Efficacy of folic acid and enalapril combined therapy on reduction of blood pressure and plasma glucose: A multicenter, randomized, double-blind, parallel-controlled, clinical trial

Guangyun Mao, M.D., Ph.D.\textsuperscript{a}, Xiumei Hong, Ph.D.\textsuperscript{b}, Houxun Xing, M.D.\textsuperscript{a}, Ping Liu, M.D., Ph.D.\textsuperscript{a}, Haipeng Liu, M.S.\textsuperscript{c}, Yunxian Yu, M.D., Ph.D.\textsuperscript{b}, Shanchun Zhang, M.D., Ph.D.\textsuperscript{b}, Shanqun Jiang, Ph.D.\textsuperscript{a}, Xiaobin Wang, M.D., Sc.D.\textsuperscript{b}, and Xiping Xu, M.D., Ph.D.\textsuperscript{a,d,*}, on behalf of the entire study group\textsuperscript{†}

\textsuperscript{a} Anhui Biomedical Institute, Anhui Medical University, Hefei, China
\textsuperscript{b} Mary Ann and J. Milburn Smith Child Health Research Program, Children’s Memorial Hospital and Children’s Memorial Research Center, Northwestern University Feinberg School of Medicine, Chicago, Illinois, USA
\textsuperscript{c} School of Life Science, University of Science and Technology of China, Hefei, China
\textsuperscript{d} Division of Epidemiology and Biostatistics, University of Illinois at Chicago School of Public Health, Chicago, Illinois, USA

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Abstract

Objective: We compared the efficacy of folic acid (FA) plus enalapril with enalapril alone on the reduction of blood pressure (BP) and fasting plasma glucose (FPG) in adult Chinese hypertensive patients.

Methods: Four hundred eighty subjects with mild to moderate BP were randomly assigned to one of three treatment groups: 1) 10 mg of enalapril (control group), 2) 10 mg of enalapril plus 0.4 mg of FA (low-FA group), or 3) 10 mg of enalapril plus 0.8 mg of FA (high-FA group) daily for 8 wk. Generalized linear mixed models were used to compare the reduction in BP and FPG level from baseline to week 8 of the treatment and the difference among the three treatment groups, adjusting for pertinent covariates.

Results: Four hundred forty-three subjects (57.3% women, 27–75 y of age) successfully completed the trial. After the 8-wk treatment, compared with baseline, all treatment groups showed significant reduction of BP but not of FPG. There was no significant difference in BP or FPG reduction among the three treatment groups. In subgroup analysis, we found that in subjects with hyperglycemia (FPG \(\geq 6.1 \text{ mmol/L} \)) at baseline, FPG reduction was significantly greater in the high-FA group (\(-0.80 \pm 1.20 \text{ mmol/L} \)) than in the low-FA group (\(-0.39 \pm 1.44 \text{ mmol/L} \)) and the control group (\(-0.23 \pm 1.30 \text{ mmol/L} \)). Regression analysis further confirmed that FPG reduction in the high-FA group was \(0.68 \pm 0.28 \text{ mmol/L} \) greater than in the control group (\(P = 0.015 \)), even after adjustment for important covariates. A dose–response trend was evident (\(P \text{ for trend} = 0.025 \)) and the test for an interaction between treatment group and baseline FPG was significant (\(P < 0.001 \)).

Conclusion: In this sample of adult Chinese hypertensive patients, FA combined with enalapril showed a greater beneficial effect on reduction of FPG in a dose-related fashion than did enalapril alone among subjects with hyperglycemia. © 2008 Elsevier Inc. All rights reserved.

Keywords: Enalapril; Folic acid; Hyperglycemia; Hypertension; Randomized clinical trial; Chinese

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* Corresponding author. Tel.: +312-413-8934; fax: +312-413-0431.

E-mail address: xipingxu18@gmail.com (X. Xu).

† The study group also includes the following individuals who had made important contributions to this trial at the stage of study design, subject recruitment, and data collection: Jianping Li, M.D., Ph.D. and Yong Huo, M.D., Ph.D. at First Hospital of Peking University, Beijing, China; Deming Guan, M.D., Ph.D. at First affiliated hospital of Ha’erbin Medical University, Ha’erbin, China; Junbo Ge, M.D., Ph.D. at Zhongshan Hospital of Fudan University, Shanghai, China; Jian Hu, M.D., Ph.D. at First affiliated hospital of Chinese Medical University, Shenyang, China; Yanni Wang, M.D., Ph.D. at First hospital of Xi’an Jiaotong University, Xi’an, China; Fuming Zhang, M.D., Ph.D. at First affiliated hospital of Nanjing Medical University, Nanjing, China.

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Introduction

The numbers of hypertensive and diabetic patients are rising at an alarming rate worldwide [1]. Hypertension and type 2 diabetes (T2D) are commonly associated conditions; 2.4% of hypertensive patients had T2D, and at least 40% patients with T2D had hypertension [2]. Hypertension and T2D each can increase the risk of stroke, and the combination of T2D and hypertension can dramatically increase the risk of stroke. It was reported that subjects with hypertension and T2D were 4.5 times more likely to have a stroke, and if they did have a stroke, they were >9 times more likely to die from it than those with neither disease [2]. Given the significant comorbidity and health consequences of hypertension and T2D, it is of great clinical and public health importance to develop and evaluate therapy that can effectively control hypertension and reduce the risk of T2D.

Among the commonly used antihypertensive agents, angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin-receptor blockers [3], both of which act on angiotensin-converting enzyme systems, may be protective against the development of diabetes [3–7]. Studies have suggested that ACEI can slow nephropathy progression and significantly increase regression to normoglycemia [3]. It is recommended that ACEI be the primary antihypertensive drug for patients with essential hypertension and diabetes [8]. Of note, not all studies have shown consistent results [9].

Among the numerous nutritional factors, folic acid (FA) and homocysteine (Hcy) have received great attention. Elevated Hcy has been associated with cardiovascular diseases [10,11] and diabetes [12,13]. Studies have suggested that Hcy may cause endothelial cell desquamation, oxidation of low-density lipoproteins, impairment of vascular responsiveness, or enhancement of monocyte adhesion. A recent meta-analysis showed a significant beneficial effect of FA supplementation on primary prevention of stroke [14]. Another study also found that Hcy thiolactone, the main circulating metabolite of Hcy, can inhibit the insulin receptor tyrosine kinase, phosphorylation of insulin receptor substrates, and insulin-stimulated phosphoinositide-3 kinase activity, thus leading to an inhibitory effect on insulin-mediated glycogen synthesis in hepatoma cells and expression of the human insulin receptor [15]. Conceivably, reduction in Hcy level is likely to have a beneficial effect on glucose metabolism. It has been well demonstrated that Hcy level can be effectively lowered by FA treatment [16]. A meta-analysis found that 0.8 mg was the lowest daily dose of FA associated with the maximal reduction in Hcy concentrations [17].

Given the potential beneficial effects of ACEI and FA on cardiovascular outcomes and glucose metabolism, we hypothesized that ACEI plus FA would confer a greater beneficial effect on blood pressure (BP) and plasma glucose than ACEI alone among patients with essential hypertension. The primary aim of this randomized controlled trial was to evaluate the efficacy of FA plus enalapril compared with enalapril alone on lowering BP and fasting plasma glucose (FPG) in adult Chinese hypertensive patients. We were also interested in exploring if there is any difference in treatment effect between those subjects with an elevated FPG and those without at baseline.

Materials and methods

Study subjects

Four hundred eighty patients with mild to moderate primary hypertension, 27–75 y of age, were recruited for the Antihypertensive and Homocysteine-Lowering Treatment Trial (clinicaltrials.gov; identifier: NCT00520247) from six Chinese university-affiliated hospitals between September 2005 and December 2005. These hospitals were clinical pharmacology study centers in China. The inclusion criteria of the study subjects were 1) an age ≥18 and <75 y, 2) a seated systolic BP (SBP) ≥140 mmHg and <180 mmHg and/or a seated diastolic BP (DBP) ≥90 mmHg and <110 mmHg, 3) women of reproductive age who agreed to use a reliable contraception method during the study, and 4) those who had not taken any medications or nutritional supplements in the week before the study. Subjects were excluded if they had chronic or present illness including severe chronic heart failure or heart failure within the past 3 mo, renal dysfunction (plasma creatinine level ≥200 μmol/L), coronary heart disease, myocardial infarction, acute coronary syndrome, severe cardiac arrhythmia, unstable angina, stroke, and hyperkalemia. In addition, subjects who were pregnant or planning to become pregnant during the study, had impaired liver function, tumor, or planned to move out of the area within the study period were excluded. The protocol was approved by the ethics committee of Peking University First Hospital, Beijing, China. The purpose and procedure of the study were carefully explained to all participants, and informed consent was obtained from all subjects.

Randomization and double blinding

After informed consent, each participant was randomly and double-blindly assigned to one of three treatment groups: 1) 10 mg of enalapril (control group), 2) 10 mg of enalapril plus 0.4 mg of FA (low-FA group), or 3) 10 mg of enalapril plus 0.8 mg of FA (high-FA group) daily for 8 wk, respectively. To achieve a balance among the three treatment groups, permuted block randomization (with a block size randomly selected as six, performed using clinical data analysis software, cDAS [version 2.0, Anhui, China]). The allocation of participants was programmed by the statistical coordinating center, encrypted, and sent to each study center. Allocation information was accessible only to staffs in the drug distribution center, for distribution of the drugs to each study center. All pills were manufactured to be indistinguishable by external color and appearance by the
Tianshili Pharmaceutical Company (Tianjin, China). No request was ever made to break the blind.

Data collection procedures

All subjects were asked to fast overnight for at least 10 h before the clinical examinations. Baseline data collection was conducted by trained research staff according to the standard operating procedure on the first morning of the study. Each participant was interviewed with a standardized questionnaire designed specifically for the study to collect information on birth date, medical history, vitamin use, and family history of hypertension, diabetes, myocardial infarction, and premature coronary heart disease. Height was measured without shoes to the nearest 0.1 cm on a portable stadiometer. Weight was measured in light indoor clothing without shoes to the nearest 0.1 kg. Body mass index (BMI) was calculated as weight (kilograms)/height (meters) squared.

All six study centers measured BP according to the standard operating procedure and used the same type of mercury column sphygmomanometer with the standard method of calibration. Seated BPs of all participants at baseline and weeks 2, 4, 6, and 8 were measured at 0800–1000 h. The participant was asked to rest for at least 15 min before BP measurement. BP was measured three times at the right arm and the mean of three readings was used for analysis. The first and fifth Korotkoff sounds were taken as indicative of SBP and DBP, respectively. The BP measurements of each participant at different time points (baseline and weeks 2, 4, and 6) were performed by the same research staff.

Laboratory tests

After 10–12 h of fasting a venous blood sample was obtained from each study participant with tubes containing ethylene-diaminetetra-acetic acid (for plasma) or none (for serum) at baseline and days 28 and 56 of the study. Plasma or serum samples were separated within 15 min of collection, which were analyzed within 30 min or stored at −80°C in a freezer. Blood samples collected at baseline and on day 56 used for measurement of FPG, serum lipids including total cholesterol, high-density lipoprotein and triglycerides, creatinine, serum urea nitrogen, alanine aminotransferase, aspartate aminotransferase, and serum Hcy and FA level. All these tests were performed in the six study center laboratories, using standard reagents and an automatic biochemistry analyzer. Low-density lipoprotein was calculated by Friedewald’s equation. Plasma total Hcy (tHcy) was determined by high-performance liquid chromatography [18] in duplicate by well-trained technicians in the central laboratory. The intra- and interassay coefficients of variation were 3.5% and 4.2%, respectively. Serum folate was determined in duplicate of frozen specimens by chemiluminescent immunoassay using a Beckman Coulter ACCESS Immunoassay System (Beckman-Coulter Canada, Inc., Mississauga, Canada). The intra- and interassay coefficients of variations were 2.3% and 3.7%, respectively.

Follow-up of study subjects

All eligible participants were followed for a total of 8 wk. Face-to-face interviews were conducted at baseline and weeks 2, 4, 6, and 8 of the study. The subjects were asked to maintain their regular dietary habits and not to take any vitamin B pills or any other antihypertensive medications during the entire study duration. Compliance evaluation (the extent to which the participants followed the study procedure including medication and diet) was performed at weeks 2, 4, 6, and 8. On day 56, all participants were asked to return to the study centers to complete the same study procedure as at baseline. “Lost to follow-up” defined those subjects who did not complete the entire study procedure.

Statistical analysis

Double data entry was performed using Epidata (version 3.1, Denmark) in the statistical coordinating center. Data cleaning was completed before unblinding, including verification of the completeness and plausibility of the source data, according to good clinical practice guidelines for clinical trials. Normality of data distribution was assessed with the Kolmogorov-Smirnov test before statistical analyses. Total Hcy and FA levels were positively skewed and thus natural log-transformed to normalize the distribution. One-way analysis of variance for continuous variables and chi-square test (or Fisher’s exact test) for categorical variables were applied to compare the characteristics of the study subjects among the three treatment groups. The Kruskal-Wallis rank-sum test was also used to compare tHcy and FA levels among the three treatment groups.

The primary outcomes of interest were changes in BP and FPG level in response to treatment. Generalized linear mixed models were applied to compare changes in BP and FPG level from baseline to the end of study and the difference among the three treatment groups, with the adjustment of corresponding baseline levels, age, gender, BMI, plasma tHcy, serum folate, and research center. We also performed subgroup analysis, stratified by baseline FPG level (<6.1 versus ≥6.1 mmol/L). All tests were two-sided and P < 0.05 was set as the significant level. Data management and all statistical analyses were performed using SAS 6.12 (SAS Institute, Cary, NC, USA) and Sigmaplot 10.0 for Windows (SYSTAT Software Inc., Richmond, CA, USA).

Results

Characteristics of study subjects by treatment groups

Figure 1 illustrates the flow of this study. A total of 480 eligible patients with mild to moderate essential hyperten-
sion recruited from the six study centers in China were randomly assigned to one of the three treatment groups. This analysis excluded 37 individuals who were lost to follow-up or did not comply with the study protocol. The demographic and clinical characteristics of study subjects in each treatment group are shown in Table 1. Of 443 subjects (57.3% women, 27–75 y of age), 98.7% were Han nationality with a mean BMI of 25.7 kg/m². Participants in each of the three groups were well balanced at baseline with regard to relevant demographic and clinical characteristics.

Of the 443 study subjects, only 8 (1.8%) reported a history of diabetes. Using an FPG level <6.1 mmol/L as the cutoff (according to the American Diabetes Association, 1997), 91 (20.5%) of the total sample, including 32 (21.5%) in control group, 31 (21.2%) in the low-FA group, and 28 (18.9%) in the high-FA group, had impaired fasting glucose at baseline. The proportions of subjects with impaired fasting glucose were well balanced among the three treatment groups (P > 0.05).

We also compared the characteristics of two subgroups: subjects with a baseline FPG level <6.1 mmol/L and subjects with a baseline FPG level ≥6.1 mmol/L. The subjects with hyperglycemia were older (60.9 ± 9.5 versus 56.1 ± 9.7 y, P < 0.01), had greater BMI (26.5 ± 3.4 versus 25.5 ± 3.3 kg/m², P = 0.013), higher SBP (156.1 ± 10.9 versus 153.3 ± 11.1 mmHg, P = 0.035), and higher triacylglycerol levels (2.0 ± 1.4 versus 1.7 ± 1.2 mmol/L, P = 0.035) compared with the subjects with a normal FPG level.

Table 2 presents SBP, DBP, plasma tHcy, and serum FA levels at baseline and on day 56 of treatment and their

Table 1
Demographic and clinical characteristics of study subjects*

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Control group† (n = 149)</th>
<th>Low-FA group† (n = 146)</th>
<th>High-FA group† (n = 148)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>57.3 ± 10.0</td>
<td>57.4 ± 10.0</td>
<td>56.6 ± 9.6</td>
</tr>
<tr>
<td>Men</td>
<td>57 (38.3)</td>
<td>63 (43.2)</td>
<td>69 (46.6)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.7 ± 3.2</td>
<td>25.5 ± 3.3</td>
<td>25.8 ± 3.6</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>153.9 ± 11.1</td>
<td>153.4 ± 10.9</td>
<td>154.4 ± 11.3</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>93.4 ± 8.1</td>
<td>92.4 ± 8.5</td>
<td>93.4 ± 8.1</td>
</tr>
<tr>
<td>FPG (mmol/L)</td>
<td>4.9 ± 0.9</td>
<td>5.1 ± 1.3</td>
<td>5.0 ± 1.1</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>1.7 ± 1.3</td>
<td>1.7 ± 1.1</td>
<td>1.8 ± 1.2</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>1.3 ± 0.4</td>
<td>1.3 ± 0.4</td>
<td>1.3 ± 0.4</td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>2.8 ± 0.7</td>
<td>2.9 ± 0.8</td>
<td>3.0 ± 0.9</td>
</tr>
<tr>
<td>Creatinine (μmol/L)</td>
<td>68.4 ± 17.1</td>
<td>70.3 ± 18.7</td>
<td>70.2 ± 17.2</td>
</tr>
<tr>
<td>tHcy (μmol/L)‡</td>
<td>12.7 ± 1.6</td>
<td>13.4 ± 1.6</td>
<td>13.6 ± 1.6</td>
</tr>
<tr>
<td>Folate (nmol/L)‡</td>
<td>12.7 ± 1.4</td>
<td>12.6 ± 1.5</td>
<td>12.5 ± 1.5</td>
</tr>
<tr>
<td>History of diabetes</td>
<td>2 (1.3)</td>
<td>1 (0.7)</td>
<td>5 (3.4)</td>
</tr>
<tr>
<td>IFG</td>
<td>32 (21.5)</td>
<td>31 (21.2)</td>
<td>28 (18.9)</td>
</tr>
</tbody>
</table>

* Data are presented as mean ± SD or number of subjects (percentage).
† The control group received 10.0 mg of enalapril daily for 8 wk; the low-FA group received 10.0 mg of enalapril plus 0.4 mg of FA daily for 8 wk; and the high-FA group received 10.0 mg of enalapril plus 0.8 mg of FA daily for 8 wk.
‡ Log-transformed before analysis; geometric means ± anti-log SDs are presented.
changes from baseline to the end of the treatment. The mean decreases in SBP were 10.5 ± 13.9, 11.1 ± 14.3, and 11.2 ± 16.8 mmHg for the control, low-FA, and high-FA groups, respectively. The mean decreases in DBP were 5.7 ± 9.3, 5.6 ± 10.1 mmHg for the control, low-FA, and high-FA groups, respectively. The decrease in DBP and DBP between baseline and the end of the treatment was statistically significant. However, no significant difference in SBP or DBP reduction was found among the three treatment groups.

The medians (quartiles 1–3) of tHcy change were 0.0 μmol/L (−1.2 to 1.4), −1.7 μmol/L (−3.3 to 0.1), and −1.9 μmol/L (−3.9 to −0.3), respectively, for the control, low-FA, and high-FA groups. The degree of tHcy reduction after the 8-wk treatment was significantly different among the three treatment groups (P < 0.01), with the greatest reduction found in the high-FA group.

The medians (quartiles 1–3) of FPG change were 1.20 mmol/L (0.0 to 1.4), 1.64 mmol/L (0.0 to 1.4), and 1.44 mmol/L (0.0 to 1.4), respectively, for the control, low-FA, and high-FA groups. The degree of FPG reduction after the 8-wk treatment was significantly different among the three treatment groups (P < 0.01), with the greatest reduction found in the high-FA group.

Treatment effect on FPG

Table 3 presents FPG at baseline and on day 56 of the treatment and their changes from baseline to the end of the treatment for the total sample and for the subgroups defined by baseline FPG level. Among the total sample and among subjects with a baseline FPG level <6.1 mmol/L, there was no significant change in FPG from baseline to the end of the treatment or any significant difference in FPG change among the three treatment groups. Interestingly, among subjects with a baseline FPG level ≥6.1 mmol/L, the FPG reduction from baseline to day 56 of the treatment achieved statistical significance, with means ± SDs of 0.39 ± 1.44 mmol/L (P < 0.05) in the low-FA group and 0.80 ± 1.20 mmol/L (P < 0.01) in the high-FA group.

No significant reduction in FPG was observed in the control group. Furthermore, as shown in Figure 2, the magnitude of reduction in FPG differed among the three treatment groups, with the greatest reduction observed in the high-FA group and the least reduction in the control group. The difference was statistically significant between the high-FA group and the control group (P < 0.05).

To eliminate the effects of potential confounding factors on the FPG change and on the above observed difference
among treatment groups, generalized linear mixed models were used to adjust for important covariates. Table 4 presents results from the two models, with model 1 adjusting for baseline FPG level, age, gender, BMI, baseline SBP, and triacylglycerol and model 2 further adjusting for baseline DBP, serum creatinine, tHcy, tHcy change, and study centers, in addition to all the variables in model 1. The results were consistent with those presented in Table 3 and Figure 2. Our data also suggested a dose–response relation between the treatment groups and FPG reduction among the subjects with baseline hyperglycemia (*P_trend test/11021/0.025). We tested the interaction by including the treatment groups (low-FA, high-FA), baseline FPG (hyperglycemia), and interaction term (high-FA by hyperglycemia) in the model, along with age, gender, and BMI. The SE for the interaction term was −0.9 ± 0.2 (P < 0.001), which suggested that folate (0.8 mg/d) plus enalapril treatment was particularly efficacious in lowering the FPG level in subjects with hyperglycemia.

Discussion

Hypertension and T2D mellitus have emerged as major health problems in developing countries such as China [19] and are often coexistent. It is of great clinical and public health importance to develop and evaluate therapy that can effectively control hypertension and reduce the risk of T2D. Consistent with previous reports and clinical observations, this multicenter, double-blind, randomized, parallel, controlled clinical trial showed that an ACEI (enalapril) is an effective antihypertensive agent for mildly to moderately hypertensive patients in the Chinese population. Although we did not detect any significant difference in the efficacy of BP lowering and FPG lowering among the three treatment groups, we found, for the first time that, in hypertensive patients with hyperglycemia (FPG ≥6.1 mmol/L), a combined therapy of FA and enalapril significantly decreased FPG levels. Such an effect appeared to be dose-related, with the greatest effect in the high-FA group (0.8 mg) and the test for interaction was significant. Our findings appeared to be robust even after adjustment for important covariates.

Potential limitations of our study should also be noted. First, this study only examined FPG as a measurement of glucose metabolism. Other relevant measurements such as fasting insulin, oral glucose tolerance testing, and glycosylated hemoglobin were not available. FPG is a commonly used screening test for diabetes. If FPG is abnormal, an oral

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Table 3
Comparison of FPG among three treatment groups in the total sample and in subgroups defined by baseline FPG

<table>
<thead>
<tr>
<th>FPG (mmol/L)</th>
<th>Control group</th>
<th>Low-FA group</th>
<th>High-FA group</th>
</tr>
</thead>
<tbody>
<tr>
<td>All subjects</td>
<td>149</td>
<td>146</td>
<td>148</td>
</tr>
<tr>
<td>n</td>
<td>149</td>
<td>146</td>
<td>148</td>
</tr>
<tr>
<td>Baseline FPG</td>
<td>5.5 ± 1.2</td>
<td>5.5 ± 1.2</td>
<td>5.4 ± 1.2</td>
</tr>
<tr>
<td>FPG change*</td>
<td>−0.03 ± 0.87</td>
<td>0.03 ± 0.97</td>
<td>−0.02 ± 0.95</td>
</tr>
<tr>
<td>Subjects with baseline FPG &lt;6.1 mmol/L</td>
<td>117</td>
<td>115</td>
<td>120</td>
</tr>
<tr>
<td>n</td>
<td>117</td>
<td>115</td>
<td>120</td>
</tr>
<tr>
<td>Baseline FPG</td>
<td>5.0 ± 0.6</td>
<td>5.1 ± 0.6</td>
<td>5.0 ± 0.6</td>
</tr>
<tr>
<td>FPG change*</td>
<td>0.02 ± 0.71</td>
<td>0.15 ± 0.77</td>
<td>0.16 ± 0.79</td>
</tr>
<tr>
<td>Subjects with baseline FPG ≥6.1 mmol/L</td>
<td>32</td>
<td>31</td>
<td>28</td>
</tr>
<tr>
<td>n</td>
<td>32</td>
<td>31</td>
<td>28</td>
</tr>
<tr>
<td>Baseline FPG</td>
<td>7.2 ± 1.2</td>
<td>7.3 ± 1.3</td>
<td>7.2 ± 1.2</td>
</tr>
<tr>
<td>FPG change*</td>
<td>−0.23 ± 1.30</td>
<td>−0.39 ± 1.44</td>
<td>−0.80 ± 1.20</td>
</tr>
</tbody>
</table>

FA, folic acid; FPG, fasting plasma glucose
* FPG change was defined as FPG at day 56 minus FPG at baseline.
† P < 0.05 versus baseline.
‡ P < 0.01 versus baseline.
§ P < 0.05 versus control group.
glucose tolerance test will be used to confirm the abnormality. Because the primary focus of this study was to evaluate how the combined therapy affects plasma glucose, FPG is an appropriate measurement. Glycosylated hemoglobin is a “weighted” average of plasma glucose levels during the preceding 120 d. Due to the relatively small treatment window (a total of 56 d) of our study, FPG should be a more sensitive indicator of therapeutic response than glycosylated hemoglobin. In this study, we did not obtain detailed information on dietary intake. However, we asked all participants not to take any nutritional supplements during the study and to keep their regular dietary habits. We measured serum folate concentrations of each subject at baseline and at the end of the trial, which provided an accurate and objective measurement of individual folate status and its change over the treatment period.

Although subdivision by fasting glucose was a prespecified exploratory analysis, this was not part of the randomization. Therefore, our findings on the subgroup analysis, although intriguing, can be considered hypothesis generating rather than a definitive evaluation of this question. Because all participants had mild to moderate essential hypertension, treatment was necessary to control their BP. We did not have a placebo (no treatment) group for ethical considerations. As such, our study could not evaluate individual effects of FA and enalapril on BP and FPG or determine if their combined effect was additive or interactive in nature. The treatment duration of our study was relatively short. Ramipril has been suggested to have a benefit in the prevention of diabetes after 3.5 y of treatment [3]. A recent meta-analysis also suggested that the beneficial effect of FA on stroke prevention was greater with longer treatment (≥36 mo) [14]. Thus, our findings remain to be further evaluated in longer-term trials.

Despite the limitations, our study findings are biologically plausible. In addition to reducing BP, previous studies have suggested that ACEI can decrease blood glucose [5, 20–22]. The combined data from three previous trials, which compared ACEIs with placebo in subjects with cardiovascular disease, suggested a risk reduction in diabetes of 14% (95% confidence interval 5–22) [23]; The Diabetes Reduction Assessment with Ramipril and Rosiglitazone trial [3] investigators reported that the use of 15 mg of ramipril daily for 3 y did not significantly prevent diabetes or death, but significantly more participants receiving ramipril had normal FPG levels and glucose tolerance than those receiving placebo and the distribution of the glucose levels had shifted downward in the ramipril group by the end of the study. However, not all previous studies have supported this glucose-lowering effect. A study [24] reported that neither enalapril nor captopril could modify insulin sensitivity, glycemic control, or lipids in normotensive non–insulin-dependent diabetic subjects. This discrepancy may possibly due to a different population background, a small sample, and/or a relatively short duration of treatment in the above-mentioned studies. Another possibility of this discrepancy may be that some previous trials, which compared ACEI with another antihypertensive agent such as a β-blocker or a diuretic [4, 25,26], overestimated the effect of ACEI, because these antihypertensive agents could increase the risk of diabetes [27]. In this clinical trial, we could not evaluate the independent effect of enalapril on FPG due to lack of a placebo (non-treatment) group.

There are growing data that FA supplementation may

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>n</th>
<th>FPG change*</th>
<th>Adjusted†</th>
<th>Adjusted‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>β</td>
<td>SE</td>
<td>P</td>
</tr>
<tr>
<td>Overall</td>
<td></td>
<td>β</td>
<td>SE</td>
<td>P</td>
</tr>
<tr>
<td>Control</td>
<td>149</td>
<td>−0.03 ± 0.87</td>
<td>0.00</td>
<td>—</td>
</tr>
<tr>
<td>Low FA</td>
<td>146</td>
<td>0.03 ± 0.97</td>
<td>0.04</td>
<td>0.11</td>
</tr>
<tr>
<td>High FA</td>
<td>148</td>
<td>−0.02 ± 0.95</td>
<td>−0.06</td>
<td>0.11</td>
</tr>
<tr>
<td>Baseline FPG &lt;6.1 mmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>117</td>
<td>0.02 ± 0.71</td>
<td>0.00</td>
<td>—</td>
</tr>
<tr>
<td>Low FA</td>
<td>115</td>
<td>0.15 ± 0.77</td>
<td>0.07</td>
<td>0.1</td>
</tr>
<tr>
<td>High FA</td>
<td>120</td>
<td>0.16 ± 0.79</td>
<td>0.09</td>
<td>0.11</td>
</tr>
<tr>
<td>Baseline FPG ≥6.1 mmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>32</td>
<td>−0.23 ± 1.30</td>
<td>0.00</td>
<td>—</td>
</tr>
<tr>
<td>Low FA</td>
<td>31</td>
<td>−0.39 ± 1.44</td>
<td>−0.20</td>
<td>0.29</td>
</tr>
<tr>
<td>High FA</td>
<td>28</td>
<td>−0.80 ± 1.20</td>
<td>−0.68</td>
<td>0.28</td>
</tr>
</tbody>
</table>

FA, folic acid; FPG, fasting plasma glucose
* FPG change was defined as FPG at day 56 minus FPG at baseline (mean ± SD).
† Regression model was adjusted for baseline FPG, age, gender, body mass index, systolic blood pressure, and triacylglycerol.
‡ Regression model was adjusted for baseline FPG, age, gender, body mass index, triacylglycerol, systolic and diastolic blood pressures, serum creatinine, plasma homocysteine, total homocysteine change, and study center.

P = 0.025, trend test.
improve insulin resistance [28]. With the treatment with FA (2.5 mg/d) for 3 mo, fasting plasma insulin concentrations decreased significantly ($P = 0.004$) and the Homeostatic Model Assessment index was also significantly reduced in the folate group [29]. A study showed that 1 mo of supplementation with FA (5 mg/d) plus vitamin B12 (0.5 mg/d) significantly reduced plasma insulin concentrations in subjects with the metabolic syndrome [30]. Another study showed that a 6-mo treatment with FA not only decreased Hcy levels but also ameliorated insulin sensitivity and endothelial dysfunction [31].

Although the mechanisms by which FA decreases blood glucose concentration are not clearly understood, several hypotheses have been suggested. FA is necessary for metabolism of Hcy and FA supplementation can lower plasma Hcy concentration and improve large-artery stiffness [32]. A significant positive association of elevated plasma tHcy levels with hypertension has been reported [33]. The tHcy level was significantly higher in hypertensives with and without coronary artery disease ($P < 0.001$). After adjusting for age, gender, medications including lipid-lowering and antihypertensive therapies and serum creatinine, elevated tHcy levels remained significantly associated with hypertension ($P = 0.004$) [33]. Recent studies have suggested that elevated Hcy is significantly associated with plasma glucose concentration [12,13]. Subjects with impaired fasting glucose had significantly higher fasting serum tHcy levels than those with normal fasting glucose [34]. There was a significant relation between tHcy and insulin sensitivity [35]. The possible mechanism of this relation may be that Hcy thiolactone, the active form of Hcy, may inhibit the insulin-stimulated tyrosine phosphorylation of insulin receptor β-subunit and its substrates and decrease the p85 regulatory subunit of phosphatidylinositol 3-kinase activity, including a reduction in insulin-stimulated glycogen synthesis [15]. This naturally leads to insulin resistance and blood glucose increase. Another possible mechanism is that FA will ameliorate endothelial dysfunction induced by elevated Hcy, convert L-arginine to nitric oxide and L-citrulline, scavenge reactive oxygen species such as $O_2^-$ and peroxynitrite, maintain a coupled endothelial nitric oxide synthase reaction, and prevent nitric oxide synthase dysfunction. All of these may be beneficial to glycometabolism. Because there was no non-drug–treated placebo group in our study, we could not evaluate if FA had an independent beneficial effect on BP or FPG in the present study.

In conclusion, in this sample of adult Chinese hypertensive patients, folate combined with enalapril showed a greater beneficial effect on the reduction of FPG in a dose-related fashion than did enalapril alone among subjects with hyperglycemia. In other words, folate (0.8 mg/d) plus enalapril treatment was particularly efficacious in lowering FPG level in subjects with hyperglycemia. Our findings, if confirmed by future studies, offer a safe and effective treatment to control hypertension and to lower FPG in mildly to moderately hypertensive patients with hyperglycemia.

Acknowledgments

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References


