Serum Folate and Risk of Fatal Coronary Heart Disease

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Objective.—To assess the relationship between serum folate level and the risk of fatal coronary heart disease (CHD) among men and women.

Design.—Retrospective cohort study with serum folate levels measured from September 1970 to December 1972, with follow-up through 1985.

Setting.—Participants in the Nutrition Canada Survey.

Participants.—A total of 5056 Canadian men and women aged 35 to 79 years with no history of self-reported CHD.

Main Outcome Measure.—Fifteen-year CHD mortality.

Results.—A total of 165 CHD deaths were observed. We found a statistically significant association between serum folate level and risk of fatal CHD, with rate ratios for individuals in the lowest serum folate level category (<6.8 ng/mL [3 nmol/L]) compared with the highest category (>13.6 ng/mL [6 nmol/L]) of 1.69 (95% confidence interval, 1.10-2.61).

Conclusions.—These data indicate that low serum folate levels are associated with an increased risk of fatal CHD.

WELL-ESTABLISHED risk factors for coronary heart disease (CHD) include cigarette smoking, diabetes, hypertension, and elevated serum cholesterol levels.12 Although there is only limited epidemiologic evidence that folate concentration is associated with risk of CHD, folate consumption has been found to be a determinant of homocysteine-related carotid artery thickening.5,6 Moderate hyperhomocysteinemia has been linked to both cerebrovascular and CHD.7,8 Several mechanisms have been proposed,9 including the toxic effects of homocysteine on vascular endothelium, and in proliferation of arterial wall smooth muscle cells. Folate is required for the conversion of homocysteine to methionine.9 A strong inverse correlation has been noted between serum folate and homocysteine levels among subjects with elevated homocysteine levels.10

The purpose of this article is to assess the relationship between serum folate level and the risk of fatal CHD among both men and women in the Nutrition Canada Survey (NCS) cohort.

METHODS

The NCS was conducted between September 1970 and December 1972 using a 3-stage stratified cluster design. A total of 12 795 people responded to the initial invitation to participate (46% response rate); 3295 unsolicited volunteers also participated.11,12 Each survey center included a laboratory for the collection and initial processing of blood and urine samples. Blood samples were centrifuged to obtain serum, and a stabilizing solution was added to a portion of the serum for later determination of vitamin C level. All samples were then immediately frozen and shipped to a central laboratory for further biochemical analyses.13

Participants were followed up retrospectively between the ages of 35 and 79 years. Thus, those younger than 35 years at interview began follow-up when they reached the age of 35 years, while those aged 80 years or older at interview were excluded.

For editorial comment see p 1929.

The results of this article are based on the mortality experience of both solicited and unsolicited respondents. Among the 16 090 NCS participants, individuals who were interviewed as members of specially targeted populations (pregnant women, Native Indians, and Inuit) were excluded (n=2828). A total of 3568 subjects were excluded because they did not contribute follow-up to the 35- to 79-year-old age category. Of the 8792 remaining individuals, an additional 3736 were excluded, either because of self-reported heart disease (n=987) or because of missing data (n=2749), resulting in a final study population of 5056 subjects. Work to extend the follow-up period to the end of 1993 is ongoing.

We examined the following survey variables in our analysis: age; sex; respondent status (solicited or volunteer); smoking habits (never, former, current); diastolic blood pressure; history of diabetes or presence of glucose in the urine (as detected with a glucose oxidase reagent strip); serum total cholesterol level; serum vitamin C, serum vitamin A, and serum vitamin E levels; dietary folic acid consumption; use of folic acid supplements; use of vitamin and/or min-
eral supplements; serum iron level; and serum folate level. Folate levels were determined microbiologically using *Lactobacillus casei*. Serum homocysteine levels were not measured.

Age was categorized as 35 to 54, 55 to 59, 60 to 64, 65 to 69, 70 to 74, and 75 to 79 years. The serum folate levels were categorized into approximate quartiles, ranging from <6.8 nmol/L (3.0 ng/mL) to ≥13.6 nmol/L (6.0 ng/mL). We assessed food consumption using a 24-hour food recall questionnaire. United States Department of Agriculture and Canadian Health Protection Branch tables were used to convert the information on diet to nutrient intake.

Diastolic blood pressure was recorded in the survey only for subjects with a reading of 100 mm Hg or greater; only subjects who had this level or reported receiving treatment for hypertension from a physician were considered hypertensive. Fifth-phase diastolic blood pressure was measured while subjects were sitting.

The mortality history of the cohort was obtained by linking the survey file to Statistics Canada's National Mortality Database for the years 1970-1985 using the generalized iterative record linkage computer system. Coronary heart disease (8th revision of the International Classification of Diseases, rubrics 410 to 414) was selected when listed as the underlying cause of death.

We created Poisson regression models that included terms for age, sex, serum folate level, cigarette smoking, hypertension, serum cholesterol level, and diabetes status. Volunteer status and serum levels of vitamins A, C, and E did not confound the folate-CHD association and were therefore not included in the final models. Sex-specific and age-specific models were also created, as were models examining serum folate interaction by serum cholesterol levels.

**RESULTS**

Table 1 displays the baseline characteristics of study subjects. Approximately 23% of the men and 27% of the women had serum folate levels less than 6.8 nmol/L (3.0 ng/mL). There was relatively poor correlation between serum folate and dietary folate (as determined by a 24-hour food recall, data not shown).

As seen in Table 2, an increased risk of fatal CHD was associated with cigarette smoking, hypertension, elevated serum cholesterol level, diabetes, and decreased serum folate levels (lowest quartile compared with highest quartile rate ratio = 1.69, 95% confidence interval [CI] = 1.10-2.61). Risk increased in a step-wise fashion as the serum folate levels decreased (Figure). Sex-specific estimated relative risks (RRs) for folate are displayed in Tables 3 and 4 for men and women, respectively. Estimated RRs for the lowest folate category compared with the highest were greater for women (RR, 2.83; 95% CI, 1.30-6.18) than for men (RR, 1.65; 95% CI, 0.80-3.31); however, there was no statistically significant interaction by sex.

Although there was no statistically significant interaction by age, the dose-response relationship appears to be slightly stronger for individuals younger than 65 years compared with those 65 years and older (Table 5).

To test whether the serum cholesterol level modifies the relationship between serum folate level and fatal CHD risk, models were fitted with serum cholesterol levels less than 6.2 mmol/L (240 mg/dL) and 6.2 mmol/L (240 mg/dL) or higher. Coronary heart disease rate ratios according to serum folate level did not differ significantly by cholesterol status (data not shown).

There were too few exposed to folate supplements to assess risk (<1% of respondents). We found no important associations between either use of vitamin and/or mineral supplements or dietary folic acid consumption and risk of fatal CHD.

**COMMENT**

Using computerized record linkage, a total of 165 CHD deaths were identified in the Canadian Mortality Database among the 5056 subjects included in the analysis. The potential for deaths having been missed is expected to have been small given the quality of the personal identifiers, the completeness of the Canadian Mortality Database, and the accuracy of the record linkage process used by Statistics Canada.

This study observed a statistically significant inverse relationship between serum folate level and risk of fatal CHD. A previous case-control study of early onset coronary artery disease noted a reduction in risk with increased serum folate levels. Folate consumption is a...
Table 3.—Serum Folate Level and Risk of Fatal Coronary Heart Disease, Nutrition Canada Survey Cohort, 1970 Through 1985, Men*

<table>
<thead>
<tr>
<th>Serum Folate, nmoL (ng/mL)</th>
<th>Deaths, No.</th>
<th>Person-Years</th>
<th>RR†</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥13.6 (≥6.0)</td>
<td>26</td>
<td>6175</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>9.1–13.6 (4.0–6.0)</td>
<td>30</td>
<td>7577</td>
<td>1.06</td>
<td>0.64-1.83</td>
</tr>
<tr>
<td>6.8–9.1 (3.0–4.0)</td>
<td>26</td>
<td>5374</td>
<td>1.24</td>
<td>0.72-2.15</td>
</tr>
<tr>
<td>&lt;6.8 (3.0)</td>
<td>30</td>
<td>5511</td>
<td>1.36</td>
<td>0.80-2.31</td>
</tr>
</tbody>
</table>

*RR indicates estimated relative risk, and CI, confidence interval. Test for trend, P<.02.
†Adjusted for age, serum cholesterol level, smoking status, diabetes status, and diastolic blood pressure.

Table 4.—Serum Folate Level and Risk of Fatal Coronary Heart Disease, Nutrition Canada Survey Cohort, 1970 Through 1985, Women*

<table>
<thead>
<tr>
<th>Serum Folate, nmoL (ng/mL)</th>
<th>Deaths, No.</th>
<th>Person-Years</th>
<th>RR†</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥13.6 (≥6.0)</td>
<td>9</td>
<td>8252</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>9.1–13.6 (4.0–6.0)</td>
<td>13</td>
<td>8995</td>
<td>1.24</td>
<td>0.53-2.91</td>
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<tr>
<td>6.8–9.1 (3.0–4.0)</td>
<td>9</td>
<td>7614</td>
<td>1.32</td>
<td>0.52-3.27</td>
</tr>
<tr>
<td>&lt;6.8 (3.0)</td>
<td>22</td>
<td>8663</td>
<td>2.83</td>
<td>1.30-6.18</td>
</tr>
</tbody>
</table>

*RR indicates estimated relative risk, and CI, confidence interval. Test for trend, P<.04.
†Adjusted for age, serum cholesterol level, smoking status, diabetes status, and diastolic blood pressure.

Table 5.—Serum Folate Level and Risk ofFatal Coronary Heart Disease Estimated Relative Risks by Age, Nutrition Canada Survey Cohort*

<table>
<thead>
<tr>
<th>Serum Folate, nmoL (ng/mL)</th>
<th>Age, &lt;65 y</th>
<th>Age, ≥65 y</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Deaths, No.</td>
<td>Person-Years</td>
</tr>
<tr>
<td>≥13.6 (≥6.0)</td>
<td>7</td>
<td>10559</td>
</tr>
<tr>
<td>9.1–13.6 (4.0–6.0)</td>
<td>12</td>
<td>12156</td>
</tr>
<tr>
<td>6.8–9.1 (3.0–4.0)</td>
<td>10</td>
<td>9747</td>
</tr>
<tr>
<td>&lt;6.8 (3.0)</td>
<td>11</td>
<td>10614</td>
</tr>
</tbody>
</table>

*RR indicates estimated relative risk, and CI, confidence interval.
†Adjusted for age, sex, serum cholesterol level, smoking status, diabetes status, and diastolic blood pressure.

Determinant of homocysteine-related carotid artery thickening. Arterial thickening has been associated with both stroke and CHD. It has 2 significant advantages over previous work. The NCS cohort was derived from a national nutritional survey. Previous studies of folate levels in men and the elderly. The current study noted significant associations between serum folate level and fatal CHD in both men and women. It did not rely on indirect measures of an intermediate health effect, such as carotid artery thickening.

Increased risks were not restricted to individuals with extremely low serum folate levels, but were observed for individuals with normal levels as well, suggesting that current definitions of appropriate serum folate levels be reassessed. Although a significant exposure-response relationship was observed, it is possible that some of the observed reduction in risk associated with serum folate levels reflects unmeasured dietary factors for which folate is a good marker. However, serum levels of vitamins A, C, and E, also markers of a good diet, did not confound the association.

Although neither interaction was statistically significant, the magnitude of the protective effect of folic acid appeared to be greater for women than for men and slightly greater for those younger than 65 years compared with those aged 65 years and older. Relative risks for most CHD risk factors decline with age. It is not known if the increased RR among women as compared with men was a chance finding, or whether it reflects true differences in risk between the sexes.

We found no clear relationship between dietary folic acid consumption estimated by 24-hour food recall and fatal CHD. Our crude measure of dietary folic acid consumption did not include the contribution from vitamin supplements. Although a 24-hour food recall questionnaire is a valid means of determining population folate levels, within-individual variation in diet severely limits its usefulness in classifying individuals. In addition, determination of dietary folate consumption was particularly problematic in the NCS, and there was only a weak correlation between estimated dietary folate consumption and serum folate levels. There were too few exposed people to adequately assess folic acid supplements. No effect was noted for individuals reporting use of vitamin and/or mineral supplements. However, it is not known how many of these were actually taking supplements that contained folic acid.

The failure to note a dietary effect should not be taken as evidence that dietary folate was inversely associated with carotid artery stenosis in the Framingham Study. Dietary folic acid supplementation has also been associated with a reduced risk of neural tube defects. Low serum folate levels have been reported to enhance the effect of risk factors for cervical dysplasia, and low folic acid intake has been associated with an increased risk of rectal cancer.

Folic acid fortification of staple foods has been recommended for consideration as a means to reduce the risk of cancer and neural tube defects. Folic acid fortification of grain to prevent neural tube defects alone has been estimated to be cost-effective. Although folic acid is generally considered safe even at high levels of intake, high intake can mask the clinical signs of pernicious anemia, and can interfere with the effectiveness of anticonvulsants. Roughly one half of US adults on a given day consume less than the newly lowered recommended dietary allowance for folate, and an estimated 88% consume less than the levels needed to produce low, stable homocysteine levels.

Efforts should be made to increase folate consumption by a modification of the diet to include more vegetables and legumes. However, in spite of health promotion efforts, not all people will increase their dietary consumption of folate and the bioavailability of folate from food is significantly less than that from folic acid supplements. The recent decision of the Food and Drug Administration to require folic acid fortification of food products to prevent spina bifida may have the additional benefit of reducing CHD risk.

References
5. Selhub J, Jacques PF, Bostom AG, et al. Association between plasma homocysteine concentra-