Increased dietary micronutrients decrease serum homocysteine concentrations in patients at high risk of cardiovascular disease¹⁻³

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ABSTRACT

Background: Elevated blood homocysteine is a risk factor for cardiovascular disease. A 5-μmol/L increase is associated with an ≈70% increase in relative risk of cardiovascular disease in adults. For patients with established risk factors, this risk is likely even greater.

Objective: Effects of increased dietary folate and recommended intakes of vitamins B-12 and B-6 on serum total homocysteine (tHcy) were assessed in individuals at high risk of cardiovascular disease.

Design: This trial was conducted at 10 medical research centers in the United States and Canada and included 491 adults with hypertension, dyslipidemia, type 2 diabetes, or a combination thereof. Participants were randomly assigned to follow a prepared meal plan (PMP; n = 244) or a self-selected diet (SSD; n = 247) for 10 wk, which were matched for macronutrient content. The PMP was fortified to provide 100% of the recommended dietary allowances for 23 micronutrients, including folate.

Results: Mean folate intakes at 10 wk were 601 ± 143 μg/d with the PMP and 270 ± 107 μg/d with the SSD. With the PMP, serum tHcy concentrations fell from 10.8 ± 5.8 to 9.3 ± 4.9 μmol/L (P < 0.0001) between weeks 0 and 10 and the change was associated with increased intakes of folate, vitamin B-12, and vitamin B-6 and with increased serum and red blood cell folate and serum vitamin B-12 concentrations. tHcy concentrations did not change significantly with the SSD.

Conclusions: The PMP resulted in increased intakes and serum concentrations of folate and vitamin B-12. These changes were associated with reduced serum tHcy concentrations in persons at high risk of cardiovascular disease. Am J Clin Nutr 1999;70:881–7.

KEY WORDS Homocysteine, dietary folate, vitamin B-12, vitamin B-6, cardiovascular disease, randomized clinical trial, humans

INTRODUCTION

An elevated serum total homocysteine (tHcy) concentration is now recognized as an important, independent risk factor for cardiovascular disease (1–3). A rapidly growing number of case-control (1), retrospective (1, 3–5), and prospective (6–10) studies have reported a relation between elevated tHcy concentrations and early–onset vascular disease in coronary, cerebral, and peripheral arteries. Graham et al (11) detected hyperhomocysteinemia in 42% of patients with cerebrovascular disease, 28% with peripheral vascular disease, and 30% with coronary vascular disease. From the Physicians' Health Study, it was reported that baseline tHcy concentrations were markedly higher in men who later had myocardial infarctions than in matched control subjects, and that ≈7% of cases could be attributed to elevated tHcy concentrations (7). In a Framingham cohort that was older and more representative of the US population, the prevalence of high tHcy concentrations was 29% (12). More recently, Nygard et al (10) observed a strong, graded association between plasma tHcy concentrations and overall mortality in patients with angiographically confirmed coronary artery disease.

Elevated tHcy concentrations can be the result of genetic disorders (13, 14) or vitamin deficiencies (15, 16). Observational
studies have reported an inverse correlation between serum tHcy concentrations and blood concentrations or dietary intakes of folate and vitamins B-12 and B-6 (12, 17, 18), which are cofactors in the metabolic processing of homocysteine. Morrison et al (19) reported a significant inverse relation between serum folate concentrations and risk of fatal coronary artery disease. Clinical studies have shown that folate and vitamin B supplementation can reduce elevated tHcy concentrations (15, 20–23) and have generally concluded that administration of supplemental folate or a combination of folate, vitamin B-6, and vitamin B-12 is an effective means of improving hyperhomocysteinemia.

In the present study, we examined the effects of increased dietary folate and B vitamins as part of a prepared meal plan (PMP) or self-selected diet (SSD) on serum tHcy concentrations in persons with hypertension, dyslipidemia, type 2 diabetes, or a combination of these disorders who were participants in the Cardiovascular Risk Reduction Dietary Intervention Trial (24). The PMP consisted of prepackaged, nutritionally balanced meals fortified to provide ≥100% of the National Academy of Sciences recommended dietary allowance (RDA) for 22 vitamins and minerals, including vitamins B-12 and B-6, and twice the RDA for folate (25). The SSD consisted of foods chosen by the participants based on the exchange list system. Both interventions were designed to meet the macronutrient recommendations of major government and health organizations. As reported previously (24), a PMP produced greater improvements in systolic blood pressure (SBP), diastolic blood pressure (DBP), and weight loss than did an SSD, whereas reductions in cholesterol and glucose were comparable with both interventions.

SUBJECTS AND METHODS

This study was part of a larger trial to evaluate the effects of a PMP and SSD on traditional cardiovascular disease risk factors. Results of that study were reported elsewhere (24). The trial was conducted at 10 clinical centers after approval of the protocol by the institutional review board at each of the centers. Written, informed consent was obtained from each participant.

Subjects

Adult men and women were recruited through outpatient clinics and advertisements. To be eligible for the study, subjects had to be 25–70 y of age, have a body mass index (BMI; in kg/m²) ≤42, and have hypertension, dyslipidemia, type 2 diabetes, or a combination thereof.

Subjects with hypertension had to be taking no antihypertensive medication and have an average sitting SBP and DBP of 140–180 and 90–105 mm Hg, respectively, or have been stabilized (average sitting SBP and DBP of 135–180 and 85–100 mm Hg, respectively) for ≥1 mo before the study after taking antihypertensive medication. Subjects with dyslipidemia had to be taking no lipid-lowering medication and have a total cholesterol concentration of 5.7–7.8 mmol/L (220–300 mg/dL), a triacylglycerol concentration of 2.3–11.3 mmol/L (200–1000 mg/dL), or both, or have been stabilized for ≥1 mo before the study after taking lipid-lowering medication and a cholesterol concentration of 5.2–6.7 mmol/L (200–260 mg/dL), a triacylglycerol concentration of 2.3–11.3 mmol/L (200–1000 mg/dL), or both. Subjects with type 2 diabetes had to be taking no hypoglycemic agents and have a fasting blood glucose concentration > 7.8 mmol/L (140 mg/dL) and a glycated hemoglobin (Hb A₁c) concentration ≤200% of the median normal value (≤15.4%), or have been stabilized [HbA₁c concentration 100–175% of the median for assay (7.7–13.4%)] for ≥1 mo before the study after taking oral hypoglycemic agents.

Those subjects who had other chronic or life-threatening diseases (including renal disease), who were receiving insulin treatment, who refused to discontinue vitamin or mineral supplement use, or who had an alcohol- or substance-abuse problem and women who were pregnant or lactating or not practicing a medically approved method of birth control were excluded.

Study design

Baseline period

A 4-wk baseline period (week −4 to week 0) preceded the 10-wk treatment period. During this time, participants were advised to maintain their usual diets, completed two 3-d food records, and were seen weekly for blood pressure and weight measurements.

Nutrition prescriptions

At week −2, before randomization, a nutrition prescription was calculated for each patient by using the Harris-Benedict equation (26) to estimate the individual’s energy needs for weight maintenance or loss. Each energy intake prescription had an 840-kJ window; the minimum prescription was 5040–5876 kJ/d. Weight loss was limited to 1 kg/wk and to a total of 11 kg for the intervention. Participants not desiring weight loss were prescribed an isocaloric diet. Nutrition counseling was provided to all participants at weeks 0 and 2, but no further diet-specific instruction was provided.

Randomization

At week −2, 560 participants were randomly assigned by the coordinating center at Oregon Health Sciences University to either the PMP or SSD, stratified by clinic site and 4 diagnostic categories: 153 with hypertension, 162 with dyslipidemia, 148 with type 2 diabetes, and 97 with any combination of these disorders.

Treatment period

Participants consumed either the PMP or SSD (described below) for 10 wk and were seen every 2 wk for measurements of blood pressure and weight and collection of 3-d food records. Fasting blood samples were collected at weeks 0 and 10 for measurement of serum tHcy, folate, and vitamin concentrations, and were frozen at −20°C until analyzed.

Dietary intervention

Both diets provided ~20% of energy as fat, 55–60% as carbohydrate, and 15–20% as protein. The meals were produced by the Campbell Soup Company (Camden, NJ) and included 6 breakfasts, 8 lunches, 10 dinners, and 6 snack selections. Participants ordered meals at clinic visits, and the meals were delivered to their homes.

Prepared meal plan

The PMP met nutritional guidelines for sodium, total and saturated fat, cholesterol, refined sugars, fiber, and complex carbohydrates and was fortified with vitamins and minerals to provide 400 µg ofolate/d (twice the RDA) and ≥100% of the RDA for adults (25, 27, 28) for 22 nutrients, with the exceptions of vitamin D and copper (77% and 91% of the RDA, respectively).
Subjects were instructed to consume daily one breakfast, one lunch, and one dinner, including at least one serving of fruit, vegetables, and low-fat dairy products. Prepared snacks or additional meals were used to adjust the energy content of the diet. One food selection was allowed daily from a bonus list; the list included one alcoholic beverage or the energy equivalent in fruit, vegetables, or low-fat dairy products.

Self-selected diet

The SSD group was instructed to consume a fixed number of servings from each of the exchange lists of the American Dietetic and American Diabetes Associations (29), consisting primarily of breads and starches, fruit, low-fat milk, vegetables, and lean meats. The number of servings was determined by the individual’s nutrition prescription. Participants were also allowed to select one serving daily from the bonus list. Participants received a weekly monetary food allowance to compensate for the free food given to the PMP group.

Outcome measurements

Serum tHcy concentrations were determined by HPLC and electrochemical detection by using the method of Smolin and Schneider (30), as modified by Malinow et al (31), at the Oregon Regional Primate Research Center, Beaverton. Serum and red blood cell (RBC) vitamin concentrations were determined at the University of California at Davis Vitamin and Mineral Laboratory. Folate and vitamin B-12 concentrations were measured by radioimmunoassay (Bio-Rad Laboratories, Richmond, CA) and vitamin B-6 by spectrophotometry with an automated analyzer and AST/GOT kit (Sigma Diagnostics, St Louis). For vitamin B-6, the enzymatic activity of D-alanine transaminase was measured in the absence and presence of added pyridoxine, and vitamin B-6 status (coefficient ratio) was reported as the ratio of activity with and without pyridoxine (32). The nutrient composition of the foods listed on the dietary records was determined by using a licensed copy of the University of Minnesota Nutrition Coordinating Center database in Minneapolis (NDS, version 2.8, 1995) and the product content of the prepackaged meals was provided by the Campbell Soup Company.

Statistical analysis

The study was part of a parallel intervention study to compare the efficacy of a PPM with that of an SSD in producing clinically relevant changes in the major endpoints associated with hypertension, dyslipidemia, and type 2 diabetes (24). Regression relations were tested by analysis of covariance, with diet as a fixed-effect factor, and were followed by post hoc tests of correlations both within and between diets. The effect of the diets on endpoints was tested by repeated-measure analysis of variance. SAS software (SAS Institute Inc, Cary, NC) was used for the analysis. Data are presented as means ± SDs.

RESULTS

Of the 560 participants randomly assigned in the main trial, 12 dropped out of the study before beginning dietary therapy. Of the remaining 548 participants (PMP: n = 274; SSD: n = 274), sufficient serum samples for tHcy analysis at weeks 0 and 10 were available from 491 subjects (PMP: n = 244; SSD: n = 247). This group included 130 with hypertension, 143 with dyslipidemia, 132 with type 2 diabetes, and 86 with a combination of these disorders. Losses due to insufficient sample availability were similar across diagnostic categories and treatments. In the PMP group, tHcy concentrations fell from 10.8 ± 5.8 μmol/L at week 0 to 9.3 ± 4.9 μmol/L at week 10 (P < 0.0001). In the SSD group, tHcy concentrations did not change significantly between weeks 0 (11.0 ± 5.6 μmol/L) and 10 (11.1 ± 5.9 μmol/L).

With the PMP, dietary intakes of folate and vitamins B-6 and B-12 increased significantly; folate from 293 ± 109 to 692 ± 132 μg/d (P < 0.0001), vitamin B-12 from 5.4 ± 4.7 to 12.8 ± 2.8 μg/d (P < 0.0001), and vitamin B-6 from 1.9 ± 0.6 to 4.0 ± 0.7 mg/d (P < 0.0001). With the SSD, folate intakes increased slightly (from 290 ± 116 to 358 ± 139 μg/d; P < 0.0001), whereas vitamin B-12 intakes tended to decrease from 5.3 ± 4.3 to 4.9 ± 2.9 μg/d (P = 0.11) between weeks 0 and 10. For all 3 vitamins, the increase in intakes over 10 wk with the PMP differed significantly from intakes with the SSD (P < 0.0001).

Differences were also observed in serum and RBC concentrations of folate and in serum concentrations of vitamin B-12 (Table 1). With the PMP, serum and RBC folate nearly doubled. With the SSD, serum folate decreased by 9%, whereas RBC folate increased by 12%. Despite an increased vitamin B-6 intake with the PMP, neither serum nor RBC vitamin B-6 concentrations changed significantly with either diet. Serum vitamin B-12 increased by 27% in the PMP group and decreased by 6% with the SSD. The change in serum and RBC folate and serum B-12 with the PMP differed significantly from the change with the SSD.

A significant inverse relation was shown between the change in serum tHcy concentrations and the change in dietary folate and vitamin B-6 in the PMP group (Figure 1). The change in vitamin B-12 did not correlate with the change in tHcy in either group.

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\begin{align*}
\text{Table 1} & \\
\text{Dietary effects on folate, vitamin B-6, and vitamin B-12} & \\
\end{align*}
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<table>
<thead>
<tr>
<th></th>
<th>Prepared meal plan (n = 244)</th>
<th>Self-selected diet (n = 247)</th>
<th>P for change between treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum folate (nmol/L)</td>
<td>17.5 ± 10.0</td>
<td>16.8 ± 9.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>RBC folate (nmol/L)</td>
<td>567 ± 338</td>
<td>555 ± 315</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Serum vitamin B-6 (^2)</td>
<td>1.9 ± 1.2</td>
<td>1.8 ± 1.0</td>
<td>0.59</td>
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<tr>
<td>RBC vitamin B-6 (^2)</td>
<td>2.5 ± 1.9</td>
<td>2.3 ± 1.1</td>
<td>0.22</td>
</tr>
<tr>
<td>Serum vitamin B-12 (pmol/L)</td>
<td>367 ± 168</td>
<td>380 ± 202</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

\(^2\)Coefficient ratio.
FIGURE 1. Relation between changes in serum total homocysteine (tHcy) concentrations and dietary folate and vitamin B-6 intakes in participants consuming the prepared meal plan.
change in serum tHcy was also inversely related to the change in the concentrations of serum folate \( (r = -0.22, P < 0.0001) \) and RBC folate \( (r = -0.14, P < 0.01) \) in the overall population. There were no significant relations between changes in serum tHcy and changes in serum or RBC vitamin B-6 or serum vitamin B-12 concentration. The change in tHcy was best explained by a model that included changes in serum folate as well as in serum vitamins B-12 and B-6 \( (r = -0.26, P < 0.001) \). This suggests that serum measurements of vitamins B-12 and B-6 explained a small proportion of the variance in tHcy, along with serum folate. None of these relations were significantly different between treatments.

As shown in Figure 2, the change in serum tHcy concentrations in response to the 2 diets correlated significantly with baseline concentrations. Individuals in the highest quartile for baseline tHcy had the greatest change in response to diet. However, differences in the tHcy response to the 2 diets were observed in all 4 quartiles. Approximately 14% of participants had baseline serum tHcy concentrations > 15 \( \mu \text{mol/L} \), which is generally regarded as high (6). In subjects with baseline concentrations > 15 \( \mu \text{mol/L} \), serum tHcy concentrations fell 5.7 ± 5.0 \( \mu \text{mol/L} \) \( (P < 0.001) \) with the PMP and 2.3 ± 10.0 \( \mu \text{mol/L} \) \( (P = 0.26) \) with the SSD. At 10 wk, 9% of the PMP group and 14% of the SSD group had tHcy concentrations > 15 \( \mu \text{mol/L} \).

**DISCUSSION**

In this study, the consumption of the PPM, which contained natural foods fortified with vitamins and minerals to provide twice the RDA for folate and the RDA for vitamins B-6 and B-12, was associated with a greater decrease in serum tHcy concentrations than was the consumption of the SSD, which was designed to meet typical nutritional recommendations for individuals at high cardiovascular disease risk. Serum tHcy concentrations fell on average by 1.5 \( \mu \text{mol/L} \) in the PMP group, but did not change significantly in those consuming the traditional “risk reduction” diet (ie, the SSD) for 10 wk.

The 400-\( \mu \text{g/d} \) concentration of folate in the PMP was chosen in light of observations that the current RDA for folate may not provide optimal intakes with respect to tHcy reduction (1, 12). In their recent meta-analysis of studies of homocysteine and cardiovascular disease risk, Boushey et al (1) showed that homocysteine concentrations decreased as dietary intakes of folate increased from 200 to 400 \( \mu \text{g/d} \) and that homocysteine concentrations reached a plateau at folate intakes > 400 \( \mu \text{g/d} \). In our study, it appeared that serum tHcy concentrations continued to fall as folate intakes exceeded 400 \( \mu \text{g/d} \) because a higher prevalence of tHcy concentrations > 15 \( \mu \text{mol/L} \) was observed in participants consuming < 400 \( \mu \text{g/d} \) (17%) than in those consuming > 400 \( \mu \text{g/d} \) (8%; \( P < 0.01 \)). Although only a small number of participants \( (n = 41) \) consumed > 800 \( \mu \text{g/d} \), no difference in the prevalence of hyperhomocysteinemia was observed relative to those consuming < 600 \( \mu \text{g/d} \). With use of the predicted equation for the line in Figure 1 (top), one would expect a 1.4-\( \mu \text{mol/L} \) decrease in tHcy with a 400-\( \mu \text{g/d} \) increase in folate intake and a 2.2-\( \mu \text{mol/L} \) decrease in tHcy with a 600-\( \mu \text{g/d} \) increase in folate. For the PMP group, estimates of folate intake far exceeded the amount of folate fortification (400 \( \mu \text{g/d} \)) as a result of the intake of the vegetable and fruit allowances included in this plan. No relation between the change in dietary intake of folate and the change in serum tHcy was observed with the SSD. This may be a result of the relatively small change in the intake of folate that occurred in this group or because folate was not as bioavailable in the SSD as it was in the fortified foods included in the PMP.

The estimated intake of vitamin B-6 also more than doubled with the PMP, although no changes in either serum or RBC vitamin B-6 concentrations were observed. The cause of this lack of an observable effect may have been due to the vitamin B-6 assay used for these measurements. Both the dietary intake and the serum concentrations of vitamin B-12 increased with the PMP and these increases may have contributed to the observed reduction in serum tHcy concentrations. Fortification in the PMP was provided by a prepared mix and therefore it is not possible to statistically separate the effects on tHcy of one vitamin from another because of multicollinearity.

The meta-analysis of Boushey et al (1) suggested that homocysteine concentrations decreased by 2 \( \mu \text{mol/L} \) for every 100-\( \mu \text{g/d} \)

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**FIGURE 2.** Response of serum total homocysteine (tHcy) concentrations to diet in relation to baseline serum tHcy concentrations. Participants consuming either the prepared meal plan (PMP) or the self-selected diet (SSD) were divided into quartiles on the basis of their serum tHcy concentrations at week 0.
increase in dietary folate, which was a greater change than seen in the present study. This difference may have been due in part to the shorter duration of our study or to the fact that most of the participants in our study had baseline tHcy concentrations within the normal range. The greatest change in tHcy concentrations in response to the PMP was seen in subjects in the upper quartile of tHcy concentration, in which the baseline concentrations of some participants were > 15 μmol/L, a generally accepted upper limit of normal (5).

With an increase in tHcy concentrations of 5 μmol/L, the relative risk of cardiovascular disease has been estimated to increase by ~60% for men and 80% for women (1). In individuals with baseline tHcy concentrations > 15 μmol/L, concentrations fell by 5.7 μmol/L with the PMP. A change of this magnitude may markedly decrease cardiovascular risk in the presence of the more traditional and commonly clustered risk factors of hypertension, dyslipidemia, and type 2 diabetes (33, 34).

This study showed that an adequate intake of nutrients known to beneficially influence tHcy concentrations, including folate and vitamins B-12 and B-6, can effectively decrease tHcy concentrations in persons with concomitant risk factors for cardiovascular disease and that appropriate intakes of these nutrients can be achieved by fortifying a prepared diet that is low in fat, cholesterol, and sodium. We showed that a comprehensive meal plan that meets all dietary recommendations of major health organizations for reducing cardiovascular risk (28, 35–38) offers an alternative and more simplified approach to dietary therapy intended to improve cardiovascular risk factors, including serum tHcy. The effectiveness of the PMP in reducing serum tHcy concentrations, in addition to improving other risk factors, including hypertension, dyslipidemia, and hyperglycemia (24), may confer further potential benefit to persons at high risk of cardiovascular disease.

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