Total Homocysteine Lowering Treatment Among Coronary Artery Disease Patients in the Era of Folic Acid–Fortified Cereal Grain Flour

Andrew G. Bostom, Paul F. Jacques, Gintaras Liaugaudas,† Gail Rogers, Irwin H. Rosenberg, Jacob Selhub

Abstract—The prevalence of deficient plasma folate status and elevated total plasma levels of homocysteine (tHcy), have been dramatically reduced after fortification of all enriched cereal grain flour products with folic acid at 140 μg/100 g flour. Against this new background fortification, we evaluated the tHcy-lowering efficacy of pharmacological dose, folic acid–based vitamin B supplementation among stable coronary artery disease (CAD) patients. Using a 2×2 factorial design, 131 stable CAD patients (mean age 60.1 years; 29.8% women) were randomly assigned to receive a combination of folic acid 2.5 mg/d, riboflavin 5 mg/d, + B₁₂ 0.4 mg/d, or placebo, with or without vitamin B₆ 50 mg/d, for 12 weeks of treatment. ANCOVA adjusted for baseline fasting tHcy levels revealed only very modest (ie, ≈1.0 μmol/L), albeit statistically significant (P<0.05), reductions in mean fasting tHcy levels afforded by the folic acid–containing treatments. Additional analyses indicated that none of the treatments provided a statistically significant reduction in the 2-hour post-methionine increase in tHcy levels, relative to placebo treatment. CAD patients exposed to cereal grain flour products fortified with high-dose, folic acid–containing vitamin B regimen, experience only very modest reductions in their mean fasting plasma tHcy levels. These findings have important implications for the statistical power of clinical trials testing the hypothesis that tHcy-lowering treatment may reduce recurrent atherothrombotic event rates. (Arterioscler Thromb Vasc Biol. 2002;22:488-491.)

Key Words: B vitamins ▪ randomized trial ▪ treatment efficacy

The prevalence of both deficient plasma folate status,¹⁻⁵ and elevated fasting total plasma levels of the putatively atherothrombotic⁶ sulfur amino acid homocysteine (tHcy),¹⁻⁵ have been dramatically reduced since the recent advent of United States⁷ and Canadian⁸ initiatives to fortify all enriched cereal grain flour products with physiological amounts (ie, 140 μg/100 g flour) of folic acid. Presently, there are three large, randomized, controlled trials of tHcy-lowering for the potential reduction of arteriosclerotic cardiovascular disease outcomes ongoing in the United States and Canada.⁹ The considerable (nutritional) biochemical effects¹⁻⁵ of cereal grain flour fortification with folic acid could substantially reduce the statistical power of these ongoing cardiovascular disease prevention studies. All three trials assume the patient groups assigned to active treatment will achieve the same mean tHcy-lowering treatment effects (ie, a mean reductions of ≈33%, or 4 to 6 μmol/L) previously reported¹⁰ in the absence of the large potential background effect of folic acid–fortified cereal grain flour. We re-examined this assumption by evaluating the tHcy-lowering efficacy of pharmacological dose, folic acid–based vitamin B supplementation among stable coronary artery disease (CAD) patients chronically exposed to cereal grain flour products fortified with folic acid at 140 μg/100 g flour.

Methods

The institutional review board at Memorial Hospital of Rhode Island (Pawtucket, RI) approved the study protocol, and all participants provided written, informed consent. Study participants were 267 stable CAD patients (ie, they were at least 3 months post-myocardial infarction or coronary angioplasty and/or at least 6 months post-coronary artery bypass graft surgery). CAD status was confirmed by established 12-lead electrocardiographic and cardiac isoenzyme (ie, creatine phosphokinase MB) criteria for definite myocardial infarction, and/or unstable angina with angiographically proven ≥50% stenosis of at least one major epicardial coronary artery. Participants lived in the Pawtucket and Providence, RI, metropolitan areas and underwent their baseline examinations between October 1997, and May 1999.⁵ Information regarding previous vitamin supplement use was obtained by standardized interview, and subjects were either nonusers of any supplements containing folic acid, or they had abstained from using such supplements for at least 6 weeks by the...
time of their examination. However, all participants were examined at least three to four months after the widespread availability in New England (John Watson, President, Watson Foods, New Haven, Conn, written communication, 1997) of cereal grain flour products fortified with folic acid at 140 μg per 100 g flour.1 Of these 267 persons examined, 131 were enrolled into the 12-week tHcy-lowering treatment phase of the study protocol based on the following criteria: a serum creatinine of 1.9 mg/dL or less; absence of clinical liver or thyroid disease, seizure disorder, uncontrolled diabetes, progressive congestive heart failure, malignancy, or cachectic disorders; chronic, stable dosing (ie, 3-months before treatment phase) of medications (as needed); a 2-hour post-methionine load increase above their fasting tHcy levels of at least 12 μmol/L; and pretreatment albumin, vitamin B12, and pyridoxal phosphate levels within normal ranges. "Normal ranges" were those established from a reference range created from the distribution of results observed in the 131 subjects participating in the study. Subjects who did not meet these criteria were excluded. Compliance with treatment was assessed by pill counts and performed by code so that treatment assignments remained concealed. Compliance with treatment was assessed by pill counts and treatment assignments remained concealed. All parentheses represent 95% confidence intervals. PML indicates post-methionine load.

**TABE 1. Baseline Characteristics**

<table>
<thead>
<tr>
<th></th>
<th>Vitamin B6</th>
<th>Folic Acid, Vitamin B12, and Riboflavin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo</td>
<td>Treatment</td>
</tr>
<tr>
<td>Age, y</td>
<td>61.4 (59.2–63.7)</td>
<td>58.7 (56.4–61.0)</td>
</tr>
<tr>
<td>Men, %</td>
<td>67.6 (56.7–78.6)</td>
<td>73.0 (61.7–84.4)</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>81.5 (77.6–85.3)</td>
<td>81.2 (77.2–85.2)</td>
</tr>
<tr>
<td>Albumin, g/L</td>
<td>45.3 (44.7–46.0)†</td>
<td>46.4 (45.6–47.1)§</td>
</tr>
<tr>
<td>Creatinine, mg/dL</td>
<td>1.03 (0.98–1.07)</td>
<td>1.05 (1.00–1.10)</td>
</tr>
<tr>
<td>Fasting tHcy, μmol/L*</td>
<td>8.7 (8.2–9.2)</td>
<td>8.7 (8.2–9.2)</td>
</tr>
<tr>
<td>PML increase in tHcy, μmol/L*</td>
<td>17.5 (16.0–19.1)</td>
<td>16.9 (15.4–18.5)</td>
</tr>
<tr>
<td>Folate, ng/mL</td>
<td>9.7 (8.8–10.8)</td>
<td>10.2 (9.2–11.4)</td>
</tr>
<tr>
<td>Vitamin B12, pg/mL*</td>
<td>344 (318–373)</td>
<td>334 (308–363)</td>
</tr>
<tr>
<td>Pyridoxal 5’-phosphate, nmol/mL*</td>
<td>51.3 (44.8–58.7)</td>
<td>45.9 (39.9–52.8)</td>
</tr>
</tbody>
</table>

*Indicates geometric mean.
†‡Pairwise comparisons between means in the same row are significantly different (P<0.05) if they do not share a common superscript.

Results

Table 1 reveals that randomization with respect to No B6 (groups II and IV combined, n=68), Any B6 (groups I and III combined, n=63), No Folic Acid (groups III and IV combined, n=66), and Any Folic Acid (groups I and II combined, n=65) was successful for the key baseline characteristics of fasting and 2-hour post-methionine load increase in tHcy levels, in addition to plasma folate, vitamin B12, and pyridoxal 5’-phosphate levels. As displayed in Table 2, none of the treatments produced a statistically significant reduction in the 2-hour post-methionine load increase in tHcy levels, relative to placebo treatment. Finally, ANCOVA adjusted for age, as well as pretreatment fasting tHcy and albumin levels, revealed only very modest (≈1.0 μmol/L), albeit statistically significant (P<0.05), reductions in mean fasting tHcy levels afforded by the folic acid containing treatments.

Discussion

We found that none of the treatments, including the vitamin B6-containing treatments, provided a statistically significant reduction in the 2-hour post-methionine load increase in tHcy levels, relative to placebo treatment. These data contrast with...
uncontrolled reports,22–24 and our earlier randomized, placebo-controlled 2×2 factorial study in renal transplant recipients,25 demonstrating that vitamin B6 treatment could significantly reduce mean post-methionine load increases in tHcy levels. However, in all these previous reports,22–25 subjects had lower mean plasma pyridoxal 5′-phosphate status, and/or significantly greater mean post-methionine load increases in their tHcy levels, at baseline. In conjunction with the current null findings, the aggregate data22–25 suggest that vitamin B6 treatment for the potential reduction of post-methionine load tHcy levels may only be effective when vitamin B6 status is marginal, emphasizing the role of vitamin B6 as a cofactor, not a substrate, for cystathionine beta synthase in the transsulfuration pathway.26

Including the current report, two controlled total homocysteine-lowering treatment studies have been completed in the United States and Canada among coronary artery disease (CAD) patient populations chronically exposed to a background of folic acid fortified cereal grain flour. Earlier, Title and colleagues27 studied 75 stable Canadian CAD patients selected to have a fasting tHcy level ≥9 μmol/L from among 166 consecutive CAD patients (ie, ~50% of the total number of CAD patients screened had total homocysteine levels <9 μmol/L). Subjects were randomly assigned to one of three groups of 25 patients each, receiving 5 mg/d folic acid, with or without 2 g/d vitamin C and 800 IU/d vitamin E (ie, 50 patients received 5 mg/d folic acid), or placebo, for 16 weeks of treatment. For the 50 patients receiving 5 mg/d folic acid, mean fasting tHcy levels were 12.1 μmol/L pretreatment and 10.9 μmol/L post-treatment, a -1.2 μmol/L difference. For the 25 patients receiving placebo, mean fasting tHcy levels were 12.1 μmol/L pretreatment and 11.8 μmol/L post-treatment, a -0.3 μmol/L difference. Preliminary data consistent with the findings of Title and colleagues27 have been presented by the PACIFIC trial investigators26 in CAD patients unexposed to mandated flour fortification with folic acid, but with comparable baseline plasma folate status. These investigators have reported that folic acid at doses of 0.2 mg/d and 2.0 mg/d for 6 months reduced mean fasting tHcy levels by only 1.2 or 1.7 μmol/L, respectively, relative to placebo among 723 individuals with stable CAD.24 Our trial further demonstrated that only very modest reductions in mean fasting tHcy levels were achieved even when CAD patients received supraphysiologic doses of folic acid, combined with high doses of vitamin B12, vitamin B6, and riboflavin.

The findings of Title and colleagues27 are directly relevant to the screening strategy of the Vitamin Intervention for Stroke Prevention (VISP) investigators,29 whereas our current data are directly relevant to the designs of the Heart Outcomes Prevention Evaluation (HOPE-2) and Women’s Antioxidant Cardiovascular Disease Study (WACS) trials,9 both of which do not include any screening tHcy level eligibility criteria. The rather meager reductions in mean fasting tHcy levels (ie, approximately ~1 μmol/L) achieved in the two CAD populations studied were well below the assumed tHcy-lowering treatment effect of a mean reduction of ~4 to 6 μmol/L.9,10 The data we have presented highlight the impact of flour fortification with folic acid in patients with established cardiovascular disease, who are free of overt chronic renal disease, ie, a tHcy-lowering treatment responsiveness to high-dose folic acid–based regimens that results in only very modest reductions in their mean tHcy levels. As a consequence, the three ongoing United States and Canadian clinical trials attempting to evaluate the hypothesis that tHcy-lowering treatment will reduce arteriosclerotic cardiovascular disease outcomes (VISP, HOPE-2, and WACS), will likely achieve only ~20% to 25% (ie, mean reductions of 1.0 to 1.5, versus 4.0 to 6.0 μmol/L) of their projected mean tHcy-lowering treatment effects. Accordingly, none of these trials would remain adequately powered to test their specific total homocysteine-lowering hypotheses identified a priori.

Acknowledgments

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References


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