.62)</td>
</tr>
<tr>
<td>≥200</td>
<td>1889</td>
<td>61(3.2)</td>
<td>1815</td>
<td>40(2.2)</td>
<td>0.68(0.46-1.01)</td>
</tr>
<tr>
<td>Homocysteine&lt;12.5 μmol/L(median)</td>
<td>2142</td>
<td>50(2.3)</td>
<td>2048</td>
<td>41(2.0)</td>
<td>0.86(0.57-1.30)</td>
</tr>
<tr>
<td>≥200</td>
<td>2899</td>
<td>89(3.1)</td>
<td>3946</td>
<td>69(2.3)</td>
<td>0.76(0.55-1.04)</td>
</tr>
<tr>
<td>Homocysteine ≥12.5 μmol/L(median)</td>
<td>2044</td>
<td>59(2.9)</td>
<td>2124</td>
<td>61(3.0)</td>
<td>1.03(0.72-1.47)</td>
</tr>
<tr>
<td>≥200</td>
<td>2994</td>
<td>148(4.9)</td>
<td>3015</td>
<td>93(3.1)</td>
<td>0.62(0.48-0.80)</td>
</tr>
<tr>
<td>Vitamin B12 &lt;380 pg/mL(median)</td>
<td>2253</td>
<td>52(2.3)</td>
<td>2235</td>
<td>53(2.4)</td>
<td>1.03(0.70-1.51)</td>
</tr>
<tr>
<td>≥200</td>
<td>2751</td>
<td>126(4.6)</td>
<td>2771</td>
<td>74(2.7)</td>
<td>0.57(0.43-0.77)</td>
</tr>
<tr>
<td>Vitamin B12 ≥380 pg/mL(median)</td>
<td>1887</td>
<td>52(2.8)</td>
<td>1847</td>
<td>48(2.6)</td>
<td>0.95(0.64-1.40)</td>
</tr>
<tr>
<td>≥200</td>
<td>3111</td>
<td>110(3.5)</td>
<td>3139</td>
<td>87(2.8)</td>
<td>0.78(0.59-1.03)</td>
</tr>
</tbody>
</table>
### Adjusted, if not stratified, for age, sex, MTHFR C677T genotypes, study centers, systolic blood pressure, and diastolic blood pressure at baseline, mean systolic blood pressure and diastolic blood pressure over the treatment period, body-mass index, baseline laboratory measurements for vitamin B12, folate, homocysteine, triglycerides, high density lipoproteins cholesterol, creatinine and glucose levels, and smoking status.

### Participants with history of diabetes and/or under treatment for diabetes at baseline were classified as having diabetes.

**Abbreviation:** SBP, Systolic blood pressure; DBP, Diastolic blood pressure.
<table>
<thead>
<tr>
<th>LDL cholesterol, mg/dL</th>
<th>Enalapril</th>
<th>Enalapril-folic acid</th>
<th>HR (95% CI)</th>
<th>Adjusted HR* (95% CI)</th>
<th>P* for interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>n. (%)</td>
<td>Total</td>
<td>n. (%)</td>
<td></td>
</tr>
<tr>
<td>Age&lt;60 years</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;100</td>
<td>1017</td>
<td>21(2.1)</td>
<td>1011</td>
<td>18(1.8)</td>
<td>0.86(0.46-1.62)</td>
</tr>
<tr>
<td>≥100</td>
<td>4096</td>
<td>113(2.8)</td>
<td>4029</td>
<td>76(1.9)</td>
<td>0.68(0.51-0.91)</td>
</tr>
<tr>
<td>Age≥60 years</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;100</td>
<td>964</td>
<td>18(1.9)</td>
<td>1005</td>
<td>26(2.6)</td>
<td>1.40(0.77-2.56)</td>
</tr>
<tr>
<td>≥100</td>
<td>3983</td>
<td>192(4.8)</td>
<td>4005</td>
<td>144(3.6)</td>
<td>0.74(0.60-0.92)</td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;100</td>
<td>934</td>
<td>23(2.5)</td>
<td>981</td>
<td>17(1.7)</td>
<td>0.70(0.38-1.32)</td>
</tr>
<tr>
<td>≥100</td>
<td>3189</td>
<td>151(4.7)</td>
<td>3111</td>
<td>96(3.1)</td>
<td>0.64(0.50-0.83)</td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;100</td>
<td>1047</td>
<td>16(1.5)</td>
<td>1035</td>
<td>27(2.6)</td>
<td>1.79(0.93-3.21)</td>
</tr>
<tr>
<td>≥100</td>
<td>4890</td>
<td>154(3.2)</td>
<td>4923</td>
<td>124(2.5)</td>
<td>0.80(0.63-1.01)</td>
</tr>
<tr>
<td>MTHFR 677 CC genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;100</td>
<td>587</td>
<td>16(2.7)</td>
<td>591</td>
<td>16(2.7)</td>
<td>1.00(0.50-2.00)</td>
</tr>
<tr>
<td>≥100</td>
<td>2166</td>
<td>82(3.8)</td>
<td>2164</td>
<td>45(2.1)</td>
<td>0.54(0.38-0.78)</td>
</tr>
<tr>
<td>MTHFR 677 CT genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;100</td>
<td>984</td>
<td>18(1.8)</td>
<td>959</td>
<td>18(1.9)</td>
<td>1.03(0.54-1.99)</td>
</tr>
<tr>
<td>≥100</td>
<td>3951</td>
<td>131(3.3)</td>
<td>3977</td>
<td>116(2.9)</td>
<td>0.88(0.68-1.13)</td>
</tr>
<tr>
<td>MTHFR 677 TT genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;100</td>
<td>410</td>
<td>5(1.2)</td>
<td>466</td>
<td>10(2.2)</td>
<td>1.77(0.60-5.18)</td>
</tr>
<tr>
<td>≥100</td>
<td>1962</td>
<td>92(4.7)</td>
<td>1893</td>
<td>59(3.1)</td>
<td>0.66(0.48-0.91)</td>
</tr>
<tr>
<td>Folate &lt;8.1 ng/mL(median)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;100</td>
<td>896</td>
<td>16(1.8)</td>
<td>904</td>
<td>15(1.7)</td>
<td>0.94(0.46-1.89)</td>
</tr>
<tr>
<td>≥100</td>
<td>4103</td>
<td>183(4.5)</td>
<td>4090</td>
<td>131(3.2)</td>
<td>0.71(0.57-0.89)</td>
</tr>
<tr>
<td>Folate ≥8.1 ng/mL(median)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;100</td>
<td>1067</td>
<td>21(2.0)</td>
<td>1088</td>
<td>29(2.7)</td>
<td>1.36(0.78-2.39)</td>
</tr>
<tr>
<td>≥100</td>
<td>3909</td>
<td>118(3.0)</td>
<td>3879</td>
<td>86(2.2)</td>
<td>0.73(0.55-0.96)</td>
</tr>
<tr>
<td>Folate ≥9.6 ng/mL(the highest tertile)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;100</td>
<td>744</td>
<td>14(1.9)</td>
<td>768</td>
<td>21(2.7)</td>
<td>1.46(0.74-2.87)</td>
</tr>
<tr>
<td>≥100</td>
<td>2621</td>
<td>80(3.1)</td>
<td>2514</td>
<td>53(2.1)</td>
<td>0.69(0.48-0.97)</td>
</tr>
<tr>
<td>Homocysteine&lt;12.5µmol/L(median)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;100</td>
<td>1041</td>
<td>19(1.8)</td>
<td>1022</td>
<td>22(2.2)</td>
<td>1.19(0.64-2.19)</td>
</tr>
<tr>
<td>≥100</td>
<td>3978</td>
<td>119(3.0)</td>
<td>3958</td>
<td>87(2.2)</td>
<td>0.73(0.55-0.96)</td>
</tr>
<tr>
<td>Homocysteine ≥12.5 µmol/L(median)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;100</td>
<td>939</td>
<td>20(2.1)</td>
<td>994</td>
<td>22(2.2)</td>
<td>1.05(0.57-1.92)</td>
</tr>
<tr>
<td>≥100</td>
<td>4095</td>
<td>186(4.5)</td>
<td>4079</td>
<td>132(3.2)</td>
<td>0.71(0.56-0.88)</td>
</tr>
<tr>
<td>Vitamin B12 &lt;380 pg/mL(median)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;100</td>
<td>1068</td>
<td>17(1.6)</td>
<td>1088</td>
<td>21(1.9)</td>
<td>1.22(0.65-2.32)</td>
</tr>
<tr>
<td>≥100</td>
<td>3928</td>
<td>161(4.1)</td>
<td>3904</td>
<td>106(2.7)</td>
<td>0.65(0.51-0.84)</td>
</tr>
<tr>
<td>Vitamin B12 ≥380 pg/mL(median)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;100</td>
<td>896</td>
<td>20(2.2)</td>
<td>904</td>
<td>23(2.5)</td>
<td>1.15(0.63-2.09)</td>
</tr>
<tr>
<td>≥100</td>
<td>4084</td>
<td>140(3.4)</td>
<td>4066</td>
<td>111(2.7)</td>
<td>0.79(0.62-1.02)</td>
</tr>
</tbody>
</table>
**SBP < 160 mmHg**

<table>
<thead>
<tr>
<th></th>
<th>&lt;100</th>
<th>10(1.2)</th>
<th>868</th>
<th>12(1.4)</th>
<th>1.20(0.52-2.79)</th>
<th>1.46(0.61-3.48)</th>
<th>0.290</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥100</td>
<td>2896</td>
<td>61(2.1)</td>
<td>2960</td>
<td>54(1.8)</td>
<td>0.86(0.60-1.24)</td>
<td>0.87(0.60-1.26)</td>
<td></td>
</tr>
</tbody>
</table>

**SBP ≥ 160 mmHg**

<table>
<thead>
<tr>
<th></th>
<th>&lt;100</th>
<th>1118</th>
<th>29(2.6)</th>
<th>1148</th>
<th>32(2.8)</th>
<th>1.08(0.65-1.79)</th>
<th>1.25(0.74-2.10)</th>
<th>0.045</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥100</td>
<td>5183</td>
<td>244(4.7)</td>
<td>5074</td>
<td>166(3.3)</td>
<td>0.69(0.57-0.84)</td>
<td>0.70(0.57-0.85)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Mean SBP over the treatment period < 140 mmHg**

<table>
<thead>
<tr>
<th></th>
<th>&lt;100</th>
<th>1146</th>
<th>7(0.6)</th>
<th>1169</th>
<th>12(1.0)</th>
<th>1.69(0.66-4.29)</th>
<th>2.02(0.74-5.47)</th>
<th>0.051</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥100</td>
<td>4452</td>
<td>93(2.1)</td>
<td>4503</td>
<td>66(1.5)</td>
<td>0.70(0.51-0.96)</td>
<td>0.69(0.50-0.95)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Mean SBP over the treatment period ≥ 140 mmHg**

<table>
<thead>
<tr>
<th></th>
<th>&lt;100</th>
<th>32(3.8)</th>
<th>847</th>
<th>32(3.8)</th>
<th>0.99(0.61-1.62)</th>
<th>1.12(0.67-1.85)</th>
<th>0.181</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥100</td>
<td>3627</td>
<td>212(5.8)</td>
<td>3531</td>
<td>154(4.4)</td>
<td>0.74(0.60-0.91)</td>
<td>0.75(0.61-0.93)</td>
<td></td>
</tr>
</tbody>
</table>

**Mean SBP < 140 mmHg and mean DBP < 90 mmHg over the treatment period**

<table>
<thead>
<tr>
<th></th>
<th>&lt;100</th>
<th>1051</th>
<th>6(0.6)</th>
<th>1090</th>
<th>11(1.0)</th>
<th>1.77(0.66-4.79)</th>
<th>1.77(0.64-4.88)</th>
<th>0.078</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥100</td>
<td>4162</td>
<td>87(2.1)</td>
<td>4197</td>
<td>60(1.4)</td>
<td>0.68(0.49-0.95)</td>
<td>0.67(0.48-0.93)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Body-mass index < 25 kg/m²**

<table>
<thead>
<tr>
<th></th>
<th>&lt;100</th>
<th>1224</th>
<th>22(1.8)</th>
<th>1234</th>
<th>22(1.8)</th>
<th>0.99(0.55-1.79)</th>
<th>1.10(0.60-2.02)</th>
<th>0.150</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥100</td>
<td>4138</td>
<td>156(3.7)</td>
<td>4099</td>
<td>100(2.4)</td>
<td>0.64(0.50-0.82)</td>
<td>0.65(0.51-0.84)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Body-mass index ≥ 25 kg/m²**

<table>
<thead>
<tr>
<th></th>
<th>&lt;100</th>
<th>756</th>
<th>17(2.3)</th>
<th>782</th>
<th>22(2.8)</th>
<th>1.27(0.67-2.39)</th>
<th>1.56(0.80-3.06)</th>
<th>0.070</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥100</td>
<td>3940</td>
<td>149(3.8)</td>
<td>3929</td>
<td>120(3.1)</td>
<td>0.80(0.63-1.02)</td>
<td>0.80(0.63-1.03)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Triglycerides < 150 mg/dL**

<table>
<thead>
<tr>
<th></th>
<th>&lt;100</th>
<th>1256</th>
<th>25(2.0)</th>
<th>1266</th>
<th>28(2.2)</th>
<th>1.12(0.65-1.92)</th>
<th>1.33(0.75-2.34)</th>
<th>0.088</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥100</td>
<td>5174</td>
<td>183(3.5)</td>
<td>5219</td>
<td>146(2.8)</td>
<td>0.79(0.63-0.98)</td>
<td>0.78(0.63-0.98)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Triglycerides ≥ 1.7 mg/dL**

<table>
<thead>
<tr>
<th></th>
<th>&lt;100</th>
<th>725</th>
<th>14(1.9)</th>
<th>750</th>
<th>16(2.1)</th>
<th>1.11(0.54-2.28)</th>
<th>1.22(0.59-2.53)</th>
<th>0.139</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥100</td>
<td>2905</td>
<td>122(4.2)</td>
<td>2815</td>
<td>74(2.6)</td>
<td>0.62(0.46-0.83)</td>
<td>0.65(0.49-0.87)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Fasting glucose < 7.0 mmol/L**

<table>
<thead>
<tr>
<th></th>
<th>&lt;100</th>
<th>1847</th>
<th>33(1.8)</th>
<th>1862</th>
<th>40(2.2)</th>
<th>1.21(0.76-1.92)</th>
<th>1.32(0.82-2.12)</th>
<th>0.030</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥100</td>
<td>7086</td>
<td>243(3.4)</td>
<td>7082</td>
<td>179(2.5)</td>
<td>0.73(0.60-0.89)</td>
<td>0.76(0.62-0.92)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Fasting glucose ≥ 5.6 & < 7.0 mmol/L**

<table>
<thead>
<tr>
<th></th>
<th>&lt;100</th>
<th>473</th>
<th>9(1.9)</th>
<th>478</th>
<th>10(2.1)</th>
<th>1.12(0.46-2.76)</th>
<th>1.27(0.46-3.50)</th>
<th>0.222</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥100</td>
<td>2783</td>
<td>99(3.6)</td>
<td>2743</td>
<td>67(2.4)</td>
<td>0.68(0.50-0.93)</td>
<td>0.70(0.51-0.96)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Fasting glucose ≥ 7.0 mmol/L or Diabetes†**

<table>
<thead>
<tr>
<th></th>
<th>&lt;100</th>
<th>134</th>
<th>6(4.5)</th>
<th>154</th>
<th>4(2.6)</th>
<th>0.57(0.16-2.03)</th>
<th>0.89(0.21-3.80)</th>
<th>0.761</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥100</td>
<td>993</td>
<td>62(6.2)</td>
<td>950</td>
<td>41(4.3)</td>
<td>0.69(0.47-1.03)</td>
<td>0.65(0.43-0.97)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---

*Adjusted, if not stratified, for age, sex, MTHFR C677T genotypes, study centers, systolic blood pressure, and diastolic blood pressure at baseline, mean systolic blood pressure and diastolic blood pressure over the treatment period, body-mass index, baseline laboratory measurements for vitamin B12, folate, homocysteine, triglycerides, high density lipoproteins cholesterol, creatinine and glucose levels, and smoking status.

†Participants with history of diabetes and/or under hypoglycemic treatment at baseline were classified as having diabetes.

**Abbreviation:** LDL, low-density lipoprotein; SBP, Systolic blood pressure; DBP, Diastolic blood pressure.
Figure I. The flow chart of the participants
Detailed Methods

This study was approved by the Ethics Committee of the Institute of Biomedicine, Anhui Medical University, Hefei, China (FWA assurance number: FWA00001263) and registered with ClinicalTrials.gov, NCT00794885. All participants gave prior written informed consent.

Participants

Briefly, the China Stroke Primary Prevention Trial (CSPPT) was a multi-community, randomized, double-blind, controlled trial conducted from May 19, 2008 to August 24, 2013 in 32 communities in China. Eligible participants were men and women aged 45-75 years with hypertension, defined as seated resting systolic blood pressure (SBP) ≥140 mmHg or diastolic blood pressure (DBP) ≥90 mmHg at both the screening and recruitment visit or, who were taking antihypertensive medication. The major exclusion criteria included history of physician-diagnosed stroke, myocardial infarction (MI), heart failure, post-coronary revascularization, and/or congenital heart disease.

Procedures

Eligible participants were randomly assigned, in a 1:1 ratio, to one of two treatment groups: a daily oral dose of one tablet containing 10mg enalapril and 0.8mg folic acid (the enalapril-folic acid group), or a daily oral dose of one tablet containing 10mg enalapril only (the enalapril only group). During the trial period, B-vitamin supplements were not allowed, however concomitant use of other antihypertensive drugs (mainly calcium channel blockers or diuretics) was allowed.

Seated blood pressure measurements were obtained by trained research staff after the patients had been seated for 10 minutes using a mercury manometer, and using the standard method and appropriately sized cuffs. Triplicate measurements on the same
arm were taken, with at least 2 min between readings. The mean SBP and DBP of the three independent measures were used in analysis.

Participants were followed up every three months. At each visit, the blood pressures were measured, the numbers of pills taken between visits were counted, and concomitant medications and adverse events were recorded.

Outcomes

The primary outcome was a first nonfatal or fatal stroke (ischemic or hemorrhagic), excluding subarachnoid hemorrhage and silent stroke. The secondary outcomes included first ischemic stroke (fatal or non-fatal); first hemorrhagic stroke (fatal or non-fatal); and a composite of cardiovascular events consisting of cardiovascular death, MI and stroke.

All outcomes were reviewed and adjudicated by an independent end-point adjudication committee whose members were unaware of study group assignments.

Statistical Analysis

Means (standard deviation) or medians (25\textsuperscript{th} percentile, 75\textsuperscript{th} percentile) and proportions were calculated for population characteristics by the treatment groups in accordance with baseline TC strata \{\geq 5.2 \text{ versus} <5.2 (desirable levels) mmol/L\}. The differences in population characteristics were compared using two-sample t-tests, signed rank tests, or chi-square tests, accordingly. Participants with a history of diabetes and/or under treatment for diabetes at baseline were classified as having diabetes. Low-density lipoprotein cholesterol (LDL-C) was estimated by the Friedewald formula as follows: LDL-C, mmol/L= TC-HDL-C (high density lipoprotein cholesterol)-[triglycerides (TG) *0.45].

The efficacy analyses were conducted according to the intention-to-treat principle. The hazard ratios (HRs) and 95% confidence intervals (CI) for the risk of the primary
outcome associated with elevated TC levels (≥5.2 versus <5.2 mmol/L) were estimated using Cox proportional hazards models stratified by treatment groups with adjustment for age, sex, MTHFR (methylene-tetrahydrofolate reductase) C677T genotypes, study centers, SBP and DBP at baseline, mean SBP and DBP over the treatment period, body mass index (BMI), baseline laboratory measurements for vitamin B12, folate, homocysteine, TG, HDL-C, creatinine and glucose levels, and smoking status.

We further assessed the effect of folic acid supplementation on the prevention of study outcomes according to different TC strata, as well as assessed the interaction between baseline TC strata and folic acid therapy on the study outcomes by means of Cox proportional hazards regression both before and after adjustment for the above covariables. Interactions were examined by including interaction terms in the Cox models. A sensitivity analysis with a composite outcome consisting of the primary outcome and all-cause death was also performed. We also assessed the consistency of the interaction effect in different subgroups of participants. A similar approach was applied to the interaction between baseline LDL-C (≥2.6 versus <2.6 (optimal levels) mmol/L) strata and folic acid therapy on the primary outcome.

A two-tailed $P<0.05$ was considered to be statistically significant in all analyses. R software, version 3.2.0 (http://www.R-project.org/) was used for all statistical analyses.