Nonfasting Plasma Total Homocysteine Levels and All-Cause and Cardiovascular Disease Mortality in Elderly Framingham Men and Women

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Background: Elevated fasting total homocysteine (tHcy) levels were recently shown to confer an independent risk for all-cause and cardiovascular disease (CVD) mortality among selected Norwegian patients with confirmed coronary heart disease. We examined whether elevated fasting plasma tHcy levels were predictive of all-cause and CVD mortality in a large, population-based sample of elderly US women and men.

Methods: Nonfasting plasma tHcy levels were determined in 1933 elderly participants (mean age, 70 ± 7 years; 58.9% women) from the original Framingham Study cohort, examined between 1979 and 1982, with follow-up through 1992. Unadjusted and adjusted (ie, for age, sex, diabetes, smoking, systolic blood pressure, total and high-density lipoprotein cholesterol, and creatinine) relative risk estimates (with 95% confidence intervals [CIs]) for total and CVD mortality were generated by proportional hazards modeling, with tHcy levels (quartiles) as the independent variable.

Results: There were 653 total deaths and 244 CVD deaths during a median follow-up of 10.0 years. Proportional hazards modeling revealed that tHcy levels of 14.26 µmol/L or greater (the upper quartile), vs less than 14.26 µmol/L (the lower three quartiles), were associated with relative risk estimates of 2.18 (95% CI, 1.86-2.56) and 2.17 (95% CI, 1.68-2.82) for all-cause and CVD mortality, respectively. The relative risk estimates after adjustment for age, sex, systolic blood pressure, diabetes, smoking, and total and high-density lipoprotein cholesterol levels attenuated these associations, but they remained significant: 1.54 (95% CI, 1.31-1.82) for all-cause mortality; 1.52 (95% CI, 1.16-1.98) for CVD mortality.

Conclusion: Elevated nonfasting plasma tHcy levels are independently associated with increased rates of all-cause and CVD mortality in the elderly.

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METHODS

The study population consisted of the original Framingham Heart Study cohort. Baseline examinations for the present analyses took place between May 1979 and May 1982, with follow-up occurring through May 1992. Of 2351 persons examined during the baseline period, 1933 had specimens available for plasma tHcy determinations. Additional baseline covariates assessed for the present analyses included age, sex, systolic blood pressure, cigarette smoking, diabetes, total and HDL cholesterol levels, and creatinine level.

Total homocysteine level was determined by high-performance liquid chromatography with fluorescence detection, using nonfasting plasma aliquots stored at −20°C from the baseline examination period, until mid-1997. Such long-term storage conditions have been validated for tHcy determinations in plasma or serum. Total and HDL cholesterol levels were assessed from fresh, nonfasting plasma samples by standard Lipid Research Clinics techniques, and creatinine level was measured in nonfasting plasma samples by the Jaffe method.

Diabetes was operationally defined as use of insulin preparations or oral hypoglycemic agents, or any recorded blood glucose level of 11.1 mmol/L or higher (≥200 mg/dL), and smoking was defined as current cigarette smoking.

Information for outcome ascertainment was obtained from records supplied by hospitals, attending physicians, pathologists, medical examiners, and families. Cardiovascular disease death consisted (primarily) of coronary heart disease death and stroke death, with a very minor contribution from "other" CVD death (eg, hypertensive heart disease). As per all events in the Framingham Study, a panel of physicians reviewed the pooled evidence to arrive at the cause of death.

The skewed tHcy data were natural log transformed, and comparisons of differences in geometric mean tHcy levels by sex, diabetes, and smoking status were performed using unpaired t tests. Spearman r was used to assess unadjusted rank-order correlations between untransformed tHcy levels, and age, creatinine, systolic blood pressure, total cholesterol, and HDL cholesterol. Unadjusted and adjusted (ie, for age, sex, diabetes, smoking, systolic blood pressure, total and HDL cholesterol) relative risk estimates (with 95% confidence intervals) for total and CVD mortality were generated by proportional hazards modeling, with tHcy levels (natural log or quartiles) as the independent variable.

RESULTS

Subject characteristics at the baseline examination are depicted in Table 1. Geometric mean tHcy levels were higher in men than in women (12.40 vs 11.27 µmol/L; P<.001), but did not differ according to the presence or absence of diabetes (11.67 vs 11.73 µmol/L, P = .86), or among current cigarette smokers vs nonsmokers (11.76 vs 11.71, P = .67). Weak, but significant Spearman correlations were observed between tHcy levels and age (+0.215, P < .001), creatinine (+0.186, P < .001), systolic blood pressure (+0.114, P < .001), HDL cholesterol (−0.114, P < .001), and total cholesterol (−0.049, P = .03). Quartiles of tHcy (in micromoles per liter) were as follows: Q1, 1.43-9.25; Q2, 9.26-11.43; Q3, 11.44-14.25; and Q4, 14.26-219.84.

There were 653 total deaths and 244 CVD deaths, during a median follow-up of 10.0 years. Although natural log tHcy (as a continuous variable) was associated with both total and CVD mortality in unadjusted and multivariable-adjusted proportional hazards analyses, the excess risk for these outcomes was largely confined to the uppermost quartile. Furthermore, the sex × tHcy (quartile analyses) interaction term was nonsignificant, so the total and CVD mortality analyses were not stratified by sex. Results of the total and CVD mortality analyses are presented in Table 2. A nonfasting plasma tHcy level of 14.26 µmol/L or greater was associated with an approximately 2.2-fold increased risk for both total and CVD mortality. Following multivariable adjustment, these associations persisted, but were attenuated to an approximately 1.5-fold increased risk for total and CVD mortality. Further adjustment for creatinine level (as a continuous variable, or as creatinine level ≥144 µmol/L vs <144 µmol/L) did not change these relationships (data not shown). Lastly, there was no evidence that the assumption of constant proportional hazards was violated (P>.2, data not shown).

COMMENT

The present findings are consistent with recently reported data from women and men participants aged 65 years and older in the Cardiovascular Health Study indicating that age, sex, elevated (brachial) systolic blood pressure, and elevated creatinine levels, were independently predictive of 5-year all-cause mortality. We further report the initial population-based evidence that elevated nonfasting tHcy levels may be independently predictive of all-cause and CVD mortal-

### Table 1. Subject Characteristics at Baseline Examination (N = 1933)*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Women, No. (%)</th>
<th>Men, No. (%)</th>
<th>Age, y</th>
<th>Systolic blood pressure, mm Hg</th>
<th>Total cholesterol, mmol/L†</th>
<th>HDL cholesterol, mmol/L†</th>
<th>Creatinine, µmol/L</th>
<th>Total homocysteine, µmol/L</th>
<th>Diabetes, No. (% of total)</th>
<th>Smoking, No. (% of total)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1138 (58.9)</td>
<td>795 (41.1)</td>
<td>70 ± t</td>
<td>141 ± 21 (86-225)</td>
<td>5.95 ± 1.06 (2.72-10.21)</td>
<td>1.29 ± 0.41 (0.41-3.46)</td>
<td>84 ± 23</td>
<td>12.55 ± 7.19 (4.13-219.84)</td>
<td>184 (9.5)</td>
<td>414 (21.4)</td>
</tr>
</tbody>
</table>

*Data are given as mean ± SD (range) unless otherwise specified.
†To convert cholesterol values to milligrams per deciliter, divide millimoles per liter by 0.2586. HDL indicates high-density lipoprotein.
ity in elderly women and men. Our findings are in turn consistent with an earlier report indicating that elevated fasting tHcy levels were a powerful independent predictor of all-cause and CVD mortality in a younger, select group of predominantly male Norwegian patients with established coronary artery disease.\(^7\) Considerable subject population differences (ie, distribution of age, sex, and other key comorbidities and CVD risk factors) likely account for the sizable disparity in the magnitude of total and CVD mortality risk conferred by tHcy levels, comparing the present and previous study.\(^7\)

The best estimate from meta-analyses\(^1,3\) updated through early 1998,\(^8\) for the increased risk of coronary heart disease morbidity and mortality comparing tHcy levels of greater than 15 \(\mu\text{mol/L}\) with levels less than 10 \(\mu\text{mol/L}\), after adjustment for the traditional CVD risk factors, appears to be 1.4. This estimate does not change when only prospective studies are included (Omenn et al\(^6\); S.A.A. Beresford, PhD, written communication, November 1998). Simultaneous pursuit of 2 related areas of investigation will be required to confirm a causal relationship between hyperhomocysteinemia and CVD: (1) randomized, placebo-controlled trials of the effect of tHcy-lowering treatment on recurrent and de novo CVD outcomes and (2) elucidation of the basic pathomechanisms linking hyperhomocysteinemia to arteriosclerosis. Recently it has been proposed that clinical or even subclinical arteriosclerosis may play an important etiologic role in the development of hyperhomocysteinemia.\(^11\) This hypothesis appears untenable in light of the following published findings from both human and animal studies: (1) Despite the absence of any traditional CVD risk factors, 50% of untreated children and young adults with homocystinuria due to cystathionine synthase deficiency experience a major atherothrombotic event by age 30 years.\(^2,13\) Furthermore, strategies designed solely to reduce tHcy levels in these patients have been shown to decrease atherothrombotic event rates.\(^2,13\) (2) In adults (\(n=38\); mean \(\pm SD\) age, 58 \(\pm 12\) years) with mild hyperhomocysteinemia, tHcy-lowering treatment appears to have reduced the rate of progression of ultrasound-determined extracranial carotid artery plaque area.\(^14\) (3) Young, healthy subjects, free of clinical arteriosclerosis or CVD risk factors, who have normal baseline flow-mediated brachial artery reactivity, experience a dramatic, “dose-response” reduction in their flow-mediated brachial artery reactivity following acute hyperhomocysteinemia produced by an oral \(L\)-methionine load.\(^15\) (4) Randomized, controlled studies have revealed that mild dietary-induced hyperhomocysteinemia resulted in abnormal vascular reactivity among nonhuman primates,\(^16\) as well as increased arterial stiffness, and frank atherothrombotic sequelae, in minipigs.\(^17\)

Our data confirm that tHcy levels are increased in elderly persons.\(^9,18\) Potential etiologic factors accounting for this age-related increase in tHcy levels include suboptimal intake and absorption of the key vitamin B cofactors or substrates for Hcy metabolism\(^18\); reduced activity of Hcy-metabolizing enzymes (for example, as demonstrated for cystathionine synthase\(^19\)); and declining renal,\(^20\) and possibly thyroid\(^21\) function. Indeed, key limitations of the present analyses include the absence of data on intake or status of folic acid, vitamin \(B_6\), vitamin \(B_{12}\), and riboflavin, and the lack of a specific index of renal function such as glomerular filtration rate. A further potential limitation of these analyses is their relevance to the current era of folic acid fortification of cereal grain flour (140 \(\mu\text{g per 100 g}\) of flour) in the United States, begun voluntarily to lower three quartiles of nonfasting plasma total homocysteine (tHcy), and other potential independent predictor variables. Relative risk estimates were adjusted for all variables listed in the table, with the exception of the unadjusted tHcy analyses. HDL indicates high-density lipoprotein.

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### References


