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 B9 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 folic acid who receive high-dose, folic acid–containing vitamin B regimens, 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),1,4,5 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

Received October 15, 2001; revision accepted January 7, 2002.

From the Division of Renal Diseases (A.G.B.), Rhode Island Hospital, Providence, and Memorial Hospital of Rhode Island, Pawtucket, RI, and Vitamin Bioavailability Laboratory (A.G.B., P.F.J., G.R., I.H.R., J.S.), The Tufts Jean Mayer USDA Human Nutrition Research Center, Boston, Mass.
†Gintaras Liaugaudas is deceased.


Address correspondence to Andrew G. Bostom, MD, MS, Division of Renal Diseases, Rhode Island Hospital, 593 Eddy St, Providence, RI 02903. E-mail abstom@lifespan.org

© 2002 American Heart Association, Inc.

Arterioscler Thromb Vasc Biol. is available at http://www.atvbaha.org DOI: 10.1161/hq0302.105369
Bostom et al 19) blood samples were collected from each participant, screenings with a potential impact 11 screening and throughout the 12-week treatment phase) of medical cachectic disorders; chronic, stable dosing (ie, 3-months before controlled diabetes, progressive congestive heart failure, malignancy, or 12 groups: (I) folic acid 2.5 mg/d, riboflavin 5 mg/d, 1.5 g/d or more; fenofibrate or 131 subjects were randomly assigned in blocks based on sex, 2-hour post-methionine load increase willingness to participate. These 131 subjects were randomly assigned in blocks based on sex, 2-hour post-methionine load increase above their fasting tHcy levels of at least 12 μmol/L; absence of clinical liver or thyroid disease, seizure disorder, uncontrollable diabetes, progressive congestive heart failure, malignancy, or cachectic disorders; chronic, stable dosing (ie, 3-months before screening and throughout the 12-week treatment phase) of medications with a potential impact11-18 on tHcy levels (ie, nicotinic acid at 1.5 g/d or more±colestil at 20 g/d or more; fenofibrate or gemfibrozil; thiazide or loop diuretics; the antiepileptic drugs carbamazepine, phenytoin, valproate, phenobarbital, or primidone; levodopa; estrogen replacement therapy, raloxifene, or tamoxifen); a willingness to participate. These 131 subjects were randomly assigned in blocks based on sex, 2-hour post-methionine load increase in tHcy levels, and fasting tHcy levels to one of four treatment groups: (I) folic acid 2.5 mg/d, riboflavin 5 mg/d, + 50 mg/d vitamin B12 (n=31); (II) folic acid 2.5 mg/d, riboflavin 5 mg/d, + vitamin B12 0.4 mg/d, + placebo folic acid, riboflavin, + vitamin B12 (n=31); (III) folic acid 50 mg/d vitamin B12, + placebo folic acid, riboflavin, + vitamin B12 (n=32); and (IV) placebo vitamin B12, + placebo folic acid, riboflavin, + vitamin B12 (n=34). Treatment assignments were made by a pharmacist who was blinded to all other aspects of the study. Laboratory analyses, data entry, and data analyses were performed by code so that treatment assignments remained concealed. Compliance with treatment was assessed by pill counts and determination of the change in plasma vitamin status.

Overnight (10 to 14 hours) fasting, as well as 2-hour post-methionine loading (100 mg L-methionine/kg body weight, per Bostom et al19) blood samples were collected from each participant, at the screening examination, on the day of enrollment into the treatment phase, and twice during the 12th week of treatment. All whole blood specimens for tHcy and vitamin B assays were collected and stored on wet ice until the plasma was separated in a refrigerated centrifuge within 2-hours of collection, aliquoted, and stored at −70°C until analyzed. Whole blood for serum was allowed to clot at room temperature for 15 to 20 minutes, followed by prompt separation of the serum, aliquoting, and storage at −70°C until analyzed. Plasma tHcy levels were determined by using high-performance liquid chromatography with fluorescence detection,20 and plasma pyridoxal 5’-phosphate levels were measured by using radioenzymatic (tyrosine decarboxylase) assay.21 Plasma folate and vitamin B12 levels were measured by radioassay (BioRad Quanta-aphase II). Serum creatinine (Jaffe method) and albumin (bromcresol method) levels were determined by using standard techniques adapted for automated clinical chemistry laboratory analyzers. To eliminate inter-assay variability, all analytes were batch assayed from thawed aliquots (cryopreserved at −70°C) obtained during each of the four study visits. All laboratory analyte values reported are based on averages of two pretreatment and post-treatment values. Descriptive statistics included arithmetic or geometric means and frequencies (percentages). Baseline continuous variables were compared by using ANOVA, and categorical variables were compared by using χ2 analysis. Continuous variables were assessed by using both untransformed and (natural log) transformed values. Treatment effects on percentage changes in fasting and the 2-hour post-methionine load increase in tHcy levels were presented as average pretreatment level—average posttreatment level and were compared by using general linear modeling with ANCOVA. To assess the relative independent effects of the treatments, the ANCOVA adjusted for age, pretreatment levels of fasting or the 2-hour post-methionine load increase in tHcy, and pretreatment albumin.

### Results

Table 1 reveals that randomization with respect to No Ba (groups II and IV combined, n=68), Any Ba (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 B9-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

### TABLE 1. Baseline Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Vitamin B6 Placebo</th>
<th>Vitamin B6 Treatment</th>
<th>Folic Acid, Vitamin B12, and Riboflavin Placebo</th>
<th>Folic Acid, Vitamin B12, and Riboflavin Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>68</td>
<td>63</td>
<td>66</td>
</tr>
<tr>
<td>Age, y</td>
<td>61.4 (59.2–63.7)</td>
<td>58.7 (56.4–61.0)</td>
<td>60.1 (57.8–62.3)</td>
<td>60.2 (57.9–62.5)</td>
</tr>
<tr>
<td>Men, %</td>
<td>67.6 (56.7–78.6)</td>
<td>73.0 (61.7–84.4)</td>
<td>69.7 (58.6–80.8)</td>
<td>70.8 (59.6–82.0)</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>81.5 (77.6–85.3)</td>
<td>81.2 (77.2–85.2)</td>
<td>81.0 (77.0–84.9)</td>
<td>81.7 (77.8–85.7)</td>
</tr>
<tr>
<td>Albumin, g/L</td>
<td>45.3 (44.7–46.0)†</td>
<td>46.4 (45.6–47.1)‡</td>
<td>46.1 (45.4–46.8)</td>
<td>45.6 (44.8–46.3)</td>
</tr>
<tr>
<td>Creatinine, mg/dL</td>
<td>1.03 (0.98–1.07)</td>
<td>1.05 (1.00–1.10)</td>
<td>1.03 (0.98–1.08)</td>
<td>1.05 (1.00–1.10)</td>
</tr>
<tr>
<td>Fastig Hcy, μmol/L*</td>
<td>8.7 (8.2–9.2)</td>
<td>8.7 (8.2–9.2)</td>
<td>8.5 (8.0–9.1)</td>
<td>8.8 (8.3–9.4)</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>
<td>16.7 (15.2–18.2)</td>
<td>17.8 (16.3–19.5)</td>
</tr>
<tr>
<td>Folate, mg/mL*</td>
<td>9.7 (8.8–10.8)</td>
<td>10.2 (9.2–11.4)</td>
<td>10.4 (9.4–11.5)</td>
<td>9.5 (8.6–10.6)</td>
</tr>
<tr>
<td>Vitamin B12, pg/mL*</td>
<td>344 (318–373)</td>
<td>334 (308–363)</td>
<td>330 (304–358)</td>
<td>350 (322–379)</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>
<td>50.2 (43.8–57.6)</td>
<td>47.0 (41.0–54.0)</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.

All parentheses represent 95% confidence intervals. PML indicates post-methionine load.
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, approximately 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 investigators28 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 supraphysiological 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 Anti-oxidant Cardiovascular Disease Study (WACS) trials,30 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.31–33 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

This manuscript is dedicated to the memory of our friend and colleague, Dr Gintaras Liaugaudas. Support for the work described was provided in part by the National Heart, Lung and Blood Institute grant No. R01-HL-56908-01A1, the United States Department of Agriculture, Agricultural Research Service agreement No.58-1950-1-310, and a grant from Roche Vitamin Division. The contents of this publication do not necessarily reflect the views or policies of the US Department of Agriculture, nor does mention of trade names, commercial products, or organizations imply endorsement by the US Government. We thank Anna Iosifidis, BS, Marie Nadeau, MS, and Bonnie Souppa, BS, for their excellent technical assistance.

Table 2. Values After 12 Weeks of Treatment, Adjusted for Age, Pre-treatment Albumin Level, and Pre-treatment Level of Each Analyte or Change in Analyte Listed

<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></td>
<td>N 12-wk Δ in folate, ng/mL*</td>
<td>Placebo N 12-wk Δ in folate, ng/mL*</td>
</tr>
<tr>
<td>68</td>
<td>13.9 (8.2 to 20.9)</td>
<td>9.1 (4.2 to 15.2)</td>
</tr>
<tr>
<td>63</td>
<td></td>
<td>–2.2 (–3.8 to –0.5)††</td>
</tr>
<tr>
<td>12-wk Δ in vitamin B6, nmol/mL*</td>
<td>1.7 (–9.1 to 6.5)†‡</td>
<td>270.0 (231.9 to 312.9)†‡</td>
</tr>
<tr>
<td>64.5 (36.4 to 94.7)</td>
<td></td>
<td>75.5 (45.5 to 107.7)†</td>
</tr>
<tr>
<td>Fasting tHcy, μmol/L</td>
<td>1.7 (–9.1 to 6.5)†‡</td>
<td>8.8 (8.5 to 9.1)†‡</td>
</tr>
<tr>
<td>6.2 (7.9 to 8.6)</td>
<td></td>
<td>7.7 (7.4 to 8.0)‡</td>
</tr>
<tr>
<td>12-wk Δ in fasting tHcy, μmol/L</td>
<td>0.4 (–0.8 to –0.1)</td>
<td>0.15 (–0.21 to 0.50)††</td>
</tr>
<tr>
<td>15.3 (14.4 to 16.2)</td>
<td></td>
<td>–0.97 (–1.3 to –0.6)‡‡</td>
</tr>
<tr>
<td>PML increase in tHcy, μmol/L</td>
<td>1.4 (4.0 to 2.2)</td>
<td>14.4 (13.6 to 15.3)</td>
</tr>
<tr>
<td>2.1 (–3.0 to –1.3)</td>
<td></td>
<td>15.1 (14.3 to 16.1)</td>
</tr>
<tr>
<td>12-wk Δ in the PML increase in tHcy, μmol/L</td>
<td>14.4 (4.0 to 2.2)</td>
<td>14.5 (13.7 to 15.4)</td>
</tr>
</tbody>
</table>

PML: Plasma methylmalonic acid level; VISP: Vitamin Intervention for Stroke Prevention; HOPE: Heart Outcomes Prevention Evaluation; WACS: Women’s Anti-oxidant Cardiovascular Disease Study; PML: Plasma methylmalonic acid level.

All parentheses represent 95% confidence intervals.

*Indicates geometric mean; square-root transformation used for 12-wk Δ in vitamin B12 (pg/mL).

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

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 Anti-oxidant Cardiovascular Disease Study (WACS) trials,30 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.31–33 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

This manuscript is dedicated to the memory of our friend and colleague, Dr Gintaras Liaugaudas. Support for the work described was provided in part by the National Heart, Lung and Blood Institute grant No. R01-HL-56908-01A1, the United States Department of Agriculture, Agricultural Research Service agreement No.58-1950-1-310, and a grant from Roche Vitamin Division. The contents of this publication do not necessarily reflect the views or policies of the US Department of Agriculture, nor does mention of trade names, commercial products, or organizations imply endorsement by the US Government. We thank Anna Iosifidis, BS, Marie Nadeau, MS, and Bonnie Souppa, BS, for their excellent technical assistance.
References

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 and Jacob Selhub

doi: 10.1161/hq0302.105369

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2002 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/22/3/488