Serum Folate and Cardiovascular Disease Mortality Among US Men and Women

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Background: Folate has been linked to cardiovascular disease (CVD) through its role in homocysteine metabolism.

Objective: To assess the relationship between serum folate and CVD mortality.

Design: In this prospective study, serum folate concentrations were measured on a subset of adults during the Second National Health and Nutrition Examination Survey (1976-1980) and vital status ascertained after 12 to 16 years.

Setting and Patients: A national probability sample consisting of 689 adults who were 30 to 75 years of age and did not have a history of CVD at baseline.

Main Outcome Measure: Vital status was determined by searching national databases that contained information about US decedents.

Results: The associations between serum folate and CVD and all-cause mortality differed by diabetes status ($P = .04$ and $P = .03$, respectively). Participants without diabetes in the lowest compared with the highest serum folate tertile had more than twice the risk of CVD mortality after adjustment for age and sex (relative risk [RR], 2.64; 95% confidence interval [CI], 1.15-6.09). This increased risk for participants in the lowest tertile was attenuated after adjustment for CVD risk factors (RR, 2.28; 95% CI, 0.96-5.40). Serum folate tertiles were not significantly associated with total mortality, although the age- and sex-adjusted risk was increased for participants in the lowest compared with highest tertile (RR, 1.74; 95% CI, 0.96-3.15). Risk estimates for participants with diabetes were unstable because of the small sample size (n=52).

Conclusion: These data suggest that low serum folate concentrations are associated with an increased risk of CVD mortality among adults who do not have diabetes.


LOW SERUM folate concentrations have been linked to carotid artery stenosis, presumably through folate’s role in homocysteine metabolism, and by association to an increased risk of atherosclerosis. Homocysteine is formed during the metabolism of methionine, an essential amino acid released during protein digestion. Remethylation of homocysteine to methionine occurs through 2 pathways, one of which requires folate as a methyl donor and vitamin B12 as a cofactor. Thus, low serum folate concentrations could lead to elevated homocysteine concentrations. Indeed, several studies have shown that serum folate concentrations are inversely related to homocysteine concentrations in healthy individuals. Elevated homocysteine levels have been associated with an increased risk of cardiovascular disease (CVD), as summarized in recent review articles. A number of potential mechanisms through which homocysteine may be atherogenic have been proposed; homocysteine may exert a direct toxic effect on endothelial cells, promote oxidation of low-density lipoprotein, increase DNA synthesis in and promote the proliferation of smooth muscle cells, or impair platelet activity.

Nevertheless, results from prospective cohort studies of serum folate and CVD have been inconsistent; some found a significant association, whereas others found a weak or nonexistent relationship. We used data from a nationally representative sample of US adults who were followed up for a median of 14 years to test whether serum folate concentrations were associated with risk of CVD mortality independent of established CVD risk factors. We also tested whether age,
METHODS

This study used data from the NHANES II Mortality Study, a prospective study of participants examined in the Second National Health and Nutrition Examination Survey (NHANES II) (1976-1980). NHANES II collected extensive demographic, medical history, nutritional, clinical, and laboratory data on a multistage probability sample of the civilian, noninstitutionalized US population. Adults 60 to 74 years of age were oversampled 4 to 1. The examination response rate was 73%. The vital status of NHANES II participants who were 30 to 74 years of age at their examination was ascertained as of December 31, 1992, resulting in 12 to 16 years of follow-up. Vital status was assessed by searching the National Death Index and the Social Security Administration Death Master File. Cause of death was obtained from the National Center for Health Statistics Multiple Cause of Death file or death certificates and coded according to the International Classification of Diseases, Ninth Revision (ICD-9). Decedents with underlying causes of death from ICD-9 code 390 to 459 were defined as having died from CVD.

BASELINE DATA

Blood was drawn from participants at baseline in mobile examination centers. Serum samples were shipped on dry ice to the Centers for Disease Control and Prevention and stored at −20°C until analyzed. Two different assays were used to measure serum folate concentrations in NHANES II. A microbiologic method was used during the first half of the survey, whereas a radioassay (Quanta-Count folate radioassay kit; Bio-Rad Laboratories, Richmond, Calif) was used during the second half because of quality control problems with the microbiologic method. Previous analysis showed that the assay methods were not comparable throughout the full range of folate concentrations. A more recent methodologic study recommended the use of assay-specific reference ranges for evaluating folate data. For this reason, we used assay-specific tertile cut points to categorize folate status (9.5 and 16.8 nmol/L and 10.6 and 16.8 nmol/L, microbiologic and radioassay, respectively).

Red blood cell folate concentrations were also measured in NHANES II, using the same 2 assays as serum folate. Although red blood cell folate may be a better indicator of long-term folate status, we do not present risk estimates because a large percentage (26%) of the 899 eligible participants were missing these data. Serum B12 concentrations were also measured; however, we do not present these data because a nonpurified porcine intrinsic factor was used as a binder (E. Gunter, e-mail communication, April 1, 1999), making it susceptible to generating false elevations in subjects who were cobalamin deficient.

Blood pressure was measured twice in the sitting position by a physician using a mercury sphygmomanometer. The average of the 2 blood pressure readings was used in our analyses. Body mass index was calculated as weight in kilograms divided by the square of height in meters. Total serum cholesterol was determined using a Lieberman

RESULTS

Selected baseline characteristics according to serum folate tertile and diabetes status are presented in Table 1. There were fewer participants with diabetes in the lowest tertile compared with the other tertiles. Participants with diabetes who were in the lowest tertile were more likely to be current smokers, have less than a high school education, and drink fewer alcoholic beverages per week compared with participants in other tertiles (P < .05). Participants without diabetes in the lowest tertile were younger compared with participants in other tertiles, whereas participants in the highest tertile were less likely to be current smokers (P < .05). Other established CVD risk factors were not significantly associated with serum folate tertile in either group.

At the end of 12 to 16 years of follow-up, 25 participants with diabetes had died, 12 of them from CVD. The RR of dying from CVD or all causes for participants in the lowest vs highest serum folate tertile appeared to be higher among participants with diabetes compared with those without diabetes (data not shown). Risk estimates for this group are not presented because they were unstable, due to the small number of participants with diabetes (n = 52).

Among participants without diabetes, 122 died during follow-up, 49 from CVD (Table 2). Participants without diabetes in the lowest compared with the highest tertile had more than twice the risk of CVD mortality after adjustment for age and sex (RR, 2.64; 95% confidence interval [CI], 1.15-6.09). This increased risk for participants in the lowest tertile was slightly attenuated after adjustment for CVD risk factors (RR, 2.28; 95% CI, 0.96-5.40). However, there was no increased risk among participants without diabetes in the middle compared with the highest tertile (RR, 0.96; 95% CI, 0.49-1.91). Serum folate tertiles were not significantly associated with total mortality, although the age- and sex-adjusted risk was increased for participants in the lowest compared with the highest tertile (RR, 1.74; 95% CI, 0.96-3.15).

COMMENT

After following up participants in this probability sample of the US population for 12 to 16 years, we found a significant association between serum folate tertile and CVD mortality that differed by whether the participant had diabetes at baseline. Participants without diabetes at baseline who had concentrations in the lowest but not the middle tertile compared with those in the highest tertile had more than a 2-fold increased risk of dying from CVD. We found a similar pattern in risk of dying from any cause, although the magnitude of the risk was not as high as that for CVD mortality, suggesting that death from CVD accounts for most of the increased total mortality risk. Although risk estimates associated with the lowest ter-
Burchard reagent.30 Diabetes status was positive if a participant reported having been told by a physician that he or she had diabetes, was using insulin at baseline, had a 2-hour post–oral glucose challenge plasma glucose level greater than or equal to 11.1 mmol/L (200 mg/dL), or had a fasting plasma glucose level (with no challenge) greater than or equal to 7.8 mmol/L (140 mg/dL). At baseline, CVD was defined as having a history of physician-reported heart attack or stroke or symptoms of angina, as determined by a modified Rose questionnaire. Participants were asked to rate their recreational physical activity as much, moderate, or little or no activity.

STUDY POPULATION

Serum folate concentrations were measured on a 10% random sample of NHANES II participants.30 Serum folate data were missing for 11% of the eligible 899 NHANES II Mortality Study participants primarily because of disruptions caused by the change in assays. There were few differences in the distributions of selected baseline characteristics among participants with missing compared with available serum folate data (data not shown). However, serum total cholesterol levels were significantly higher (P=.05) among participants with missing data compared with those with available data. An additional 2% of participants were missing data for established CVD risk factors. Finally, another 10.5% were excluded because they had a history of CVD at baseline. After all exclusions, there were 689 participants for the main analyses.

**DATA ANALYSIS**

Relative risks (RRs) of CVD and total mortality were estimated using Cox proportional hazards models. Person-years of follow-up for each participant were calculated from baseline examination to the date of death or December 31, 1992. The proportional hazards assumption was met as judged by including time-dependent variables in initial Cox models. The RR estimates were initially adjusted for age (years) and sex. Multivariate models also included race (African American vs other), education level (<12 vs ≥12 years), smoking status (current vs past and never smokers), leisure-time activity level (light, moderate, heavy), alcohol consumption (weekly frequency of beer, wine, and liquor consumption), diabetes status (yes, no), serum total cholesterol level, systolic blood pressure, and body mass index. All analyses were run using SUDAAN31 and sample weights to account for the complex sample design,21 except those involving time-dependent variables, which were run without weights using the Statistical Analysis System.32

Interactions between serum folate concentration and selected covariates were assessed by including interaction terms in preliminary multivariate models. There were no significant interactions with age, sex, race, or alcohol consumption. In contrast, the associations between serum folate tertile and CVD and total mortality differed by whether the participant had diabetes at baseline (P=.04 and P=.03, respectively). For this reason, we performed analyses stratified by diabetes status.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Serum Folate Tertile for Participants With Diabetes (n = 52)</th>
<th>Serum Folate Tertile for Participants Without Diabetes (n = 637)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low (n = 8)</td>
<td>Middle (n = 24)</td>
</tr>
<tr>
<td>Age, y</td>
<td>60.2 ± 2.0</td>
<td>55.6 ± 3.0</td>
</tr>
<tr>
<td>Male, %</td>
<td>22.2 ± 11.5</td>
<td>49.0 ± 12.7</td>
</tr>
<tr>
<td>White, %</td>
<td>69.3 ± 15.4</td>
<td>74.1 ± 13.5</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>30.9 ± 3.0</td>
<td>28.3 ± 0.7</td>
</tr>
<tr>
<td>Serum total cholesterol, mmol/L†</td>
<td>8.6 ± 1.0</td>
<td>5.8 ± 0.2</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>139.1 ± 5.4</td>
<td>149.7 ± 7.2</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>79.9 ± 4.9</td>
<td>89.8 ± 4.4</td>
</tr>
<tr>
<td>Current cigarette smoker, %</td>
<td>58.5 ± 17.5</td>
<td>11.7 ± 7.9</td>
</tr>
<tr>
<td>Alcohol consumption, drinks per week</td>
<td>0.5 ± 0.2</td>
<td>3.3 ± 1.3</td>
</tr>
<tr>
<td>Less than high school education, %</td>
<td>65.9 ± 16.3</td>
<td>17.8 ± 5.7</td>
</tr>
<tr>
<td>Little or no leisure time activity, %</td>
<td>6.1 ± 0.3</td>
<td>10.6 ± 7.4</td>
</tr>
</tbody>
</table>

*Data are presented as mean or percentage ± SE. Serum folate tertiles were derived from all eligible participants, including those with cardiovascular disease at baseline, using cut points dependent on laboratory method (microbiologic: 9.5 and 16.8 nmol/L; radioassay: 10.6 and 16.8 nmol/L). NHANES II indicates Second National Health and Nutrition Examination Survey.

†To convert serum total cholesterol from millimoles per liter to milligrams per deciliter, divide by 0.0259.

‡Tertiles differed from each other (P<.05).

Our finding that the association between serum folate and mortality differed by diabetes status is consistent with those from previous studies that show an interaction between diabetes status and serum folate or hyperhomocysteinemia.20 Our results concerning adults without diabetes are consistent with some but not all of the findings from previous studies that investigated the link between serum folate and CVD. The only other prospective study17 to examine serum folate and CVD mortality found a 40% increased but nonsignificant risk as-
associated with folate concentrations below 9.3 nmol/L compared with those above this cutpoint using data from the NHANES I Epidemiologic Follow-up Study. This RR was lower than that estimated for participants without diabetes in our study using essentially the same cutpoint (microbiologic) as our lowest serum tertile. Another study found a 70% increased risk of dying from coronary heart disease (CHD) among participants with serum folate concentrations below 6.8 nmol/L compared with those above 13.6 nmol/L (microbiologic). Three other studies used a combination of CHD incidence and mortality as their end points. Two of these studies used lower cut points for their low serum folate group and shorter follow-up periods than ours, but neither found a significantly increased CHD risk. The third study, based on data from the NHANES I Epidemiologic Follow-up Study, found a more than 2-fold increased CHD risk among persons 35 to 55 years of age with serum folate concentrations less than 9.9 nmol/L compared with those above 21.8 nmol/L (microbiologic). Finally, plasma folate concentrations were inversely associated with carotid artery stenosis in a cross-sectional study.

One limitation of this study is that our measure of folate status is based on a single serum folate concentration. Serum folate is labile and may not be indicative of long-term folate status, because it is sensitive to fluctuations in recent intake and metabolism, potentially leading to misclassification of some individuals. Such misclassification could have weakened the associations found in this study. In addition, 2 different laboratory methods were used in NHANES II to measure serum folate concentration. We used assay-specific cut points for tertiles to reduce potential misclassification error arising from the use of 2 methods.

Another limitation is the relatively small sample size. When combined with the large SEs resulting from the complex sample design, the small sample may have contributed instability to risk estimates and could account for the borderline significance among participants without diabetes after adjustment for CVD risk factors. When we repeated the analysis to include baseline CVD cases, we found almost identical results that were statistically significant with a larger sample size and more statistical power (RR, 2.17; 95% CI, 1.02-4.62). Even though inclusion of baseline CVD cases, who may have changed their dietary or other health behaviors as a result of their disease, could introduce a bias, apparently no bias was introduced, because the results did not materially change. The small sample also prohibited stratification by smoking status, so we cannot exclude the possibility of residual confounding by cigarette smoking. In addition, the assessment of CVD used to exclude participants at baseline was based on self-report, which could have biased our findings in either direction. Finally, mortality was probably underascertained in the NHANES II Mortality Study because of the passive methods used, resulting in misclassification of some participants. Because serum folate concentration was unlikely to be related to such misclassification, risk estimates were probably unaffected; however, the statistical power may have been reduced. Such a reduction in power could account for the borderline significant risk associated with the lowest tertile among participants without diabetes.

Nonetheless, the RR of CVD mortality associated with low serum folate concentrations among participants without diabetes was greater than 2 in this study, which used a nationally representative sample, controlled for established CVD risk factors, and had a relatively long follow-up period. Our finding that the risk associated with serum folate and CVD mortality differs by diabetes status needs to be confirmed in larger studies in which risks can be reliably estimated among adults with diabetes. Among participants without diabetes, serum folate concentrations below 9.5 or 10.6 nmol/L, using microbiologic and radioassay methods, respectively, compared with those above 16.8 nmol/L were associated with a more than 2-fold increased risk of dying from CVD. Our cut points for the lowest tertile are consistent with estimates of serum folate concentrations needed to prevent an elevation of homocysteine from other studies: 9.2* and 9.1 nmol/L using a microbiologic assay and radioassay, respectively. Although the cut points used in this study were statistically derived, they suggest that a considerable proportion of the adult US population between 1976 and 1980 had serum folate concentrations that might have increased their risk of dying from CVD. Given this relatively large proportion potentially at risk, clinical trials are needed to confirm that low folate status does confer increased CVD risk and to establish adequate intake and serum concentrations for preventing CVD. If the folate-CVD relationship can be confirmed, then we will need to assess whether recent efforts to fortify the US food supply with folic acid have increased folate intakes and serum concentrations enough to decrease the proportion at risk of CVD.

| Table 2. Relative Risks and 95% Confidence Intervals of Cardiovascular Disease (CVD) Mortality and Total Mortality According to Tertile of Serum Folate Concentration Among Participants Without a History of Baseline CVD and Diabetes at Baseline, NHANES II Mortality Study, 1976-1992* |
|----------------|----------------|----------------|
| Serum Folate Tertile† | CVD mortality | Total mortality |
|                  | Deaths | Person-years | Relative risk (95% CI) | Deaths | Person-years | Relative risk (95% CI) |
| Low             | 14     | 2848         | 1.0 (Reference)       | 40     | 2848         | 1.0 (Reference)       |
| Middle          | 11     | 2809         | 0.99 (0.59-1.65)      | 33     | 2809         | 1.08 (0.50-2.27)      |
| High            | 24     | 2931         | 2.28 (0.96-5.40)      | 49     | 2931         | 2.64 (1.15-6.09)      |

*NHANES II indicates Second National Health and Nutrition Examination Survey; CI, confidence interval.
†Serum folate tertiles were derived from all eligible participants, including those with CVD at baseline, using cut points dependent on laboratory method (microbiologic: 9.5 and 16.8 nmol/L; radioassay: 10.6 and 16.8 nmol/L).
‡Adjusted for age at baseline examination, sex, race (African American, other), highest attained education level (<12, ≥12 years), current cigarette smoker (yes, no), leisure time activity level (little or none, moderate, much), weekly frequency of consuming alcohol, serum total cholesterol level, systolic blood pressure, and body mass index.
REFERENCES

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Correction

Mislabeled Headings in Table. In the Original Investigation by Loria et al titled “Serum Folate and Cardiovascular Disease Mortality Among US Men and Women,” published in the November 27, 2000, issue of the ARCHIVES (2000; 160:3258-3262), in Table 2 on page 3261, under the column heading “Serum Folate Tertile,” the column subheadings “Low” and “High” should have been transposed.