Nonfasting Plasma Total Homocysteine Levels and Stroke Incidence in Elderly Persons: The Framingham Study

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Background: Total homocysteine levels are associated with arteriosclerotic outcomes.

Objective: To determine whether total homocysteine levels predict incident stroke in elderly persons.

Design: Prospective population-based cohort study with 9.9 years of follow-up.

Setting: Framingham, Massachusetts.

Patients: 1947 Framingham Study participants (1158 women and 789 men; mean age ± SD, 70 ± 7 years).

Measurements: Baseline total homocysteine levels and 9.9-year stroke incidence.

Results: The quartiles of nonfasting total homocysteine levels were as follows: quartile 1, 4.13 to 9.25 μmol/L; quartile 2, 9.26 to 11.43 μmol/L; quartile 3, 11.44 to 14.23 μmol/L; quartile 4, 14.24 to 219.84 μmol/L. During follow-up, 165 incident strokes occurred. In proportional hazards models adjusted for age, sex, systolic blood pressure, diabetes, smoking, and history of atrial fibrillation and coronary heart disease, relative risk (RR) estimates comparing quartile 1 with the other three quartiles were as follows: quartile 2 compared with quartile 1—RR, 1.32 (95% CI, 0.81 to 2.14); quartile 3 compared with quartile 1—RR, 1.44 (CI, 0.89 to 2.34); quartile 4 compared with quartile 1—RR, 1.82 (CI, 1.14 to 2.91). The linear trend across the quartiles was significant ($P < 0.001$).

Conclusion: Nonfasting total homocysteine levels are an independent risk factor for incident stroke in elderly persons.

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Meta-analyses (1, 2) strongly suggest that mildly to moderately elevated circulating levels of the sulfur amino acid homocysteine, either fasting or nonfasting, confer an independent risk for clinical arteriosclerotic outcomes, including stroke. However, just four reports (3–6) have provided somewhat limited, conflicting prospective data on the potential relation between total homocysteine levels and stroke incidence. Only one of these studies (3) used a population-based sample. Samples from two studies were limited to middle-aged men (4, 5): One consisted of U.S. physicians responding to a survey (4), and the other was derived from family practice registries in Great Britain (5). The fourth study (6) was a longitudinal investigation that primarily included women with systemic lupus erythematosus (mean age at baseline, 35 years).

No prospective studies have specifically evaluated mild hyperhomocysteinemia as a potential risk factor for stroke in men and women 60 years of age or older, an age group that experiences the most pronounced morbidity and mortality from cerebrovascular disease (7). Accordingly, we examined the association between baseline nonfasting plasma total homocysteine levels and incident stroke in a well-characterized, population-based cohort of elderly women and men who at baseline had not had stroke.

Methods

The study sample consisted of the original Framingham Study cohort (8). Baseline examinations for the current analyses took place between May 1979 and May 1982, with follow-up occurring through May 1992. Of 2351 persons examined during the baseline period, 1947 had not previously had stroke and had specimens available for measurement of plasma total homocysteine levels. Additional baseline covariables assessed for the current analyses were age, sex, cigarette smoking, diabetes, history of atrial fibrillation, history of coronary heart disease, systolic blood pressure, and creatinine levels. Detailed operational definitions for all these covariables are provided elsewhere (8). Stroke outcome ascertainment and definition methods used in the Framingham Study, including subtype classification, have been described in detail previously (8, 9).

Total homocysteine levels were determined by high-performance liquid chromatography with fluorescence detection (10). Nonfasting plasma aliquots were stored at −20 °C from the baseline examination period until mid-1997. Data from long-term storage studies conducted at −20 °C have confirmed both the biochemical stability and long-term within-person reproducibility of total homocysteine determinations (10). Creatinine levels were measured in

nonfasting plasma by the Jaffe method, adapted for autoanalyzers.

The skewed total homocysteine data were natural log–transformed, and differences in geometric mean total homocysteine levels according to sex, diabetes, history of atrial fibrillation or coronary heart disease, and smoking status were compared by using unpaired *t*-tests. The Spearman rho was used to assess unadjusted rank-order correlations between untransformed total homocysteine levels and age, creatinine level, and systolic blood pressure. Unadjusted and adjusted (for age, sex, history of atrial fibrillation or coronary heart disease, diabetes, smoking, systolic blood pressure, and creatinine level) relative risk estimates (hazards ratios with 95% CIs) for total stroke, nonhemorrhagic stroke, and atherothrombotic brain infarction were generated by proportional hazards modeling. Total homocysteine level (natural log–transformed or expressed in quartiles) was the independent variable. All statistical analyses were performed by using SAS software, version 6.12 (SAS Institute, Inc., Cary, North Carolina).

Results

The study sample ($n = 1947$) consisted of 1158 women (59.5%) and 789 men (40.5%). Four hundred thirteen patients (21.2%) were cigarette smokers, 340 (17.5%) had a history of coronary heart disease, 182 (9.3%) were diabetic, and 81 (4.2%) had a history of atrial fibrillation. Arithmetic means ($\pm$SD) for key continuous variables were as follows: age, 70 ± 7 years (range, 59 to 91 years); systolic blood pressure, 141 ± 21 mm Hg (range, 86 to 225 mm Hg); creatinine level, 97.2 ± 26.5 μmol/L (range, 35.3 to 442 μmol/L); and total homocysteine level, 12.65 ± 7.19 μmol/L (range, 4.13 to 219.84 μmol/L).

Geometric mean total homocysteine levels were higher in men than in women (12.35 compared with 11.32 μmol/L; $P < 0.001$), in patients with a history of atrial fibrillation than in those without (13.17 compared with 11.66 μmol/L; $P = 0.003$), and in patients with a history of coronary heart disease than in those without (12.49 compared with 11.57 μmol/L; $P < 0.001$). However, these levels did not differ according to the presence or absence of diabetes (11.83 compared with 11.71 μmol/L; $P > 0.2$) or between current cigarette smokers and nonsmokers (11.67 compared with 11.74 μmol/L; $P > 0.2$). Weak but significant Spearman correlations were observed between total homocysteine levels and age ($r = 0.212$; $P < 0.001$), creatinine level ($r = 0.178$; $P < 0.001$), and systolic blood pressure ($r = 0.111$; $P < 0.001$). Quartiles of total homocysteine were as follows: quartile 1, 4.13 to 9.25 μmol/L; quartile 2, 9.26 to 11.43 μmol/L; quartile 3, 11.44 to 14.23 μmol/L; quartile 4, 14.24 to 219.84 μmol/L.

Table 1. Predictors of Incident Total Stroke in Elderly Women and Men in the Framingham Study Cohort

Variable	Relative Risk Estimate (95% CI)*
Age, per 1-year increase	1.06 (1.04–1.09)
Systolic blood pressure, per 20–mm Hg increase	1.16 (1.01–1.34)
Smoking	1.52 (1.03–2.24)
Diabetes	1.90 (1.25–2.89)
Atrial fibrillation	2.29 (1.29–4.04)
Coronary heart disease	1.49 (1.04–2.16)
Total homocysteine level, quartile 4 compared with quartile 1	1.82 (1.14–2.91)

* From multivariable-adjusted proportional hazards regression analysis, including all variables listed in the table.

During a median follow-up of 9.9 years, 165 incident total strokes occurred; 153 of these were incident nonhemorrhagic strokes, and 100 were incident atherothrombotic brain infarctions. Five hundred twenty-four persons were censored during follow-up because of death from causes other than stroke. Age, systolic blood pressure, current smoking, diabetes, and history of atrial fibrillation or coronary heart disease were independently predictive of total stroke occurrence (**Table 1**). Levels of total homocysteine (natural log) as a continuous variable (data not shown) and across quartiles ($P < 0.001$ for linear trend) were associated with all stroke outcomes in unadjusted and multivariable-adjusted proportional hazards analyses (**Tables 1** and **2**). The interaction term between sex and total homocysteine level (quartile analyses) was nonsignificant ($P = 0.1$); thus, the stroke incidence analyses were not stratified by sex. Further adjustment for creatinine level did not change the results of any of these analyses (data not shown).

Discussion

Our findings are consistent with previously reported data, derived primarily from elderly female and male participants in the Framingham Study (9, 11), indicating that age, systolic blood pressure, diabetes, cigarette smoking, and history of atrial fibrillation or coronary heart disease were independently predictive of stroke incidence. We report population-based evidence that elevated nonfasting total homocysteine levels are also independently associated with stroke incidence among elderly women and men.

Four earlier studies (3–6) have examined the relation between total homocysteine levels and stroke incidence. Alfthan and colleagues (3) did not find an association between total homocysteine levels and incident stroke among Finnish men and

Table 2. Relative Risk Estimates in Elderly Women and Men in the Framingham Study Cohort: Comparison of Each of the Upper Three Quartiles to the Lowest Quartile of Nonfasting Plasma Total Homocysteine Level

Type of Stroke	Quartile Comparisons	Relative Risk Estimate (95% CI)	
		Unadjusted	Multivariable-Adjusted*
Total stroke (165 events)	Q2 vs. Q1	1.44 (0.88–2.33)	1.32 (0.81–2.14)
	Q3 vs. Q1	1.62 (1.01–2.62)	1.44 (0.89–2.34)
	Q4 vs. Q1	2.53 (1.61–3.98)	1.82 (1.14–2.91)†
Nonhemorrhagic stroke (153 events)	Q2 vs. Q1	1.34 (0.81–2.21)	1.22 (0.73–2.01)
	Q3 vs. Q1	1.48 (0.90–2.43)	1.31 (0.79–2.16)
	Q4 vs. Q1	2.52 (1.59–4.00)	1.79 (1.11–2.89)†
Atherothrombotic brain infarction (100 events)	Q2 vs. Q1	1.40 (0.73–2.66)	1.30 (0.68–2.49)
	Q3 vs. Q1	2.02 (1.10–3.70)	1.82 (0.99–3.36)
	Q4 vs. Q1	2.51 (1.38–4.58)	1.90 (1.02–3.51)†

* Adjusted for age, sex, diabetes, cigarette smoking, systolic blood pressure, and history of coronary heart disease or atrial fibrillation.
† $P < 0.001$ for trend across quartiles.

women 40 to 64 years of age (total events, 76). Similarly, Verhoef and coworkers (4) found only a weak, nonsignificant association between total homocysteine level and stroke incidence (total events, 109) in a cohort of male physicians whose mean age was 59.7 years (upper quintile compared with lower four quintiles: odds ratio, 1.2 [CI, 0.7 to 2.0]). In contrast, Perry and colleagues (5) reported a robust, independent association between total homocysteine levels (across quartiles) and incident stroke (total events, 107) among British men whose mean age was 54.0 years (quartile 3 compared with quartile 1: odds ratio, 3.3 [CI, 0.9 to 11.5]; quartile 4 compared with quartile 1: odds ratio, 7.4 [CI, 1.9 to 29.0]). More recently, elevated total homocysteine levels were independently linked to the development of stroke outcomes in a cohort of predominantly younger women (mean age, 34.9 years) with systemic lupus erythematosus (6). The two negative studies (3, 4) were characterized by modest stroke event rates and overall exposure to lower total homocysteine levels on the basis of sound nutritional (4) or, possibly, favorable genetic (3) influences. More widespread exposure to elevated total homocysteine levels, due perhaps to worse nutritional status, may have accounted for the strong association between total homocysteine level and incident stroke reported by Perry and colleagues (5). The positive relation between total homocysteine level and stroke occurrence described by Petri and associates in the Hopkins Lupus Cohort (6) was confined to patients with the highest total homocysteine levels (>14 μmol/L). Our analyses, which revealed an independent association between total homocysteine level and incident stroke (total events, 165), were performed in an elderly sample characterized, as expected, by a relatively higher stroke event rate (7, 9, 11, 12). This higher rate occurred in conjunction with an increased prevalence of mild hyperhomocysteinemia (that is, in approximately 25% of patients with total homocysteine level > 14 μmol/L) at the baseline examination.

Despite the lack of substantiation by either proven or biologically plausible mechanisms, it has nevertheless been proposed that hyperhomocysteinemia is an epiphenomenon of clinical or even subclinical arteriosclerosis (13, 14). This hypothesis appears untenable in view of the following published findings from both human and animal studies. First, despite the absence of any traditional arteriosclerotic risk factors, 50% of untreated children and young adults with homocystinuria due to cystathionine synthase deficiency experience a major atherothrombotic event by 30 years of age (15). Strategies designed solely to reduce total homocysteine levels in these patients have been shown to decrease atherothrombotic event rates (15, 16). Second, in adults with mild hyperhomocysteinemia, treatment to decrease total homocysteine levels seems to have reduced the rate of progression of ultrasonographically determined extracranial carotid artery plaque area (17).

Third, young, healthy persons who do not have clinical arteriosclerosis or arteriosclerotic risk factors and have normal baseline flow-mediated brachial artery reactivity experience a dramatic, dose-response reduction in their flow-mediated brachial artery reactivity after acute hyperhomocysteinemia produced by an oral L-methionine load (18). Finally, randomized, controlled studies have revealed that mild dietary-induced hyperhomocysteinemia resulted in abnormal vascular reactivity among nonhuman primates (19) and in increased arterial stiffness and frank atherothrombotic sequelae among minipigs (20).

We conclude that the plasma level of nonfasting total homocysteine is an independent risk factor for incident stroke in elderly persons. Ultimately, proof of a causal association between homocysteine level and arteriosclerotic outcomes, including stroke, will require 1) elucidation of basic pathomechanisms by use of appropriate animal models (19, 20) and 2) evidence from controlled clinical trials in humans that interventions decreasing total homocysteine levels reduce arteriosclerotic events.

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