Low Dietary Folate Intake Is Associated With an Excess Incidence of Acute Coronary Events
The Kuopio Ischemic Heart Disease Risk Factor Study

Sari Voutilainen, PhD, RD; Tiina H. Rissanen, MSc, RD; Jyrki Virtanen, MSc, RD; Timo A. Lakka, MD, PhD; Jukka T. Salonen, MD, PhD, MScPH

Background—Although several prospective studies have shown that low folate intake and low circulating folate are associated with increased risk of coronary heart disease (CHD), the findings are inconsistent.

Methods and Results—We studied the associations of dietary intake of folate, vitamin B\textsubscript{6}, and vitamin B\textsubscript{12} with the risk of acute coronary events in a prospective cohort study of 1980 Finnish men 42 to 60 years old examined in 1984 to 1989 in the Kuopio Ischemic Heart Disease Risk Factor Study. Nutrient intakes were assessed by 4-day food record. During an average follow-up time of 10 years, 199 acute coronary events occurred. In a Cox proportional hazards model adjusted for 21 conventional and nutritional CHD risk factors, men in the highest fifth of folate intake had a relative risk of acute coronary events of 0.45 (95% CI 0.25 to 0.81, \textit{P}=0.008) compared with men in the lowest fifth. This association was stronger in nonsmokers and light alcohol users than in smokers and alcohol users. A high dietary intake of vitamin B\textsubscript{6} had no significant association and that of vitamin B\textsubscript{12} a weak association with a reduced risk of acute coronary events.

Conclusions—The present work in CHD-free middle-aged men is the first prospective cohort study to observe a significant inverse association between quantitatively assessed moderate-to-high folate intakes and incidence of acute coronary events in men. Our findings provide further support in favor of a role of folate in the promotion of good cardiovascular health. (\textit{Circulation}. 2001;103:2674-2680.)

Key Words: cardiovascular diseases ■ diet ■ epidemiology ■ follow-up studies ■ nutrition

Folate is the generic term for compounds that have vitamin activity similar to that of pteroylmonoglutamic acid.\textsuperscript{1} Mammals lack the ability to synthesize folates de novo and require preformed folates in the diet. Naturally occurring folates are reduced derivates, and consequently, fully oxidized folic acid is found only in pharmaceutical products or in foodstuffs supplemented with folic acid. Synthetic folic acid is heat stable and approximately twice as bioactive as the folate that occurs naturally in food.\textsuperscript{2,3} The role of folates in the human body is, almost without exception, to carry 1-carbon units, eg, to interconvert homocysteine to methionine and to take part in the synthesis of DNA.\textsuperscript{4}

Homocysteine is a sulfur-containing amino acid formed from the essential amino acid methionine.\textsuperscript{5} Defects in intracellular homocysteine metabolism lead to the elevation of plasma total homocysteine (tHcy) levels. These metabolic defects can have a genetic or a nutritional background, ie, an inadequate intake of folate or vitamin B\textsubscript{6} or B\textsubscript{12} that serve as cofactors or substrates to the enzymes involved in homocysteine metabolism.\textsuperscript{5} Approximately two thirds of the cases of elevated tHcy levels have been estimated to be due to low or moderate concentrations of these vitamins,\textsuperscript{6} of which folate is considered the most important.\textsuperscript{7} In a number of cross-sectional studies, higher concentrations of plasma tHcy have been observed in patients with cardiovascular disease than in control subjects,\textsuperscript{8,9} suggesting that elevated plasma tHcy concentration is a risk factor for cardiovascular disease. This association has been confirmed in some but not in all prospective epidemiological studies.\textsuperscript{10}

Several previous epidemiological studies have addressed the inverse association between folate levels and the risk of cardiovascular disease.\textsuperscript{11–18} In these studies, subjects with lower circulating folate concentrations\textsuperscript{11–14} or lower dietary intake of folate\textsuperscript{15} have had increased risk of coronary events compared with others, although not all studies have found this association.\textsuperscript{16–18} In a recent large European multicenter case-control study with 750 cases and 800 controls, low circulating concentrations of folate and vitamin B\textsubscript{6} conferred an increased risk of atherosclerotic diseases.\textsuperscript{19}

The purpose of this prospective cohort study was to test the hypothesis that high folate intakes are associated with a decreased risk of acute coronary events in middle-aged Finnish men free of prior coronary heart disease (CHD).
Study Population
The Kuopio Ischemic Heart Disease Risk Factor Study (KIHD) is an ongoing prospective population-based cohort study designed to investigate risk factors for cardiovascular disease, atherosclerosis, and related outcomes in middle-aged men from eastern Finland.26 The study population with one of the highest recorded rates of CHD. The study protocol was approved by the Research Ethics Committee of the University of Kuopio. All subjects gave informed consent. The study population is a random sample of men living in the Kuopio city and neighboring rural communities, stratified and balanced into 4 strata: 42, 48, 54, or 60 years old at the baseline examination. A total of 2682 participants (82.9% those eligible) were enrolled in the study between 1984 and 1989. Because previous disease affects the diet, men with a prevalent CHD (n=677) were excluded from the main analyses. Of the remaining 2005 men, food record data were available for 1980 men.

Ascertainment of Follow-Up Events
The province of Kuopio participated in the multinational MONItoring of Trends and Determinants of Cardiovascular Disease (MONICA) project FINMONICA.20b The diagnostic classification applied in this study was described previously.21 According to the diagnostic classification of the events, there were 104 definite and 61 probable acute myocardial infarctions and 34 typical prolonged chest pain episodes. Of these 199 acute coronary event cases, 46 were fatal (death within next 30 days after acute coronary event) and 153 nonfatal.

Assessment of Food Consumption
Dietary intake of nutrients was assessed quantitatively with a 4-day food recording at the KIHD baseline examinations. Intake of nutrients was calculated by use of Nutrica version 2.5 software, which uses mainly Finnish values of nutrient composition of foods and takes into account food preparation losses of vitamins. The nutrient compositions of foods in Nutrica software version 2.5 were analyzed mainly in the 1990s, and it contains the latest data on vitamin contents of fruits and vegetables. Nutrica contains a comprehensive database on 1300 food items and dishes and 30 nutrients, including dietary total folate. Nutrica software is developed at the Research Center of the Social Insurance Institution of Finland.

All nutrients were adjusted for dietary energy intake by the residual method.15-22 Energy adjustment is based on the notion that a larger, more physically active person requires a higher caloric intake, which is associated with a higher absolute intake of all nutrients. Therefore, energy adjustment takes into account differences in energy requirements among individuals. The residuals were standardized by the mean nutrient intake of a subject consuming 10 MJ/d, the approximate average total energy intake in the present study population.

Assessment of Covariates
An assessment of covariates was performed as described previously.23-26 Diabetes was defined as either a previous diagnosis of diabetes or fasting whole-blood glucose concentration ≥6.7 mmol/L.

Statistical Methods
Baseline characteristics of the cohort members in folate intake fifths were expressed as means and compared by ANOVA. After energy adjustment, subjects were classified into fifths according to their mean intake of folate, B6, and B12 vitamin intake. The relationships of folate, B6, and B12 vitamins with the risk of acute coronary events were analyzed with the Cox proportional hazards models in SPSS 10.0 for Windows. Risk factor–adjusted relative hazards (risks) were estimated as the antilogarithms of coefficients in multivariate models. The CIs were estimated on the basis of the assumption of asymptotic normality of the estimates. All tests of significance were 2-sided.

Results
During the 10-year follow-up, 199 men experienced an acute coronary event. The baseline characteristics of the cohort members who developed an acute coronary event and the subjects who did not are shown in Table 1. The mean daily intake was 256 μg for folate, 1.9 mg for vitamin B6, and 9.6 μg for vitamin B12. The subjects were divided into fifths of the mean daily folate intake (<211, 211 to 236, 237 to 261, 262 to 297, and >297 μg) (Table 2). The proportion of subjects who suffered an acute coronary event during the follow-up was 12.0% among persons in the lowest folate intake fifth and 8.1% among persons in the highest folate intake fifth.

In a Cox proportional hazards model adjusted for age, examination years, coronary risk factors (serum total, LDL, and HDL cholesterol and triglyceride; urinary excretion of nicotine metabolites; maximal oxygen uptake in exercise test; body mass index; systolic blood pressure; CHD in family; and diabetes), and nutritional factors (intake of vitamins C and E, and B-carotene, fiber, and saturated fatty acids), the residuals were standardized by the mean nutrient intake of a subject consuming 10 MJ/d, the approximate average total energy intake in the present study population.

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Discussion
This prospective cohort study in middle-aged men from eastern Finland indicates that moderate-to-high daily intake
of folate is associated with greatly reduced incidence of acute coronary events. The association is strong, and adjustment for other dietary factors or traditional coronary risk factors did not attenuate the observed association. The risk-lowering effects of folate seem to be restricted mainly to study subjects without previous CHD at the study baseline.

There are several previous prospective studies concerning the association between dietary intake of folate or circulating folate concentration and CHD (Table 3). In the prospective Physicians’ Health Study,11 men with the lowest fifth of plasma folate concentrations had a 40% increased relative risk of myocardial infarction compared with those in the top 80% after adjustment for common cardiovascular risk factors. Although the association was not statistically significant, the authors concluded that low dietary intake of folate contributes to the risk of myocardial infarction. They repeated the analyses by adding plasma tHcy in the model, and the relative risk remained unchanged. The authors suggested that the increased risk for myocardial infarction at low plasma folate levels may be partly independent of homocysteine elevation. In the Nutrition Canada Survey cohort study,14 low serum folate concentrations were associated with an increased 15-year CHD mortality. In the Nurses’ Health Study,15 after control for cardiovascular risk factors, the relative risk of CHD comparing the extreme folate intake (measured by food frequency questionnaire) fifths was 0.69. The authors also found the strongest apparent benefit of a high-folate diet among women who consumed alcohol. In the Atherosclerosis Risk in Communities Study, with a short average follow-up period of 3.3 years, neither plasma folate concentration nor the dietary intake of folate had an association with CHD.16 In the prospective First National Health and Nutrition Examination Survey (NHANES I) Epidemiologic Follow-Up Study,17 with a mean 19 years of follow-up, there was a nonsignificant 41% difference in mortality from circulatory diseases for participants in the lowest fifths of serum folate concentration. In the same study, with an average 20 years of follow-up, the relative risk of CHD was greatest for persons in the lowest serum folate quarter among persons 35 to 55 years old, whereas among persons ≥55 years old, the relative risk for CHD was the greatest in persons in the highest serum folate quarter.18 The authors suggested that adults ≥55 years old are more likely to already have an atherosclerotic disease, limiting the role of low serum folate levels in the promotion of atherosclerosis. Our results agree with the previous prospective studies showing that low dietary intakes of folate or low circulating folate concentrations are associated with increased risk of CHD.

The problem with the studies based on plasma/serum folate measurements is that folate is unstable in frozen plasma and serum samples,27 and circulating folate level may not be a good indicator of the long-term dietary intake. Serum/plasma folate measurements that are usually used to assess dietary intake reflect recent dietary intake of folate.28 Red blood cell
folate is a more reliable indicator for folate intake because red blood cell folate is supposed to be an index of liver, and thus tissue, stores and reflects a 3- to 4-month intake of folate. The increased intraindividual measurement variability due to the use of plasma or serum folate measurement tends to dilute any observed association with disease risks. None of the previous studies assessed folate intake by the more reliable red blood cell folate measurements or by quantitative prospective food recording.

The mean daily dietary intake of folate in our study population was only 259 μg/d, and the mean daily intake reached the current recommended daily allowance in Nordic countries of 300 μg/d for only 20% of the subjects. The mean daily folate intake in our study is smaller than that reported

![Figure 1. Adjusted relative risk of acute coronary events in fifths of energy-adjusted folate and vitamins B6 and B12 intake in middle-aged eastern Finnish men.](http://circ.ahajournals.org/)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Folate Intake Fifths, μg/d</th>
<th>P for Trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary factors</td>
<td>&lt;211</td>
<td>211–236</td>
</tr>
<tr>
<td>Folate, μg/d*</td>
<td>187.7</td>
<td>223.6</td>
</tr>
<tr>
<td>Vitamin B&lt;sub&gt;6&lt;/sub&gt;, mg/d*</td>
<td>1.65</td>
<td>1.82</td>
</tr>
<tr>
<td>Vitamin B&lt;sub&gt;12&lt;/sub&gt;, μg/d*</td>
<td>6.91</td>
<td>7.76</td>
</tr>
<tr>
<td>Vitamin E, mg/d*</td>
<td>7.87</td>
<td>8.48</td>
</tr>
<tr>
<td>Vitamin C, mg/d*</td>
<td>45.4</td>
<td>55.1</td>
</tr>
<tr>
<td>β-Carotene, mg/d*</td>
<td>1.32</td>
<td>1.92</td>
</tr>
<tr>
<td>Fiber, g/d*</td>
<td>19.5</td>
<td>22.9</td>
</tr>
<tr>
<td>Saturated fatty acids, % of total energy</td>
<td>20.3</td>
<td>18.8</td>
</tr>
</tbody>
</table>

Biochemical and other risk factors

<table>
<thead>
<tr>
<th>Variable</th>
<th>Folate Intake Fifths, μg/d</th>
<th>P for Trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>52.1</td>
<td>52.8</td>
</tr>
<tr>
<td>Body mass index, kg/m&lt;sup&gt;2&lt;/sup&gt;</td>
<td>26.9</td>
<td>26.8</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>134.4</td>
<td>135.5</td>
</tr>
<tr>
<td>Serum total cholesterol, mmol/L</td>
<td>5.98</td>
<td>5.92</td>
</tr>
<tr>
<td>Serum LDL cholesterol, mmol/L</td>
<td>4.11</td>
<td>4.06</td>
</tr>
<tr>
<td>Serum HDL cholesterol, mmol/L</td>
<td>1.32</td>
<td>1.30</td>
</tr>
<tr>
<td>Serum triglyceride, mmol/L</td>
<td>1.22</td>
<td>1.26</td>
</tr>
<tr>
<td>Maximal oxygen uptake in exercise test, L/min</td>
<td>2.47</td>
<td>2.49</td>
</tr>
<tr>
<td>Urinary excretion of nicotine metabolites, mg/d</td>
<td>7.94</td>
<td>6.87</td>
</tr>
<tr>
<td>Coronary heart disease in family, %</td>
<td>46.0</td>
<td>43.4</td>
</tr>
<tr>
<td>Diabetes, %</td>
<td>3.0</td>
<td>3.5</td>
</tr>
<tr>
<td>Smoking, %</td>
<td>45.5</td>
<td>35.1</td>
</tr>
</tbody>
</table>

Values are means, unless indicated.
*Adjusted for energy intake.
earlier in other populations from Europe\textsuperscript{29} and the United States before\textsuperscript{15} and after\textsuperscript{28} cereal grain products began to be fortified with folic acid. We previously reported serum folate levels of KIHD study subjects measured at the KIHD study 4-year follow-up visits.\textsuperscript{13} At this visit, the mean level of serum folate concentration was 10.4 nmol/L, which is slightly lower than the approximate average concentrations of folate (12 nmol/L) in Western populations.\textsuperscript{30} The fact that in our study, subjects also have low values of folate intake increases the range of folate intake and thus statistical power to detect the association between the folate and the risk of coronary events. In populations with high intakes only, it is impossible to find an association between nutrients and disease.

The recommended daily allowance of folate in the United States had been 180 $\mu$g/d until it was changed to 400 $\mu$g/d in 1997. We believe that the new higher recommendations in both Nordic countries and the United States are more appropriate for achieving benefits for cardiovascular health. Among middle-aged Finnish men, it would be very difficult to reach the recommended 400 $\mu$g/d without food fortification or the use of supplements, because only 48 men (2.4%) in our study baseline had a mean intake of folate from normal food items of $\geq$400 $\mu$g/d. The use of vitamin supplements in Finland is very low. In this study population, only 13% of subjects used antioxidant supplements regularly. Because the commonly used vitamin supplements in the 1980s did not include folic acid, vitamin users were not excluded in the present analyses.

We found that the association between low daily folate intake and the increased risk of acute coronary events existed primarily in nonsmokers and was weak in smokers. There are 2 explanations for this difference. First, the mean daily intake of folate was significantly lower in smokers than in nonsmokers. When the subjects with high intakes are absent, it may be difficult to find an association between folate intake and the risk of acute coronary events. An alternative explanation is that because smoking is such a strong risk factor itself, smokers do not benefit from folate intake as much as nonsmokers. It was reported earlier that smokers have lower levels of serum folate\textsuperscript{31} and higher levels of plasma tHcy\textsuperscript{32,33} than nonsmokers. We think that higher plasma tHcy levels in smokers could be partly due to their lower intake of sources of folate such as fresh vegetables, not only due to the direct effects of smoking.

Our results do not support the finding of the Nurses’ Health Study\textsuperscript{15} that the strongest apparent benefit of a high-folate diet is among subjects who consume alcohol. Lower serum folate levels have been noticed in chronic alcoholics.\textsuperscript{34} Although this could be a result of low folate intake, alcohol also affects several aspects of folate metabolism. First, the absorption of folate has been noticed to be decreased by use of alcohol and chronic alcoholism. This results from decreased hydrolysis of food polyglutamates in jejunal brush border. Alcohol may also increase urinary folate excretion and impair folate storage in the liver.\textsuperscript{34} Thus, higher dietary intakes of folate are needed to compensate the reduced absorption and increased excretion in alcohol users. Oxidation of acetaldehyde that is formed in alcohol catabolism can inactivate folate.\textsuperscript{15} Acetaldehyde also binds and inactivates the methionine synthase enzyme, which takes part in folate-dependent remethylation of homocysteine back to methionine.\textsuperscript{15} Thus, it would be plausible that the need of folate is higher among alcohol consumers. In abstainers and light alcohol users, even lower intakes may be sufficient, and consequently, a stronger association can be observed between folate intake and the risk of CHD in this group. Morrison and colleagues, in their reply letter to the Nurses’ Health Study, report that in the Nutrition Canada survey cohort, as in our...
study, those who abstain from alcohol experience the CHD benefits of folate. 36

Together with previous findings, our present observations support the theory that folate and possibly vitamin B12 have a role in the prevention of CHD. The results of this study confirm previous findings showing that a diet dominated by plant-derived foods promotes good cardiovascular health. Results of the ongoing large randomized trials10 are needed to conclusively verify the effect of folic acid supplementation in cardiovascular health.

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References


**TABLE 3.** Prospective Studies on the Association of Circulating Folate Concentration or Dietary Folate Intake With the Risk of CHD in Subjects Free of CHD in Study Baseline Examinations

<table>
<thead>
<tr>
<th>Study, Publication Year</th>
<th>Follow-Up, y</th>
<th>Study Population, n</th>
<th>Cases or Events/Controls, n</th>
<th>Age, y</th>
<th>Folate Outcome</th>
<th>Risk-Factor Adjusted Relative Risk (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physicians’ Health Study Chasan-Taber et al, 1996</td>
<td>7.5</td>
<td>14 916</td>
<td>333/333</td>
<td>M</td>
<td>40–84</td>
<td>Plasma folate MI</td>
</tr>
<tr>
<td>Nutrition Canada Survey Morrison et al, 1996</td>
<td>15</td>
<td>5056</td>
<td>165</td>
<td>M+F</td>
<td>35–79</td>
<td>Serum folate Fatal CHD</td>
</tr>
<tr>
<td>Nurses Health Study Rimm et al, 1998</td>
<td>14</td>
<td>80 082</td>
<td>939</td>
<td>F</td>
<td>30–55</td>
<td>Folate intake CHD</td>
</tr>
<tr>
<td>ARIC Study Voutilainen et al, 1998</td>
<td>3.3</td>
<td>15 792</td>
<td>232/537</td>
<td>M+F</td>
<td>45–64</td>
<td>Plasma folate CHD</td>
</tr>
<tr>
<td>NHANES I Loria et al, 1998</td>
<td>19</td>
<td>2657</td>
<td>873</td>
<td>M+F</td>
<td>25–74</td>
<td>Serum folate CHD</td>
</tr>
<tr>
<td>Ford et al, 1998</td>
<td>19</td>
<td>2657</td>
<td>215</td>
<td>M+F</td>
<td>25–74</td>
<td>Serum folate Fatal CHD</td>
</tr>
<tr>
<td>NHANES I Morrison et al, 1996</td>
<td>20</td>
<td>1921</td>
<td>284</td>
<td>M+F</td>
<td>25–74</td>
<td>Serum folate CHD</td>
</tr>
<tr>
<td>KIHDA Giles et al, 1998</td>
<td>5.3</td>
<td>734</td>
<td>34</td>
<td>M</td>
<td>46–64</td>
<td>Serum folate ACE</td>
</tr>
<tr>
<td>Voutilainen et al, 2000</td>
<td>14</td>
<td>689</td>
<td>49</td>
<td>M+F</td>
<td>30–75</td>
<td>Serum folate Fatal CVD</td>
</tr>
</tbody>
</table>

**Notes:** MI indicates myocardial infarction; ACE, acute coronary events; and CVD, cardiovascular disease.


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