Folate Improves Endothelial Function in Coronary Artery Disease
An Effect Mediated by Reduction of Intracellular Superoxide?

Sagar N. Doshi, Ian F.W. McDowell, Stuart J. Moat, Derek Lang, Robert G. Newcombe, Mahmud B. Kredan, Malcolm J. Lewis, Jonathan Goodfellow

Abstract—Homocysteine is a risk factor for coronary artery disease (CAD). Folic acid lowers homocysteine and may improve endothelial function in CAD, although the mechanism is unclear. We investigated the effect of folic acid on endothelial function, homocysteine, and oxidative stress in patients with CAD. We also examined the acute effect of 5-methyltetrahydrofolate (5-MTHF), the principal circulating folate, on endothelial function in vivo and on intracellular superoxide in cultured endothelial cells. A randomized crossover study of folic acid (5 mg daily) for 6 weeks was undertaken in 52 patients with CAD. Ten further patients were given intra-arterial 5-MTHF. Endothelial function was assessed by flow-mediated dilatation (FMD). Folic acid increased plasma folate \( P<0.001 \), lowered homocysteine by 19\% \( P<0.001 \), and improved FMD \( P<0.001 \). FMD improvement did not correlate with homocysteine reduction. Malondialdehyde and total plasma antioxidant capacity, markers of oxidative stress, were unchanged. 5-MTHF acutely improved FMD \( P<0.001 \) without altering homocysteine \( P=0.47 \). In vitro, 5-MTHF abolished homocysteine-induced intracellular superoxide increase \( P<0.001 \); this effect was also observed with folic acid and tetrahydrobiop- terin. Our data support the beneficial effect of folic acid on endothelial function in CAD but suggest that the mechanism is independent of homocysteine. Reduction of intracellular endothelial superoxide may have contributed to the effect. 

Key Words: folic acid ■ homocysteine ■ coronary artery disease ■ endothelial function ■ 5-methyltetrahydrofolate

Elevated total plasma homocysteine (tHcy) is associated with an increased risk of cardiovascular disease and appears to be largely independent of other conventional risk factors. However, whether the increased risk is mediated directly by homocysteine or whether it may simply be a marker remains controversial.

The metabolism of folate and homocysteine are interrelated, and increasing folate intake augments remethylation of homocysteine, leading to a reduction of up to 25\% in its plasma concentration. This effect occurs despite normal plasma folate and can be achieved by folic acid in doses of 400 \( \mu \)g to 5 mg/d. This has led to the proposal that folic acid treatment may reduce cardiovascular risk by reducing tHcy.

In homocystinuria, a rare inborn error with markedly elevated tHcy concentrations (>100 \( \mu \)mol/L), folic acid and pyridoxine (vitamin B\(_6\)) lower tHcy and reduce cardiovascular events, the major cause of mortality. This benefit is observed despite residual tHcy concentrations that are often above the upper limit of “normal,” which suggests a benefit from B group vitamins that is independent of homocysteine. However, in the general population, in which tHcy concentrations are much lower (5 to 15 \( \mu \)mol/L), there are, as yet, only limited data on the effect of folic acid treatment on cardiovascular outcome.

Endothelial dysfunction is a key process in atherosclerosis and has been reported in chronic mild fasting hyperhomocysteinemia in subjects free of vascular disease and in experimental hyperhomocysteinemia, which is induced by methionine loading in normal subjects. Precisely how homocysteine may promote endothelial dysfunction is unclear; however, generation of reactive oxygen species is proposed to be an important mechanism. Recent studies suggest that B group vitamins enhance endothelial function in coronary artery disease (CAD) or hyperhomocysteinemia. However, the data are limited, and the mechanism underlying this improvement has not been established.

5-Methyltetrahydrofolate (5-MTHF), the main circulating folate, can improve endothelial function in subjects with hypercholesterolemia who are free of vascular disease and not receiving lipid-lowering treatment. However, the effect of 5-MTHF in subjects with severe CAD on lipid-lowering treatment is unknown.
We sought to determine whether high-dose folic acid supplementation can improve endothelial dysfunction, a surrogate of cardiovascular risk, in patients with significant CAD on standard therapy, and we endeavored to correlate this effect, if any, with changes in plasma homocysteine, plasma folate, and oxidant stress. To further investigate mechanisms, we examined the acute effect of 5-MTHF on endothelial function in a similar group of 10 patients and its effect on intracellular levels of superoxide in cultured porcine aortic endothelial (PAE) cells.

Methods

Subjects

Patients with CAD aged <70 years were recruited. We defined CAD as either angiographically proven coronary disease (≥50% luminal stenosis) or a history of myocardial infarction (creatine kinase rise >2-fold normal with ECG changes). Plasma homocysteine was not an entry criterion, and levels were not made known to the investigators during the course of the study. Patients were excluded if an acute coronary event had occurred <3 months before entry or if there was diabetes mellitus, uncontrolled hypertension, fasting plasma cholesterol >6.5 mmol/L, impaired renal function (creatinine >120 μmol/L), or clinically significant heart failure. Patients actively smoking or who had recently ceased smoking (<6 months), patients taking antioxidant vitamins (E or C), folic acid, or fish oils, and women on hormone replacement were also excluded.

The present study was designed to recruit a minimum of 10 subjects, to achieve 80% power to detect an improvement in flow-mediated dilatation (FMD) from 40 μmol/L on placebo to 80 μmol/L on folic acid at the α=0.05 level. Of 534 patients screened, 52 were eligible for entry to the folic acid study, and 10 similar subjects were identified for the intra-arterial study. All selected subjects were tested to exclude vitamin B12 deficiency, which precludes folic acid treatment, before entry.

Study Design

Folic Acid Study

The study was a randomized, double-blind, placebo-controlled crossover design. It involved two 6-week treatment periods of folic acid (5 mg daily) or matched placebo separated by a washout period of 4 months.

Intra-Arterial 5-MTHF Study

The study was open label and investigated the acute effects of 5-MTHF on brachial artery FMD and its NO component.

Withdrawals, Medication Changes, and Compliance

Two subjects were withdrawn from the folic acid study: 1 with a nonfatal myocardial infarction and 1 with atrial fibrillation. During the study, all efforts were made to hold medication constant, but clinical considerations forced changes in 6 patients. Treatment with saline was infused at 0.5 mL/min, and this infusion rate was kept constant throughout. The brachial artery was imaged 7- to 10-cm distal to the puncture site, and FMD was measured. After this, 5-MTHF (Sigma Chemical Co) was then infused at 50 μg/min to achieve a plasma concentration of at least 457 μmol/L (1 μmol/L), which has been shown to improve endothelial function in untrained hypercholesterolemia. After 30 minutes of infusion, venous blood samples were drawn from the ipsilateral antecubital vein, and FMD was reassessed. N5-Monomethyl-L-arginine (L-NMMA), an inhibitor of endothelial NO synthase (eNOS), was then coinjected (3 mg/min) with 5-MTHF to assess the NO component of the observed FMD. The L-NMMA infusion was then stopped, and during infusion with 5-MTHF, the response to NTG was reassessed.
folic acid was well tolerated, and no side effects were reported. Compliance assessed by a tablet count was 96%.

### Statistical Analysis
The main statistical analyses of the folic acid study results were based on 50 subjects after removal of the 2 withdrawals. Prefolate to postfolate changes in biochemical and vascular measurements were compared with corresponding preplacebo to postplacebo changes by using the Hills-Armitage method.26 The relationships between changes in FMD, homocysteine, and other parameters on folate administration were characterized by the Spearman rank correlation, with 95% CIs calculated by the tanh-1 method. In the intra-arterial study, paired t tests were used. The main outcome variables of FMD, folate, and homocysteine were little changed after removal of the 6 subjects in whom medication changes took place.

For the in vitro experiments, data are expressed as mean±SEM (n=10) and compared by ANOVA, followed by an appropriate multiple range test. All differences were considered significant at P<0.05.

### Results
#### Baseline Characteristics
The folic acid study group consisted of 52 patients aged 57±8 years (males 57±8 years, females 58±8 years). The 5-MTHF study group consisted of 10 similar subjects, aged 55±9 years (all male). Baseline characteristics were similar in the 2 populations (Table 1).

### Folic Acid Study
**Effects on Biochemical Parameters**
Biochemical parameters are shown in Table 2. Folic acid significantly decreased THcy (9.3±2.4 versus 10.8±2.4 [placebo] μmol/L, P<0.001) and markedly increased plasma folate (310±234 versus 9.1±3.4 [placebo] μg/L, P<0.001). MDA and plasma TAOC were not significantly altered by folic acid. Vitamin B12, lipids, glucose, and creatinine were unchanged by folic acid compared with placebo.

**Effects on Flow-Mediated Dilatation and Vascular Measurements**
Vascular data involving folic acid treatment are shown in Table 3. The coefficient of variation for the measurement of FMD in our laboratory was 5.6%. FMD was impaired in the 10 subjects at baseline compared with published normal values (FMD 43±15 μm, 0.96±0.34% baseline EDD).27 FMD significantly improved after folic acid compared with placebo (110±43 versus 47±35 μm, respectively; P<0.001; Figure 1), and in addition, the time course of vessel diameter change after cuff release was significantly altered (Figure 2). Heart rate, blood pressure, baseline brachial artery EDD, peak hyperemic flow, and NTG response did not differ significantly after folic acid.

### Intra-Arterial 5-MTHF Study
**Effects on Biochemical Parameters**
After 30 minutes of infusion, plasma 5-MTHF markedly increased (from 20.2 to 1595 μg/L, P<0.001), whereas no change in plasma homocysteine was observed (from 10.50±2.46 to 10.53±2.52 μmol/L, P=0.47).

**Effects on Flow-Mediated Dilatation and Vascular Measurements**
Vascular data during control, 5-MTHF infusion, and 5-MTHF/L-NMMA coinfusion are shown in (Table 4). FMD was impaired in the 10 subjects at baseline compared with published normal values (FMD 43±15 μm, 0.96±0.34% baseline EDD).27 5-MTHF acutely improved FMD compared with control (80±20 versus 43±15 μm, respectively; P<0.001), an effect that was completely suppressed by coinfusion with L-NMMA (Figure 3). Heart rate, blood pressure, baseline brachial artery EDD, peak hyperemic flow, and NTG response did not differ significantly during 5-MTHF infusion compared with control.

### In Vitro Effect of 5-MTHF, Folic Acid, and BH₄ on Intracellular Superoxide Levels
Intracellular superoxide content was 21.0±2.4 mV · s per 10⁶ cells in the control. Exposure to homocysteine resulted in a significant (P<0.001) increase in superoxide levels to 53.4±2.9 mV · s per 10⁶ cells. This increase was abolished (P<0.001) by coinfusion with either 5-MTHF, folic acid, or BH₄ (Figure 4).
The oral folate study, statistically the most powerful to our knowledge, confirms and extends the findings of recent parallel group studies of folic acid (5 mg daily) alone or in combination with other B group vitamins on endothelial function in CAD.14,15 In previous studies,14,15 mean baseline tHcy concentration was higher than that in the present study: 13 μmol/L14 and 12.3 μmol/L15 compared with 11.2 μmol/L in the present study. Furthermore, improvement in endothelial function in the present study was not confined to those with higher baseline homocysteine concentrations. Indeed, there was a trend toward greater FMD enhancement (r = −0.21, P = 0.15, 95% CI −0.46 to 0.08). FMD improvement was weakly and negatively correlated with baseline homocysteine (r = −0.23, P = 0.11, 95% CI −0.48 to 0.06). No correlation was found between improvement in FMD and increase in plasma folate (r = 0.09, P = 0.54, 95% CI −0.20 to 0.36) or with changes in MDA or TAOC.

**Discussion**

The present study demonstrated a significant improvement in endothelial function after 6 weeks of treatment with folic acid (5 mg daily) in subjects with significant CAD on standard therapy and with good lipid control (mean cholesterol 4.7 mmol/L). The majority (88%) of patients were taking statins, which have previously been shown to improve endothelial function,28 indicating additive benefit.

The oral folate study, statistically the most powerful to our knowledge, confirms and extends the findings of recent parallel group studies of folic acid (5 mg daily) alone or in combination with other B group vitamins on endothelial function in CAD.14,15 In previous studies,14,15 mean baseline tHcy concentration was higher than that in the present study: 13 μmol/L14 and 12.3 μmol/L15 compared with 11.2 μmol/L in the present study. Furthermore, improvement in endothelial function in the present study was not confined to those with higher baseline homocysteine concentrations. Indeed, there was a trend toward greater FMD enhancement (r = −0.21, P = 0.15, 95% CI −0.46 to 0.08). FMD improvement was weakly and negatively correlated with baseline homocysteine (r = −0.23, P = 0.11, 95% CI −0.48 to 0.06). No correlation was found between improvement in FMD and increase in plasma folate (r = 0.09, P = 0.54, 95% CI −0.20 to 0.36) or with changes in MDA or TAOC.

**Correlates of Improved FMD**

Although homocysteine was significantly decreased by folic acid, a positive correlation between improvement in FMD and reduction in plasma homocysteine was not found. To the contrary, there was a trend toward a negative correlation (r = −0.21, P = 0.15, 95% CI −0.46 to 0.08). FMD improvement was weakly and negatively correlated with baseline homocysteine (r = −0.23, P = 0.11, 95% CI −0.48 to 0.06). No correlation was found between improvement in FMD and increase in plasma folate (r = 0.09, P = 0.54, 95% CI −0.20 to 0.36) or with changes in MDA or TAOC.

**Table 2. Biochemical Parameters at Baseline, Before Placebo and Folic Acid, and After 6-wk Administration**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline (n = 50)</th>
<th>Before Placebo (n = 50)</th>
<th>After Placebo (n = 50)</th>
<th>Before Folic Acid (n = 50)</th>
<th>After Folic Acid (n = 50)</th>
<th>P *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homocysteine, μmol/L</td>
<td>11.2 ± 2.7</td>
<td>10.5 ± 2.5</td>
<td>10.8 ± 2.4</td>
<td>11.1 ± 2.8</td>
<td>9.3 ± 2.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Vitamin B6, ng/L</td>
<td>432 ± 116</td>
<td>430 ± 125.75</td>
<td>428 ± 128</td>
<td>435 ± 123</td>
<td>411 ± 128</td>
<td>0.23</td>
</tr>
<tr>
<td>Plasma folate, μg/L</td>
<td>8.8 ± 3.4</td>
<td>9.3 ± 2.9</td>
<td>9.1 ± 3.4</td>
<td>8.9 ± 3.5</td>
<td>310 ± 235</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>4.7 ± 0.8</td>
<td>4.6 ± 0.7</td>
<td>4.6 ± 0.7</td>
<td>4.8 ± 0.7</td>
<td>4.7 ± 0.7</td>
<td>0.87</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.2 ± 0.3</td>
<td>1.1 ± 0.3</td>
<td>1.1 ± 0.4</td>
<td>1.2 ± 0.4</td>
<td>1.2 ± 0.4</td>
<td>0.18</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>2.8 ± 0.7</td>
<td>2.8 ± 0.6</td>
<td>2.8 ± 0.7</td>
<td>2.8 ± 0.6</td>
<td>2.8 ± 0.6</td>
<td>0.95</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.6 ± 0.9</td>
<td>1.7 ± 0.9</td>
<td>1.6 ± 1.0</td>
<td>1.7 ± 0.9</td>
<td>1.8 ± 1.1</td>
<td>0.06</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>5.3 ± 0.6</td>
<td>5.3 ± 0.6</td>
<td>5.2 ± 0.6</td>
<td>5.3 ± 0.7</td>
<td>5.3 ± 0.7</td>
<td>0.46</td>
</tr>
<tr>
<td>Creatinine, μmol/L</td>
<td>98.6 ± 13.2</td>
<td>98.7 ± 13.6</td>
<td>98.8 ± 14.8</td>
<td>98.9 ± 13.7</td>
<td>98.0 ± 13.3</td>
<td>0.47</td>
</tr>
<tr>
<td>MDA, nmol/mL</td>
<td>0.49 ± 0.12</td>
<td>0.50 ± 0.13</td>
<td>0.47 ± 0.10</td>
<td>0.50 ± 0.12</td>
<td>0.49 ± 0.11</td>
<td>0.57</td>
</tr>
<tr>
<td>TAOC, nmol/mL</td>
<td>1.56 ± 0.13</td>
<td>1.56 ± 0.11</td>
<td>1.56 ± 0.12</td>
<td>1.56 ± 0.13</td>
<td>1.54 ± 0.11</td>
<td>0.13</td>
</tr>
</tbody>
</table>

Values are mean ± SD.

*Comparing changes with placebo vs changes with folic acid.

**Table 3. Vascular Data at Baseline, Before Placebo and Folic Acid, and After 6-wk Administration**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline (n = 50)</th>
<th>Before Placebo (n = 50)</th>
<th>After Placebo (n = 50)</th>
<th>Before Folic Acid (n = 50)</th>
<th>After Folic Acid (n = 50)</th>
<th>P *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vessel EDD, mm</td>
<td>4.37 ± 0.72</td>
<td>4.36 ± 0.73</td>
<td>4.38 ± 0.72</td>
<td>4.39 ± 0.70</td>
<td>4.39 ± 0.70</td>
<td>0.89</td>
</tr>
<tr>
<td>FMD, μm</td>
<td>50 ± 33</td>
<td>46 ± 33</td>
<td>47 ± 35</td>
<td>52 ± 34</td>
<td>110 ± 43</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NTG diameter change, μm</td>
<td>333 ± 78</td>
<td>340 ± 72</td>
<td>340 ± 72</td>
<td>340 ± 76</td>
<td>340 ± 77</td>
<td>0.79</td>
</tr>
<tr>
<td>Baseline blood flow, mL/min</td>
<td>41 ± 21</td>
<td>40 ± 20</td>
<td>40 ± 19</td>
<td>40 ± 19</td>
<td>40 ± 18</td>
<td>0.79</td>
</tr>
<tr>
<td>Peak hyperemic flow, mL/min</td>
<td>198 ± 73</td>
<td>196 ± 68</td>
<td>196 ± 71</td>
<td>202 ± 67</td>
<td>198 ± 66</td>
<td>0.51</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>59 ± 11</td>
<td>59 ± 10</td>
<td>60 ± 10</td>
<td>59 ± 10</td>
<td>59 ± 10</td>
<td>0.64</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>133 ± 16</td>
<td>132 ± 16</td>
<td>133 ± 14</td>
<td>133 ± 17</td>
<td>133 ± 14</td>
<td>0.11</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>73 ± 9</td>
<td>73 ± 9</td>
<td>71 ± 8</td>
<td>74 ± 9</td>
<td>73 ± 9</td>
<td>0.49</td>
</tr>
</tbody>
</table>

Values are mean ± SD.

*Comparing changes with placebo vs changes with folic acid.
observational data linking homocysteine and cardiovascular risk has been based on total, not free, homocysteine. Second, of the free component, only ≈1% is in the reduced form (i.e., possesses a free sulfhydryl group), which can support autooxidation and therefore superoxide generation, with the remaining component being oxidized. The pathological significance of such low levels of free reduced homocysteine on oxidative burden in the plasma has been questioned recently.

The lack of a positive correlation between the enhancement in endothelial function and tHcy reduction suggests that the beneficial effect of folic acid is unlikely to be mediated principally via tHcy lowering. This proposal is supported by the intra-arterial study, which demonstrated acute improvement in endothelial function after 30 minutes of infusion with 5-MTHF independent of a change in tHcy. This effect has not been previously reported in subjects with CAD. The improvement was abolished by coinfusion with the NO synthase inhibitor L-NMMA, indicating that it was mediated by an increase in NO bioavailability.

Generation of reactive oxygen species is proposed to be an important mechanism of homocysteine-induced endothelial injury. This view is supported by methionine loading in normal subjects, which raises tHcy to ≈30 to 35 μmol/L and which acutely impairs endothelial function. The mechanism is believed to involve oxidant stress and is abrogated by treatment with antioxidant agents. However, it cannot be assumed that moderately elevated homocysteine levels in the general population exert oxidative stress, because methionine loading results in large unphysiological increases in free reduced homocysteine and methionine. Although some observational studies have shown a correlation between tHcy levels and markers of oxidant stress in the general population, a causal relationship has not been established. In the present study, although tHcy was significantly reduced by 19%, there was no reduction in MDA or any increase in TAOC, a marker of the antioxidant capacity of plasma. This suggests either that homocysteine does not directly exert oxidative stress in the plasma or that measurement of MDA and TAOC are not sensitive enough indicators. However, MDA and TAOC were selected because methionine loading in normal subjects is associated with an increase in MDA and decrease in TAOC, indicating that under these conditions, they can detect changes in plasma oxidant stress.

The in vitro study used concentrations of homocysteine that were far higher than those experienced in vivo (1 mmol/L versus 10 to 15 μmol/L, respectively). The high concentration of homocysteine used was similar to previously published reports of in vitro work. Higher pharmacological concentrations are sometimes required in vitro to reproduce the in vivo situation, in part, because the exposure times used are much shorter than those experienced in hyperhomocysteinemia in humans. The main aim of the present study was to establish homocysteine-induced endothelial dysfunction and investigate possible mechanism(s) by which folate may reverse this and not to mimic the in vivo situation exactly. We have recently reported that exposure of cultured endothelial cells to homocysteine (≥30 μmol/L) stimulates intracellular generation of superoxide. The in vitro study confirmed this finding and further revealed that 5-MTHF can reduce levels of intracellular superoxide, suggesting a possible explanation for improvement in FMD observed in the human study. A number of mechanisms may account for this: (1) intracellular homocysteine was decreased, (2) 5-MTHF scavenged superoxide directly, and/or (3) 5-MTHF reduced superoxide production. Given that it takes some weeks before levels of

![Figure 1](http://atvb.ahajournals.org/)

**Figure 1.** FMD before and after 6 weeks of placebo and folic acid (5 mg daily). Data are presented as mean±SEM. FMD was defined as the greatest (absolute) increase in EDD during the first 3 minutes after cuff release. Comparing change with placebo vs change with folic acid.

![Figure 2](http://atvb.ahajournals.org/)

**Figure 2.** Time course of EDD change (ΔEDD, mean±SEM) after cuff release, before and after placebo and folic acid.

![Figure 3](http://atvb.ahajournals.org/)

**Figure 3.** FMD during control (saline), 5-MTHF infusion (50 μg/min), and 5-MTHF/L-NMMA coinfusion (mean±SEM). FMD was defined as the greatest (absolute) increase in EDD during the first 3 minutes after cuff release. Comparing 5-MTHF vs control. Comparing 5-MTHF/L-NMMA coinfusion vs 5-MTHF alone. Comparing control vs 5-MTHF/L-NMMA coinfusion. All comparisons were made by 2-tailed paired t test.
TABLE 4. Vascular Data During Control (Saline), 5-MTHF Infusion, and 5-MTHF/L-NMMA Coinfusion

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control (n=10)</th>
<th>5-MTHF (n=10)</th>
<th>5-MTHF/L-NMMA (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vessel EDD, mm</td>
<td>4.53±0.36</td>
<td>4.53±0.37</td>
<td>4.50±0.37</td>
</tr>
<tr>
<td>FMD, μm</td>
<td>43±15</td>
<td>80±20</td>
<td>7±6</td>
</tr>
<tr>
<td>NTG diameter change, μm</td>
<td>430±30</td>
<td>440±50</td>
<td>24±9</td>
</tr>
<tr>
<td>Baseline blood flow, ml/min</td>
<td>32±12</td>
<td>31±11</td>
<td>0.67</td>
</tr>
<tr>
<td>Peak hyperemic flow, ml/min</td>
<td>234±96</td>
<td>233±88</td>
<td>227±95</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>55±7</td>
<td>55±8</td>
<td>0.47</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>136±12</td>
<td>135±12</td>
<td>0.32</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>71±11</td>
<td>70±11</td>
<td>0.11</td>
</tr>
</tbody>
</table>

Values are mean±SD.

*Comparing control vs 5-MTHF infusion (50 μg/min).
†Comparing 5-MTHF infusion vs 5-MTHF/L-NMMA (3 mg/min) coinfusion. All comparisons were made by paired t test (2-tailed).

homocysteine fall significantly with folic acid, a reduction in homocysteine after 24 hours seems unlikely, and other direct actions of 5-MTHF more likely explain this observation. In vitro, 5-MTHF has recently been demonstrated to be capable of directly scavenging superoxide, increasing NO production by eNOS, and also reducing superoxide generation by eNOS. The in vitro study also demonstrated that BH4 is capable of inhibiting the homocysteine-induced increases in endothelial superoxide. BH4 is an essential cofactor for eNOS, and its depletion results in uncoupling of eNOS activity and a switch from production of NO, from l-arginine, to generation of superoxide. 5-MTHF is essential in the redox cycling of the inactive quinonoid BH4 (qBH2) back to the active form, BH4. Furthermore, oral BH4 supplementation can improve endothelial function in CAD. Thus, the ability of 5-MTHF to regenerate intracellular BH4 from qBH2 may, at least in part, explain the improvement in NO bioavailability observed in the human studies.

Implications of the Present Study

The oral folic acid study supports the finding that high-dose supplementation improves endothelial function in patients with CAD. Furthermore, this improvement is observed in subjects already treated with statins and is independent of baseline tHcy or its reduction. In contrast to earlier reports, the present data do not support the view that improvement is due to a pharmacological, with plasma levels far in excess of the normal range, and it cannot be assumed that the effects demonstrated will apply to low-dose folic acid (400 μg/d) or improved dietary intake. In conclusion, folic acid is safe and offers a simple, well-tolerated, and inexpensive therapeutic option for improving endothelial function in subjects with ischemic heart disease.

Acknowledgments

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References


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