ORIGINAL ARTICLE

Homocysteine as a Predictive Factor for Hip Fracture in Older Persons

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ABSTRACT

BACKGROUND

The increased prevalence of osteoporosis among people with homocystinuria suggests that a high serum homocysteine concentration may weaken bone by interfering with collagen cross-linking, thereby increasing the risk of osteoporotic fracture. We examined the association between the total homocysteine concentration and the risk of hip fracture in men and women enrolled in the Framingham Study.

METHODS

We studied 825 men and 1174 women, ranging in age from 59 to 91 years, from whom blood samples had been obtained between 1979 and 1982 to measure plasma total homocysteine. The participants in our study were followed from the time that the sample was obtained through June 1998 for incident hip fracture. Sex-specific, age-adjusted incidence rates of hip fracture were calculated for quartiles of total homocysteine concentrations. Cox proportional-hazards regression was used to calculate hazard ratios for quartiles of homocysteine values.

RESULTS

The mean (±SD) plasma total homocysteine concentration was 13.4±9.1 μmol per liter in men and 12.1±5.3 μmol per liter in women. The median duration of follow-up was 12.3 years for men and 15.0 years for women. There were 41 hip fractures among men and 146 among women. The age-adjusted incidence rates per 1000 person-years for hip fracture, from the lowest to the highest quartile for total homocysteine, were 1.96 (95 percent confidence interval, 0.52 to 3.41), 3.24 (0.97 to 5.52), 4.43 (1.80 to 7.07), and 8.14 (4.20 to 12.08) for men and 9.42 (5.72 to 13.12), 7.01 (4.29 to 9.72), 9.58 (6.42 to 12.74), and 16.57 (11.84 to 21.30) for women. Men and women in the highest quartile had a greater risk of hip fracture than those in the lowest quartile — the risk was almost four times as high for men and 1.9 times as high for women.

CONCLUSIONS

These findings suggest that the homocysteine concentration, which is easily modifiable by means of dietary intervention, is an important risk factor for hip fracture in older persons.

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Homocysteine is an amino acid intermediate formed during the metabolism of methionine. Homocystinuria, a rare autosomal recessive biochemical abnormality, causes elevated plasma concentrations of homocysteine and severe occlusive vascular disease.[1] This observation has led to studies that implicate plasma homocysteine as a risk factor for cardiovascular disease.[2,3] In patients with homocystinuria, there is an increased prevalence of skeletal deformities, including osteoporosis,[1,4,5] which is a primary risk factor for hip fracture. Thus, elevated plasma homocysteine concentrations may be associated with osteoporosis and may increase the risk of hip fracture, which can lead to substantial disability,[6] high medical costs,[7] and death.[8] We examined the association between plasma total homocysteine concentrations and the risk of hip fracture in a group of older men and women enrolled in the Framingham Study.

METHODS

PARTICIPANTS

The Framingham Study was begun in 1948 with the primary goal of evaluating risk factors for heart disease. A total of 5209 men and women who ranged in age from 28 to 62 years were recruited from a sample of two thirds of the residences in Framingham, Massachusetts, and have been examined biennially for more than 50 years.[9] The participants in the present study included 2043 older subjects from whom blood samples were obtained at the 16th biennial examination (between 1979 and 1982). Forty-four subjects with prior hip fractures were excluded, leaving 1999 participants (mean age, 70 years; range, 59 to 91) who were followed for an incident hip fracture from the date when the blood sample was obtained.

HOMOCYSTEINE MEASUREMENT

Blood samples were collected while the subjects were in a nonfasting state and were frozen immediately and stored at or below −20°C. In 1997, the samples were thawed and the plasma total homocysteine concentrations were measured with the use of high-performance liquid chromatography with fluorometric detection.[10] The stability of measurements of total homocysteine from plasma or serum stored at temperatures at or below −20°C has been validated previously.[11] The coefficient of variation for this assay was 9 percent.[12]

ASSESSMENT OF HIP FRACTURE

As reported previously,[13] all records of hospitalizations and deaths for the study participants were systematically reviewed for occurrences of hip fracture. Beginning in 1983 (18th biennial examination in the Framingham Study), hip fractures were reported by interview at each biennial examination or by telephone interview for participants unable to attend an examination. Reported hip fractures were confirmed by a review of medical records and radiographic and operative reports. Hip fracture was defined as a first-time fracture of the proximal femur that occurred in the absence of overwhelming trauma (e.g., a motor vehicle accident).

COVARIABLES

Potential confounders obtained from data from the 16th biennial examination (except where noted) included sex, age, height, weight, smoking status, caffeine consumption, alcohol consumption, educational level (≥12 years or <12 years), and current use or nonuse of estrogen among women. Height without shoes was measured to the nearest quarter inch (0.6 cm). Weight in pounds (without shoes) was measured with the use of a standard balance-beam scale at each examination. Smoking was measured at the 15th examination (between 1977 and 1979) as the average number of cigarettes smoked per day in the previous two years. Caffeine consumption in the form of tea and coffee was quantified as previously described.[14] At the 15th examination alcohol consumption (beer, wine, or spirits) was calculated as the number of ounces consumed per week, as previously reported.[15] Participants were classified at baseline (16th biennial examination) as either having a prior diagnosis of cardiovascular disease or not, according to previously published diagnostic criteria.[16] A score for physical function was calculated as a weighted sum of the participants' self-reported responses to nine questions selected from those developed by Nagi to measure physical function,[17] which were asked at the 14th biennial examination (between 1975 and 1978),[18] and a score for cognitive status was obtained with the use of the Folstein Mini–Mental State Examination,[19] which was administered at the 17th examination (between 1981 and 1984), as previously described.[20]

STATISTICAL ANALYSIS

Owing to a skewed distribution, total homocysteine concentrations were analyzed as quartiles and as

continuous, natural-log–transformed values. Incidence rates of hip fracture (the number of incident cases divided by the number of person-years at risk for fracture) were calculated for quartiles and were standardized for age in five-year age groups on the basis of sex-specific age distributions of all the study participants. For each participant, the accumulation of person-years at risk started from the collection of the blood sample and continued until the first occurrence of hip fracture, death, last contact with the participant, or the end of follow-up (June 30, 1998, for all study participants). Cox proportional-hazards regression was used to calculate the hazard ratios and the 95 percent confidence intervals that were used to estimate the relative increase in the risk of hip fracture for each of the three higher quartiles as compared with the lowest quartile (referent); it was also used to test for a linear trend in the hazard ratios across all the quartiles. To estimate the absolute association between homocysteine concentrations and hip fracture, differences in risk between the highest and lowest quartiles were calculated with 95 percent confidence intervals.

The risk of hip fracture within each quartile was calculated with the use of the Cox regression model as 1 minus the probability of survival without a hip fracture to the median follow-up time. Cox regression was also used to estimate the hazard ratio for hip fracture for each increase of 1 SD in continuous, log-transformed total homocysteine values. All analyses were conducted separately for men and women. Regression analyses used to calculate the hazard ratios were adjusted for potential confounders by including data on age, height, weight, smoking status, caffeine consumption, alcohol consumption, educational level, and current use or nonuse of estrogen among women. To estimate the risk of fracture within quartiles in the Cox regression models, potential confounders were assigned mean levels for continuous covariates and the most prevalent category for dichotomous variables.

People with higher homocysteine concentrations may have an increased risk of cardiovascular disease and cognitive dysfunction,[21] conditions that limit activity and result in greater frailty and an increased risk of hip fracture. To address cardiovascular disease as a potential confounder, we performed additional analyses adjusted for the presence or absence of cardiovascular disease and for measures of physical function and cognitive status. Because the scores for cognitive status were not collected until the 17th examination, analyses involving this covariate started at that examination. To investigate the possible effect of changes in weight, we adjusted a model for time-varying weight. To determine whether a subgroup of participants with extremely high homocysteine concentrations who were in the highest quartile might be driving the association between homocysteine and hip fracture, we repeated our original analysis but excluded subjects considered to have extreme homocysteine values within the highest quartile. For all statistical analyses, we used SAS/STAT software, version 8.1 (SAS Institute).

Table 1. Baseline Characteristics of the Study Participants.*

Characteristic	Men (N=825)	Women (N=1174)
Age (yr)		
Mean	69.5±6.9	70.3±7.1
Range	59–90	59–91
Height (in.)	67.1±2.9	61.4±2.6
Weight (lb)	172.7±27.4	141.8±26.3
Smoking (no. of cigarettes/day)	4.5±10.4	3.6±8.0
Alcohol intake (oz/wk)	4.9±6.4	1.8±3.0
High-school graduate (%)	61.9	62.4
Total homocysteine (μmol/liter)		
Mean	13.4±9.1	12.1±5.3
Range	5.8–219.8	4.1–59.3
Current use of estrogen (%)	—	3.5
Median follow-up (yr)	12.3	15.0

* Plus–minus values are means ±SD. To convert values for height to centimeters, multiply by 2.54. To convert values for weight to kilograms, multiply by 0.45. To convert values for ounces to milliliters, multiply by 29.6.

RESULTS

Table 1 lists the baseline characteristics of the participants according to sex. The mean (±SD) age among the 825 men was 69.5 years, and among the 1174 women it was 70.3 years. The men were taller than the women, weighed more, smoked more cigarettes, consumed more alcohol, and had higher mean total homocysteine concentrations. Among the men the median follow-up period was 12.3 years, and among the women it was 15.0 years. During follow-up, 41 of the men and 146 of the women sustained hip fractures.

The characteristics of the study participants across the quartiles of total homocysteine concentrations are shown in Table 2. Among men, the mean total homocysteine concentration in the low-

est quartile (quartile 1) was 8.5±0.9 μmol per liter, and among women it was 7.6±1.0. In the highest quartile (quartile 4), among men the total homocysteine concentrations ranged from 15.01 to 219.80 μmol per liter, with a mean of 20.8±15.7. Among women in quartile 4, concentrations ranged from 13.47 to 59.27 μmol per liter, with a mean of 18.6±6.4. Mean age increased from the lowest to the highest quartile among men (quartile 1, 67.9 years; and quartile 4, 71.8) and among women (quartile 1, 68.3 years; and quartile 4, 73.3). The age ranges for men and women were similar in all quartiles.

Table 3 lists the age-adjusted incidence rates of hip fracture according to the quartile of total homocysteine concentration. Among men, the number of hip fractures increased monotonically from 5 in quartile 1 to 17 in quartile 4. Among women, however, the number of hip fractures was similar from quartile 1 to quartile 3 (range, 26 to 36) and then increased to 54 in quartile 4. Age-adjusted incidence rates among men increased across the quartiles, from 1.96 fractures per 1000 person-years in quartile 1 to 8.14 per 1000 person-years in quartile 4. Among women, although the incidence rates were similar from quartile 1 to quartile 3 (range, 7.01 to 9.58 fractures per 1000 person-years), the rate of fracture was higher in quartile 4 (16.57 per 1000 person-years).

The results of the multivariable-adjusted Cox proportional-hazards regressions for quartiles of homocysteine concentrations are shown in Figure 1. Whereas men in quartiles 2 and 3 tended to have a higher risk of hip fracture than those in quartile 1 (hazard ratio for quartile 2, 1.67; 95 percent confidence interval, 0.54 to 5.14; hazard ratio for quartile 3, 2.07; 95 percent confidence interval, 0.70 to 6.09), the risk of fracture among men in quartile 4 was almost four times the risk among men in quartile 1 (hazard ratio for quartile 4, 3.84; 95 percent confidence interval, 1.38 to 10.70). The test for a linear trend in hazard ratios across the quartiles was statistically significant (P<0.01), suggesting a linear association between the quartile of homocysteine concentration and the risk of hip fracture. In quartile 4 as compared with quartile 1, there were 8.8 excess fractures per 100 men (95 percent confidence interval, 0.4 to 17.3) for the mean levels of the covariates at 14 years of follow-up.

Among the women, there was no apparent increase in the risk of fracture in quartiles 2 and 3 as compared with quartile 1 (hazard ratio for quartile 2, 0.78; 95 percent confidence interval, 0.45 to 1.33; hazard ratio for quartile 3, 1.07; 95 percent confidence interval, 0.64 to 1.78) (Fig. 1). Among women in quartile 4, however, the risk of fracture was nearly twice that among women in quartile 1 (hazard ratio for quartile 4, 1.92; 95 percent confidence interval, 1.18 to 3.10). The test for a linear trend in hazard ratios was statistically significant (P<0.01). For women who were not currently receiving estrogen therapy, the difference in the risk of hip fracture between quartiles 4 and 1 for the mean levels of their covariates at 14 years of follow-up was 9.5 excess fractures per 100 participants (95 percent confidence interval, 1.2 to 17.9).

For each increase of 1 SD in the log-transformed total homocysteine concentration, the risk of hip fracture increased by 59 percent in men (1 SD in log-transformed homocysteine concentration, 0.34; hazard ratio, 1.59; 95 percent confidence interval, 1.31 to 1.94) and by 26 percent in women (1 SD, 0.35; hazard ratio, 1.26; 95 percent confidence interval, 1.08 to 1.47). Additional adjustments for

Table 2. Homocysteine Concentrations and Ages of Participants.

Characteristic	Men				Women			
	Quartile 1 (N=206)	Quartile 2 (N=206)	Quartile 3 (N=207)	Quartile 4 (N=206)	Quartile 1 (N=293)	Quartile 2 (N=294)	Quartile 3 (N=293)	Quartile 4 (N=294)
Total homocysteine (μmol/liter)								
Mean ±SD	8.5±0.9	11.0±0.6	13.4±0.9	20.8±15.7	7.6±1.0	9.9±0.7	12.2±0.7	18.6±6.4
Range	5.83–9.83	9.84–12.04	12.05–14.99	15.01–219.80	4.13–8.92	8.93–11.13	11.15–13.74	13.47–59.27
Age (yr)								
Mean ±SD	67.9±5.8	68.6±6.3	69.8±7.1	71.8±7.4	68.3±6.3	69.1±6.6	70.6±6.8	73.3±7.7
Range	59–83	60–87	60–88	60–90	60–90	59–91	60–90	59–91

Table 3. Age-Adjusted Incidence Rates of Hip Fracture According to Quartile of Homocysteine Level.

Variable	Men				Women			
	Quartile 1	Quartile 2	Quartile 3	Quartile 4	Quartile 1	Quartile 2	Quartile 3	Quartile 4
No. of person-years	2478	2415	2374	1860	3930	3861	3675	2861
No. of hip fractures	5	8	11	17	30	26	36	54
Incidence rate per 1000 person-years (95% CI)*	1.96 (0.52–3.41)	3.24 (0.97–5.52)	4.43 (1.80–7.07)	8.14 (4.20–12.08)	9.42 (5.72–13.12)	7.01 (4.29–9.72)	9.58 (6.42–12.74)	16.57 (11.84–21.30)

* CI denotes confidence interval.

cardiovascular disease, physical function, cognitive status, and weight modeled as a time-varying covariate did not change the effect of the homocysteine concentration on the risk of hip fracture for men or women. Among the men in quartile 4, two had homocysteine values (96 and 220 μmol per liter) that were more than double the next highest value. The effect of the homocysteine concentration on the risk of hip fracture was essentially unchanged when our analysis was repeated with these two outliers excluded. There were no extreme homocysteine values among the women.

DISCUSSION

We found that plasma homocysteine concentrations were associated with the risk of hip fracture in both men and women. The study participants in the highest quartile of values for total homocysteine had a significantly higher risk of hip fracture than those in the lowest quartile: by a factor of almost four in men and by a factor of 1.9 in women. The risk of hip fracture was elevated by 59 percent in men and by 26 percent in women for each increase of 1 SD in the log-transformed total homocysteine concentration. The increase in risk across quartiles in men appeared to be monotonic. Despite the statistically significant test for a linear trend in the risk among women, only those in the highest quartile were at increased risk for hip fracture, which suggests a possible threshold effect.

The apparent differences according to sex in the gradient of risk from the lowest to the highest quartile of homocysteine concentrations may be explained by the lower background incidence of hip fracture in men. Because the magnitude of the hazard ratio depends on the risk in the reference group, we provided an absolute measure of the effect of the homocysteine concentration on the risk of hip fracture. The differences in absolute risk between the highest and lowest quartiles for men and women were more similar (8.8 and 9.5 fractures, respectively, per 100 participants at 14 years of follow-up) than the hazard ratios (3.84 and 1.92, respectively). Therefore, the effect of the homocysteine concentration on the risk of hip fracture is most likely very similar in men and women.

To explain the increased prevalence of osteoporosis among patients with homocystinuria, McKusick first proposed that homocysteine interferes with the cross-linking of collagen.[22] Later studies supported this hypothesis with evidence of reduced

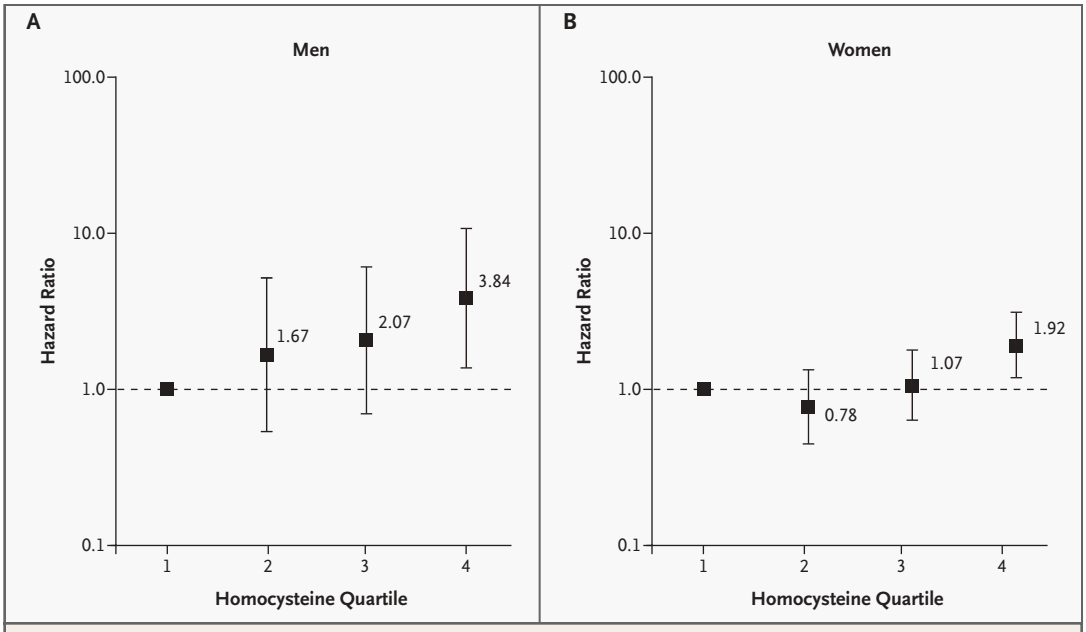

Figure 1. Multivariable-Adjusted Hazard Ratios for the Risk of Hip Fracture, According to the Quartile of Total Homocysteine Concentration.

The y axis is on a log scale. The reference group is quartile 1. The I bars denote 95 percent confidence intervals.

collagen cross-linking in patients with homocystinuria.[23,24] Whether these findings in studies of patients with the congenital condition of homocystinuria are directly applicable to normal variations in homocysteine concentrations among adults is unclear.

There is little evidence that homocysteine has a direct effect on bone. One study showed that chicks fed a homocysteine-supplemented diet had altered bone growth, bone matrix, and bone composition as compared with control chicks[25] but had similar mechanical strength and indexes of bone formation. This finding suggested that changes in bone geometry may compensate for any weakness caused by possible defects in collagen cross-linking. Future studies are needed to examine the association between homocysteine and the material and structural properties of bone in humans.

Although one previous report failed to find a relation between homocysteine concentrations and bone mineral density,[26] studies of genetic association support the concept that homocysteine is involved in the development of osteoporosis. The reduced activity of the enzyme methylenetetrahydrofolate reductase (MTHFR), which is determined by the *MTHFR* gene, can interfere with the methylation of homocysteine to methionine, possibly resulting in abnormal plasma homocysteine concentrations. A common mutation in the *MTHFR* gene[27] is associated with elevated plasma homocysteine concentrations in patients with reduced plasma folate concentrations.[28] The results of studies of bone mass[29-32] and fracture[30] are not consistent, yet they suggest that there may be a relation between the *MTHFR* gene and both bone mineral density and the risk of fracture.

If the homocysteine concentration truly is a causal mechanism for the risk of fracture, the public health implications could be substantial. The 1996 mandate of the Food and Drug Administration to fortify enriched grain products with folic acid[33] has helped to reduce the prevalence of low folate concentrations (<7 nmol per liter) in persons who are not taking vitamin supplements from 22.0 percent to 1.7 percent and to reduce the prevalence of homocysteine concentrations higher than 13 μmol per liter from 18.7 percent to 9.8 percent.[34] It remains to be seen whether this intervention will affect future rates of hip fracture in the United States.

Our study has several potential limitations. First, our findings in white men and women may not be generalizable to other racial and ethnic groups.

Second, because blood samples obtained from nonfasting subjects tend to yield higher total homocysteine concentrations than samples from fasting subjects,[35] the concentrations in our study may not be comparable to those in other studies in which samples were obtained from fasting subjects. Third, owing to the within-person variability of homocysteine concentrations, the use of a single measurement performed during the 20-year follow-up period may have led to regression dilution, resulting in an underestimate of the relative risk of hip fracture according to the homocysteine concentration.[36] Fourth, we were unable to assess potential confounding due to dietary factors, because no dietary information was available at baseline. Because folate and vitamins B_{12} and B_6 are major determinants of homocysteine concentrations in older persons,[12,37] the inadequacy of one or more of these vitamins, rather than the homocysteine concentration itself, may be responsible for the observed effect on the risk of hip fracture. Patients with pernicious anemia have decreased bone mineral density at the lumbar spine,[38] and in comparison with the general population they have almost double the risk of hip fracture.[39] A recent, population-based study showed that older women, but not men, with low bone mineral density had significantly lower vitamin B_{12} concentrations than older women with higher bone density.[40]

It is also possible that the effect of homocysteine on the risk of hip fracture is mediated through other nutrients, apart from B vitamins, that we were unable to measure. If there is a direct effect of these dietary factors on the risk of hip fracture, the homocysteine concentration may simply reflect nutritional status. Thus, dietary factors may explain the relation between the plasma homocysteine concentration and the risk of hip fracture observed in our study. Finally, because no data on bone mineral density were available for the study participants at baseline, we were unable to assess whether the effect of homocysteine on hip fracture may be mediated through bone mineral density.

We were not able to establish causality definitively, because plasma homocysteine concentrations may only be a marker for nutritional or metabolic differences that are the real causal factors in hip fracture. Furthermore, we cannot determine whether the association observed in our study population is a result of the same mechanisms that lead to an increased prevalence of osteoporosis among patients with homocystinuria.

This study suggests that the total homocysteine concentration is strongly associated with the risk of hip fracture. If the relationship proves to be one of cause and effect, this finding may have important implications for the development of interventions to prevent hip fractures, because total homocysteine concentrations can be effectively and easily modified by dietary intake of folic acid and vitamins B_6 and B_{12}. Further population-based research is needed to examine the role of homocysteine in osteoporosis and osteoporotic fracture and to determine whether nationwide folic acid fortification of food will help to reduce rates of hip fracture in the United States.

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