Enhanced Reduction of Fasting Total Homocysteine Levels With Supraphysiological Versus Standard Multivitamin Dose Folic Acid Supplementation in Renal Transplant Recipients

Andrew J. Beaulieu, Reginald Y. Gohh, Haewook Han, David Hakas, Paul F. Jacques, Jacob Selhub, Andrew G. Bostom

Abstract—The mild fasting hyperhomocysteinemia commonly observed in chronic (ie, ≥6 months posttransplantation) renal transplant recipients (RTRs) can be effectively treated with combined B-vitamin supplementation featuring supraphysiological doses of folic acid. There are no controlled data evaluating the comparative efficacy of supraphysiological versus standard multivitamin dose folic acid supplementation in reducing fasting total homocysteine (tHcy) levels among RTRs. We block-randomized 60 chronic, stable RTRs on the basis of their screening fasting tHcy level to 3 groups of 20 subjects treated for 12 weeks with folic acid at either 2.4 (group 1), 0.4 (ie, standard multivitamin dose) (group 2), or 0.0 (group 3) mg/d. All 60 study participants also received 50 mg/d vitamin B6 and 0.4 mg/d vitamin B12. The mean percent reductions (±SEM) in fasting tHcy were as follows: group 1, 32.3±2.4%; group 2, 23.4±2.3%; and group 3, 19.1±2.3%. ANCOVA accounting for the pretreatment matching and adjusted for pretreatment levels of fasting tHcy, folate, and albumin; change in creatinine during the study; and cyclosporine A use revealed significant overall group differences (P=0.005) and significant differences between groups 1 and 2 (P=0.038) and groups 1 and 3 (P=0.001), but not between groups 2 and 3 (P=0.153). Moreover, a χ² analysis of participants with pretreatment tHcy levels ≥15 μmol/L (n=29) indicated that a significantly greater proportion of those in group 1 achieved posttreatment levels <12 μmol/L: group 1, 5 of 10 (50%); group 2, 1 of 11 (9%); and group 3, 0 of 8 (0%) (P=0.016; test of trend P=0.007). We conclude that a supraphysiological dose of folic acid is superior to standard multivitamin dosing for the reduction of fasting tHcy levels in chronic RTRs. (Arterioscler Thromb Vasc Biol. 1999;19:2918-2921.)

Key Words: hyperhomocysteinemia ■ renal insufficiency ■ treatment ■ controlled trial
may require greater than standard US multivitamin amounts of folic acid to optimally lower fasting tHcy levels. To address this question, we performed a block-randomized, controlled study of the comparative efficacy of supraphysiological dose (2.4 mg/d) versus standard US multivitamin dose (0.4 mg/d) and placebo dose (0.0 mg/d) folic acid supplementation in reducing fasting tHcy levels among chronic (ie, ≥6 months posttransplantation), stable RTRs. Previously, we demonstrated the significant independent effect of 50 mg/d vitamin B6 on the postmethionine loading increase in tHcy levels among RTRs. Others have highlighted the potential adjunctive therapeutic role of oral vitamin B12 at 0.4 to 2.0 mg/d for the reduction of fasting tHcy, particularly in persons ≥55 years of age. In light of these collective data, the most appropriate tHcy-lowering regimen for clinical trials designed to test the hypothesis that such treatment may reduce arteriosclerotic outcomes among RTRs would be a combination of folic acid and vitamins B6 and B12. Accordingly, we conducted our folic acid dosing study among RTRs uniformly assigned 50 mg/d vitamin B6 and 0.4 mg/d vitamin B12.

### Methods

The institutional review board at Rhode Island Hospital (Providence, RI) approved the study protocol, and all participants provided written informed consent. Participants were 60 stable RTRs (ie, they were ≥6 months posttransplantation with no clinical evidence of acute renal graft rejection) who did not use supplements or had abstained from taking any supplements containing folic acid, vitamin B12, or vitamin B6 for ≥6 weeks before the screening visit for the study. No subjects had taken trimethoprim/sulfamethoxazole for ≥2 months before this screening visit. Participants were matched on the basis of their screening (initial) fasting tHcy levels according to the following algorithm: tHcy <15 μmol/L, matched within ±2 μmol/L; tHcy 15 to 25 μmol/L, matched within ±3 μmol/L; and tHcy >25 μmol/L, matched within ±4 μmol/L. They were then randomly assigned in blocks of 1 of 3 regimens: group 1, folic acid 2.4, vitamin B6 50, and vitamin B12 0.4 mg/d; group 2, folic acid 0.4, vitamin B6 50, and vitamin B12 0.4 mg/d (n=20); and group 3, folic acid 0.0, vitamin B6 50, and vitamin B12 0.4 mg/d (n=20). 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.

Fasting (10 to 14 hours) blood samples were collected twice before treatment and twice during week 12 of treatment, as described elsewhere. Plasma tHcy levels were determined by high-performance liquid chromatography with fluorescence detection, plasma folate levels were measured by a microbiological (Lactobacillus casei) assay, plasma pyridoxal 5'-phosphate (PLP) levels were measured by radioenzymatic (tyrosine decarboxylase) assay, and plasma vitamin B12 levels were ascertained by radioassay. Serum creatinine and albumin were measured by standard automated clinical chemistry laboratory techniques. To eliminate interassay variability, all analytes were batch-assayed from aliquots (which had been cryopreserved at −70°C) obtained during each of the 4 study visits.

Using fasting tHcy data obtained from all 60 participants at the initial pretreatment screening, with 20 subjects block-randomized to each of the 3 groups, we estimated that there was 80% power at a 2-tailed a value of 0.05 to detect a 10% absolute difference between the 2.4- and 0.4-mg/d folic acid treatments, as well as a 10% absolute difference between the 0.4- and 0.0-mg/d folic acid treatments. All laboratory analyte values reported are based on averages of 2 pretreatment and posttreatment values. Descriptive statistics included means (±SEM), or 95% confidence intervals (CI) and frequencies (percentages). Baseline continuous variables were compared by ANOVA, and categorical variables by χ2 analysis. Continuous variables were assessed with both untransformed and natural log–transformed values. Treatment effects on percentage changes in fasting tHcy levels were presented as (average pretreatment level minus average posttreatment level) divided by average pretreatment level) times 100 and were compared by general linear modeling with ANCOVA. To assess the relative independent effects of the 3 treatments, the ANCOVA accounted for the pretreatment matching and adjusted for the pretreatment levels of fasting tHcy, folate, and albumin; the change in creatinine during the study; and use of cyclosporine A immunosuppression. A χ2 analysis was performed among participants with pretreatment tHcy levels ≥15 μmol/L to assess the relative proportion of such individuals in each treatment group who achieved posttreatment levels <12 μmol/L. Furthermore, an adjusted logistic regression analysis was conducted to compare the relative proportion of individuals (odds ratio, with 95% CI) with pretreatment levels of ≥15 μmol/L in the high-dose versus standard multivitamin dose folic acid groups who achieved posttreatment levels <12 μmol/L. Overall compliance with the study capsules was confirmed by assessing the mean increase (percentage change) in plasma PLP and vitamin B12 levels among all 60 participants by

### TABLE 1. Baseline Characteristics by Treatment Group

<table>
<thead>
<tr>
<th>Group</th>
<th>Female sex, No. (%)</th>
<th>Cyclosporine use, No. (%)</th>
<th>Age, y</th>
<th>tHcy, μmol/L</th>
<th>Folate, ng/mL</th>
<th>PLP, nmol/L</th>
<th>B12, pg/mL</th>
<th>Creatinine, mg/dL</th>
<th>Albumin, mg/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7 (35)</td>
<td>16 (80)</td>
<td>46 (2)</td>
<td>16.7 (1.7)†</td>
<td>10.0 (1.0)†</td>
<td>41.9 (4.3)†</td>
<td>453 (37)†</td>
<td>2.0 (0.1)†</td>
<td>4.1 (0.1)†</td>
</tr>
<tr>
<td>2</td>
<td>7 (35)</td>
<td>16 (80)</td>
<td>50 (2)</td>
<td>17.5 (1.7)†</td>
<td>8.8 (1.0)</td>
<td>42.5 (4.3)</td>
<td>432 (37)</td>
<td>1.9 (0.1)</td>
<td>4.2 (0.1)†</td>
</tr>
<tr>
<td>3</td>
<td>7 (35)</td>
<td>17 (85)</td>
<td>44 (2)</td>
<td>17.3 (1.7)</td>
<td>9.1 (1.0)</td>
<td>43.6 (4.3)</td>
<td>392 (37)</td>
<td>1.8 (0.1)</td>
<td>4.3 (0.1)</td>
</tr>
</tbody>
</table>

*Based on χ² or ANOVA.
†Mean ± SEM.
‡Mean (95% CI).

### TABLE 2. Treatment Effects on Fasting tHcy Levels

<table>
<thead>
<tr>
<th>Group</th>
<th>Final On-Treatment Fasting tHcy Levels, μmol/L; Percent Reduction in Fasting tHcy</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11.3* (10.5–12.1); 32.3%† (2.4%)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>13.4* (12.6–14.2); 23.4%† (2.3%)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>14.0* (13.2–14.8); 19.1%† (2.3%)</td>
<td></td>
</tr>
</tbody>
</table>

* Mean (95% CI).
† Mean ± SEM.

P values based on “matched” ANOVA adjusted for baseline tHcy, folate, and albumin; change in creatinine; and cyclosporin A use, from overall F test and Fisher’s least significant difference test for a priori hypothesized between-group differences (see text for details).
paired t tests. Reported probability values were based on 2-tailed calculations. All statistical analyses were performed with SYSTAT software (version 7.0.1, SPSS).

Results
As depicted in Table 1, block-randomization was successful with respect to the baseline covariables listed. All 60 patients completed the entire study protocol. Average compliance by pill count was 95.2%, a finding confirmed by marked, significant (P<0.001) increases in the mean plasma levels of both PLP (+438.4%) and vitamin B_{12} (+62.9%). ANCOVA (see Table 2) accounting for the pretreatment matching and adjusted for pretreatment levels of fasting tHcy, folate, and albumin; change in creatinine during the study; and cyclosporin A use revealed significant (by F test) overall group differences (P=0.005) in tHcy-lowering treatment responsiveness, with significant (by Fisher’s least significant difference tests) between-group differences comparing groups 1 and 2 (group 1, 32.3±2.4% reduction versus group 2, 23.4±2.3% reduction; P=0.038) and groups 1 and 3 (group 1, 32.3±2.4% reduction versus group 3, 19.1±2.3% reduction; P=0.001), but not groups 2 and 3 (group 2, 23.4±2.3% reduction versus group 3, 19.1±2.3% reduction; P=0.153).

We have presented ANCOVA results based on the untransformed continuous-variable data only because use of the transformed data did not alter the findings. A simple χ² analysis of participants with pretreatment tHcy levels ≥15 μmol/L (n=29) indicated that a significantly greater proportion of those in group 1 achieved posttreatment levels <12 μmol/L; group 1, 5 of 10 (50%); group 2, 1 of 11 (9%); and group 3, 0 of 8 (0%) (Fisher’s exact test P=0.016; Cochran’s test of linear trend P=0.007). Finally, in a direct post hoc comparison of group 1 (2.4 mg folic acid) and group 2 (0.4 mg folic acid), a logistic regression analysis revealed that the odds ratio for achieving a posttreatment fasting tHcy level of <12 μmol/L among those with pretreatment levels of ≥15 μmol/L was 7.7 (95% CI, 1.1 to 57.5; P=0.047), group 1 relative to group 2, after adjustment for pretreatment tHcy, folate, and creatinine levels or change in creatinine levels during the study.

Discussion
Our findings represent the initial controlled evidence of a dose response to supplemental folic acid, in terms of reductions in fasting tHcy levels, among chronic, stable RTRs. Specifically, we have demonstrated that a supraphysiological dose (2.4 mg/d) of folic acid, relative to a standard US multivitamin dose (0.4 mg/d), affords significantly greater reductions in fasting tHcy levels, gauged as either changes in mean levels or the proportion of individuals with mild pretreatment hyperhomocysteinemia whose tHcy levels were normalized by treatment.

Cereal grain flour products fortified voluntarily by the manufacturer with 140 μg folic acid/100 g flour began appearing in the United States after March, 1996. The availability of such products (ie, all enriched wheat, corn, or rice flour goods) was widespread in southeast New England by July 1997 (John Watson, President, Watson Foods, New Haven, Conn, personal communication) and was mandated throughout the United States by January 1, 1998. All the RTRs participating in the present investigation had been consuming such products for ≥6 months before their initial screening examination and throughout the course of the study. Findings from the population-based Framingham Offspring Study indicate a dramatic impact of folic acid fortification in the general population among non–supplement users: a doubling of plasma folate levels, with a >90% decline in the prevalence of low plasma folate (ie, <3 ng/mL) status and a 50% decline in the prevalence of mild (ie, tHcy >13 μmol/L) fasting hyperhomocysteinemia. The very low point prevalence of plasma folate <3 ng/mL (ie, 2 of 60, or 3.3%) in the renal transplant recipients examined in the present study is completely consistent with the prevalence of folate <3 ng/mL (1.7%; 95% CI, 0.0% to 5.4%) among 248 nonusers of supplements in the Framingham Offspring Study similarly examined after the advent of fortification.

Moreover, we recently reported postfortification-era data comparing fasting plasma tHcy levels determined in a total of 86 RTRs with stable allograft function and 175 coronary artery disease patients whose serum creatinine was ≤1 mg/dL. The prevalence of fasting tHcy levels ≥12 μmol (69.8% versus 10.9%, P<0.001) was markedly increased in the RTRs despite a much younger mean age and a relative preponderance of women. The odds ratio (95% CI) for a tHcy level ≥12 μmol, when the RTRs were compared with coronary artery disease patients, after adjustment for potential confounding by age, sex, albumin, and vitamin status, was 20.3 (7.9 to 52.2). These findings prompted us to conclude that in the present era of folic acid–fortified cereal grain flour, hyperhomocysteinemia is much more common in stable RTRs than in coronary artery disease patients. Consequently, we contend that RTRs may be a preferable high-risk target population for controlled trials conducted in the United States evaluating the tenable hypothesis that lowering tHcy levels will reduce arteriosclerotic outcomes. The results from the folic acid dosing study reported here lend further support to this contention, from another perspective. The present data argue strongly that in the context of a controlled clinical outcomes trial, the RTR population, relative to any US target population with normative renal function, would be much less

| Table 3: Comparison of Final On-Treatment tHcy Values for Maintenance Dialysis Patients and RTRs |
|-----------------|-----------------|-----------------|-----------------|
| Group           | Treatment Regimen, Oral Dose/d | tHcy, μmol/L, Mean±SEM | Proportion (%) of Subjects With tHcy <12 μmol/L |
| Maintenance dialysis* (n=15) | 16.0 mg FA; 1.0 mg B_{12}; 110 mg B_{6}† | 21.9±0.5 | 1/15 (6.7) |
| Renal transplant‡ (n=20) | 2.4 mg FA; 0.4 mg B_{12}; 50 mg B_{6}† | 11.3±0.4 | 13/20 (65.0) |

FA indicates folic acid.
*From Bostom et al.†
‡Includes total contents of routine dialysis multivitamin, plus study capsule.
responsive to “drop-in” effects of over-the-counter multivitamin usage. However, RTRs would be very responsive to supraphysiological-dose folic acid supplementation, particularly when assessed by the overall percentage who achieve normal fasting tHcy levels. Last, the ability to normalize fasting tHcy levels with supraphysiological-dose folic acid–based supplementation among the preponderance of RTRs with fasting hyperhomocysteinemia distinguishes this patient population from the ESRD population, who are largely refractory to such therapy.10,11,22 For example, Table 3 illustrates final on-treatment tHcy values of the 20 renal transplant recipients in the present study compared with 15 ESRD patients on maintenance dialysis we studied earlier.10 Thirteen (65.0%) of the renal transplant recipients had final on-treatment tHcy levels maintained at <12 μmol/L, versus only 1 (6.7%) of the dialysis patients, despite a treatment regimen in the latter group that included 6-fold greater amounts of folic acid and 2-fold greater amounts of both vitamins B12 and B6.

The pathogenesis of the persistent mild hyperhomocysteinemia characteristic of patients with chronic renal insufficiency, including RTRs, remains unknown.22 Impaired homocysteine metabolism in chronic renal insufficiency could result from losses of normal intrarenal homocysteine metabolism, the adverse effect of even subclinical uremia on extrarenal homocysteine metabolism, or combined intrarenal and extrarenal defects. Ultimately, whatever specific metabolic abnormalities in homocysteine metabolism occur among individuals with chronic renal insufficiency, they appear to cause a markedly increased folate requirement to maintain normative fasting tHcy levels in this patient population.

In conclusion, we have demonstrated that a supraphysiological dose of folic acid is superior to standard multivitamin dosing for the reduction of fasting tHcy levels in chronic RTRs. These findings have important implications for the design of clinical trials testing the tenable hypothesis that lowering tHcy levels may reduce arteriosclerotic outcomes among RTRs and patients with chronic renal insufficiency in general.

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