Homocysteine Levels and Risk of Hip Fracture in Postmenopausal Women

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Background: Recent studies suggest that high homocysteine levels are associated with an increased risk of fractures. Homocysteine levels are known to be influenced by vitamin B and folate supply or status, and poor renal function can result in higher levels independent of nutritional adequacy.

Objective: The aim of the study was to determine the associations between fasting homocysteine levels and incident hip fractures, and the effects of other factors on hip fracture risk.

Design: We conducted a case-control study in the Women's Health Initiative Observational Study, a study of postmenopausal women (n = 93,676) recruited in the United States. We selected 400 incident cases of hip fracture and 400 controls matched on age, ethnicity, and blood draw date among women not on osteoporosis therapies. Outcome measures included physician-adjudicated, incident hip fractures. Baseline lifestyle and nutritional questionnaires were performed.

Results: The risk of hip fracture increased 1.38-fold [95% confidence interval (CI), 1.14, 1.66] for each SD increase in serum homocysteine level after adjustment for fracture risk factors. This association was not affected by adjustment for dietary folate, B6, or B12 intake, but it diminished after adjustment for cystatin-C level (odds ratio, 1.08; 95% CI, 0.66–1.79), a measure of renal function not affected by muscle mass. Among women in the highest quartile of homocysteine and cystatin-C compared to those without elevations in either biomarker, the risk of hip fracture was substantially elevated (odds ratio, 2.8; 95% CI, 1.61–4.87).

Conclusions: This study indicates that high homocysteine levels are associated with an increased risk of hip fracture, which could be accounted for by poor renal function. **(*J Clin Endocrinol Metab* 94: 1207–1213, 2009)**

Osteoporosis is a major public health problem in the United States, especially in women. Nutritional, lifestyle, hormonal, and genetic factors contribute to the risk of fractures. The risk of osteoporotic fractures rises exponentially with age, resulting in 2 million cases of fractures annually in the United States, including 300,000 hip fractures, the most serious fracture (1). The health consequences of hip fractures are enormous and include progressive loss of function, inability to ambulate independently in more than half of those affected, and mortality in up to 24% of women (2). Although 44 million Americans currently have osteoporosis and/or low bone mass, the recent Surgeon General's Report on Osteopo-

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Abbreviations: BMI, Body mass index; CI, confidence interval; MET, metabolic equivalent; OR, odds ratio.

rosis projects that the number of Americans affected will rise to 52.4 million by the year 2010 (3).

High homocysteine levels have been associated with an increased risk of fractures, although the factors that contribute to this fracture risk are not fully elucidated. Homocysteine is an intermediary amino acid formed by the conversion of methionine to cysteine. Homocystinuria is a rare autosomal recessive disease characterized by increased circulating homocysteine levels, with clinical manifestations involving the eyes, vasculature and nervous system, and skeleton including the development of early osteoporotic fractures (4–6). Adults without homocystinuria who have high homocysteine levels are also at risk for fractures (7–9). Nutritional factors such as vitamin B12, B6, and folate are cofactors in homocysteine metabolism, and vitamin intakes may inversely affect plasma homocysteine levels (10–12). Gene polymorphisms related to homocysteine metabolism also may result in high homocysteine levels. Thus, nutritional factors such as B12/folate deficiency and genetic factors may affect homocysteine levels and contribute to fracture risk.

Chronic kidney disease affects an estimated 19 million Americans (13). Recent data show that glomerular filtration rate as measured by cystatin-C is an important determinant of plasma homocysteine. Lewerin *et al.* (14) conducted a population-based study among 209 community-dwelling subjects with mean age of 76 yr. They found that cystatin-C levels were correlated with plasma homocysteine concentrations ($r = 0.45$; $P < 0.001$) independent of vitamin B (12) and folate status (14). Additionally, Fried *et al.* (15) recently reported that kidney dysfunction, as assessed by cystatin-C, is associated with an increased risk of hip fracture.

To advance our understanding of the mechanisms that lead to osteoporotic hip fractures, we used the Women's Health Initiative Observational Study (WHI-OS) to determine the relationship between homocysteine levels and the risk of incident hip fractures in the largest study of postmenopausal women with hip fractures. We then examined both dietary intakes of vitamin B6, B12, and folate and renal function as measured by cystatin-C levels, as potential explanatory factors for any observed association.

Subjects and Methods

The WHI-OS is a multicenter; multiethnic study designed to address the common causes of death, disability, and impaired quality of life among postmenopausal women in the United States. The study includes 93,676 women aged 50 to 79 yr at entry between 1993 and 1998 from 40 clinical centers in the United States. Details of the scientific rationale, eligibility requirements, and other aspects of the study design of the WHI-OS have been published previously (16). Women completed screening and enrollment questionnaires and underwent a physical examination, and a fasting blood specimen was collected at baseline. Blood was processed for long-term storage at −70 C. The study was reviewed and approved by human subjects review committees at each participating institution, and written, informed consent was obtained from all women enrolled in the study.

For this current analysis, we excluded participants with a history of hip fracture and those who were on certain medications including estrogen therapy, androgens, and selective estrogen receptor modulators, bisphosphonates, calcitonins, and PTH at baseline. In addition, we excluded women with confirmed or possible pathological cause for hip fractures occurring during the study, hip fractures with only local adjudication, unknown ethnicity, and inadequate available plasma.

Assessment of outcome

Data on the incidence of total fractures or radiologically confirmed fractures of the hip, spine, forearm, or wrist were collected at baseline and annually to ascertain the occurrence of a new fracture. Participants were asked annually whether they had a fracture since they last completed the medical history questionnaire and, if so, to identify the location (vertebral, shoulder, upper arm, lower arm, wrist, hip, upper leg, lower leg, or foot). All hip fractures were centrally adjudicated. A validation study of self-reported fractures was conducted on the WHI-OS and found good agreement between self-report and adjudicated fracture for hip (78%) (17).

Nested case-control design

Using this baseline population, we conducted a nested case-control study within the WHI-OS. The study population included 400 cases of incident hip fracture and 400 controls. For each case we selected one control using the risk set sampling technique described by Prentice and Breslow (18); *i.e.* for each incident hip fracture case, a control was selected at random from a list of all women at risk of the outcome. In addition, controls were matched on race/ethnicity, age (within 1 yr), and date of blood draw (within 4 months).

Data collection

Laboratory measurements

Total homocysteine levels (fasting samples) were measured at baseline in serum using a HPLC assay (Medical Research Laboratories International, Highland Heights, KY). The coefficient of variation was 7.3–7.6%, with a range of 5–15 μmol/liter. The laboratory personnel were blinded to case-control status, and samples were analyzed in random order.

Serum cystatin-C was measured using the Dade Behring BN-II nephelometer with a particle-enhanced immunonepholometric assay (GMI, Inc., Ramsey, MN) (interassay coefficient of variation, 5.7%; sensitivity, 0.02 mg/liter).

Hip fracture risk factors and dietary variables

Cohort members completed detailed self-administered questionnaires on demographic information, medical history, reproductive history, nutrition, smoking and alcohol, family history, personal habits, and recreational physical activity. Ethnicity was self-identified as white not of Hispanic origin, African-American, Hispanic, American Indian, Alaskan Native, Asian or Pacific Islander, or unknown. Smoking status was determined from lifetime smoking of at least 100 cigarettes, current daily cigarette smoking, and self-report of smoking cessation. Alcohol consumption was computed from the personal habits questionnaire. Total dietary and supplemental intake of folate and vitamins B6 and B12 was determined from the food frequency questionnaires and the supplement use questionnaires; nutrient intake analysis from the food frequency questionnaire data was adjusted with folic acid fortification of the food supply, which was initiated in 1996 (19).

Anthropometric measurements were obtained at baseline by trained and certified Clinical Center staff. Weight was measured to the nearest 0.1 kg, and height was recorded to the nearest 0.1 cm. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Physical activity was assessed by questions on the frequency and duration of several types of recreational activity, and metabolic equivalent (MET) scores were computed as the product of days per week, minutes per day, and the MET value for each activity (20).

Statistical analysis

All statistical analyses were performed with SAS version 9.1 (SAS Institute, Inc., Cary, NC). For all analyses, we categorized homocysteine into quartiles based on the distribution in the control group. The homocysteine levels greater than 50 μmol/liter were determined to be biolog-

TABLE 1. Comparison of baseline characteristics among cases of hip fractures and controls

Characteristics	Control	Case	*P* value
Age at screening (yr)	70.8 ± 6.2	70.8 ± 6.2	0.986
BMI (kg/m^2)	27.4 ± 5.1	26.0 ± 5.2	0.000
Total energy expended from physical activity (MET)	13.9 ± 15.3	10.7 ± 12.7	0.002
Years since menopause	22.0 ± 9.0	22.3 ± 8.8	0.644
Total calcium intake (mg)	1167.0 ± 683.9	1072.5 ± 694.2	0.053
Total vitamin D intake (μg)	9.3 ± 6.9	9.6 ± 9.9	0.707
Total folate intake (μg)	443.9 ± 267.6	450.4 ± 320.9	0.757
Total B12 intake (μg)	22.8 ± 80.9	24.6 ± 76.7	0.753
Homocysteine (μmol/liter)	10.7 ± 3.2	11.7 ± 4.7	0.001
Cystatin-C (mg/liter)	1.06 ± 0.24	1.10 ± 0.30	0.023
Alcohol intake (>1 drink per day) (%)	10.6	9.8	0.608
Current smokers (%)	2.5	9.1	0.000
History of treated diabetes (%)	4.8	6.0	0.433
History of myocardial infarction (%)	3.5	5.5	0.170
History of stroke (%)	2.0	4.5	0.046
Parental history of hip fracture (%)	16.0	20.0	0.141

Data are expressed as mean ± SD unless indicated otherwise.

ically implausible for postmenopausal women and were set to missing during the analysis.

Conditional logistic regression model was used to examine the association between the quartiles of homocysteine and the risk of hip fracture. Multivariate models were used to account for the potential confounders including BMI, parental history of hip fracture, treated diabetes, alcohol use, smoking, and history of stroke.

We estimated the odds ratios (OR) and the 95% confidence intervals (CI) using the lowest quartile as the reference category. Tests for trend were conducted based on continuous scale of the log-transformed plasma homocysteine value and also by the ordinal value of each quartile. All *P* values reported are two-tailed, and values less than 0.05 were considered statistically significant. We also examined the association between hip fracture, homocysteine, and other possible mediators by the additional adjustment for folate, vitamins B6 and B12, and cystatin-C one at a time and jointly. The relationship between homocysteine and cystatin-C was further explored using models including both biomarkers as continuous variables and then as quartiles.

Results

Table 1 shows the comparison of baseline characteristics in cases and controls. As expected, controls were slightly heavier but were more physically active compared with cases. There were no differences in baseline characteristics between cases and controls and treated diabetes or a history of myocardial infarction, which are also associated with high homocysteine levels (Table 1). Cases had lower calcium intake, smoked more, and tended to have an increased stroke history at baseline compared with controls in the study. The mean baseline homocysteine concentrations were higher in hip fracture cases as compared with controls (11.7 *vs.* 10.7 μmol/liter; $P = 0.001$).

Table 2 shows the OR and 95% CI for the risk of incident hip fracture by quartiles of homocysteine levels. The OR for a hip fracture for each increase in SD of plasma homocysteine was 1.36 (95% CI, 1.13, 1.63). In the conditional logistic regression model accounting for the matched design and additionally adjusted for BMI, the OR between extreme quartiles of homocysteine was 1.49 (95% CI, 0.98, 2.29). Further adjustment of several potential confounders including parental history of hip fracture, treated diabetes, alcohol use, smoking, history of stroke, and total calcium intake resulted in an OR of 1.37 (95% CI, 0.86, 2.19). The test of linearity based on log-transformed continuous scale and across quartiles produced somewhat different *P* for

TABLE 2. OR (95% CI) for the risk of hip fracture (cases/controls) by quartiles of plasma homocysteine and the relative risk for each quartile compared to the lowest quartile

Homocysteine[a]	Unadjusted	BMI-adjusted	MV-adjusted[b]
Per SD (4.06 μmol/liter) increase	1.31 (1.12, 1.53)	1.36 (1.16, 1.60)	1.36 (1.13, 1.63)
P^1 for linear trend (log-transformed)	$P = 0.002$	$P < 0.001$	$P = 0.002$
Per μmol/liter increase	1.07 (1.03, 1.11)	1.08 (1.04, 1.12)	1.07 (1.02, 1.13)
No. of missing pairs (total = 400)	7	11	24
Quartiles (according to control group)			
P^2 for linear trend across quartiles	$P = 0.078$	$P = 0.033$	$P = 0.102$
1st quartile (<8.4 μmol/liter) (reference)	1	1	1
2nd quartile (8.4 to <10.0 μmol/liter)	0.88 (0.58, 1.32)	0.91 (0.60, 1.39)	0.85 (0.54, 1.35)
3rd quartile (10.0 to <12.5 μmol/liter)	1.10 (0.75, 1.63)	1.12 (0.75, 1.68)	1.10 (0.71, 1.69)
4th quartile (≥12.5 μmol/liter)	1.34 (0.89, 2.01)	1.49 (0.98, 2.29)	1.37 (0.86, 2.19)

P^1, Test of linear trend based on continuous scale and log-transformed plasma homocysteine value; P^2, test of linear trend based on ordinal values of the homocysteine quartile; MV, multivariate.

[a] Hip fracture case and control selection matched on age, ethnicity, and blood draw date.

[b] Multivariate adjustment includes BMI, parental history of hip fracture, treated diabetes, alcohol use, smoking, history of stroke, and total calcium intake.

TABLE 3. Adjusted ORs for hip fractures, according to baseline homocysteine quartiles (μmol/liter)

(Total pairs = 400)	r[b]	1st (<8.4)	2nd (8.4 to <10.0)	3rd (10.0 to <12.5)	4th (≥12.5)	P-trend[1]	P-trend[2]
Base analysis[c]							
OR (95% CI) (n = 376)[a]		1.0	0.85 (0.54–1.35)	1.10 (0.71–1.69)	1.37 (0.86–2.19)	0.002	0.102
P			0.500	0.670	0.189		
Base analysis[c] + adjusted for total folate intake[d]							
OR (95% CI) (n = 376)[a]	−0.248	1.0	0.86 (0.54–1.36)	1.12 (0.73–1.73)	1.40 (0.87–2.25)	0.002	0.085
P	<0.0001		0.513	0.608	0.164		
Base analysis[c] + adjusted for total vitamin B6 intake[d]							
OR (95% CI) (n = 376)[a]	−0.041	1.0	0.87 (0.55–1.37)	1.10 (0.72–1.70)	1.38 (0.87–2.21)	0.002	0.098
P	0.254		0.540	0.651	0.175		
Base analysis[c] + adjusted for total vitamin B12 intake[d]							
OR (95% CI) (n = 376)[a]	−0.057	1.0	0.86 (0.54–1.36)	1.11 (0.72–1.71)	1.38 (0.86–2.20)	0.003	0.096
P	0.111		0.515	0.636	0.181		
Base analysis[c] + adjusted for plasma cystatin-C							
OR (95% CI) (n = 372)[a]	0.452	1.0	0.79 (0.50–1.26)	0.96 (0.62–1.49)	1.08 (0.66–1.79)	0.070	0.580
P	<0.0001		0.326	0.849	0.756		
Base analysis[c] + adjusted for total folate,[d] B6,[d] B12,[d] and cystatin-C							
OR (95% CI) (n = 372)[a]		1.0	0.81 (0.51–1.28)	0.98 (0.62–1.53)	1.11 (0.67–1.85)	0.050	0.516
P			0.367	0.923	0.678		
Base analysis[c] + adjusted for RAND 36 physical functioning >90							
OR (95% CI) (n = 352)[a]	−0.161	1.0	0.91 (0.57–1.46)	1.12 (0.72–1.75)	1.44 (0.88–2.35)	0.003	0.088
P	<0.0001		0.691	0.622	0.144		

P-trend[1], Test of linear trend based on continuous scale and log-transformed plasma homocysteine value; P-trend[2], test of linear trend based on ordinal values of the homocysteine quartile.

[a] n indicates number of case-control pairs included in the analysis.

[b] Pearson correlation coefficients with homocysteine.

[c] Matched on age, ethnicity, blood draw date; controlled for BMI, parental history of hip fracture, treated diabetes, smoking, alcohol use, history of stroke, and total calcium intake.

[d] Total intake from diet and supplement.

linear trend (Table 2). Therefore, the results from both methods are included in the table. The CIs for the quartiles show the precision of the quartiles. The significance of our results was determined according the *P* value for trend.

Table 3 shows the ORs for risk of hip fracture by quartiles of homocysteine with additional adjustment for levels of cystatin-C and intakes of folate and vitamins B6 and B12. The addition of folate and vitamins B6 and B12 intake in the multivariate model did not change the OR for homocysteine substantially. Adjustment for cystatin-C attenuated the association of homocysteine and risk of hip fractures. The table also presents the correlations between homocysteine and folate, vitamins B6 and B12, and cystatin-C. As anticipated, there was a negative linear correlation between homocysteine levels and folate intake ($r = -0.25$; $P < 0.0001$) and B6 and B12 intakes. There was also a positive correlation between homocysteine and cystatin-C ($r = 0.45$; $P < 0.0001$).

We also conducted a secondary analysis that shows the ORs for risk of hip fracture by quartiles of homocysteine and cystatin-C (Table 4). The odds ratio for women in the highest quartiles of both homocysteine and cystatin-C is 2.8 (1.61, 4.87; *P* for interaction = 0.026) compared with women in the lower three quartiles of both homocysteine and cystatin-C after adjusting for BMI, parental history of hip fracture, treated diabetes, alcohol use, smoking, history of stroke, total calcium intake, and total intake of folate and vitamins B6 and B12. The interaction of homocysteine and cystatin-C levels was not statistically significant when the biomarkers were studied as continuous variables or in quartile form.

Discussion

The present study supports a modest association between high homocysteine levels and increased hip fracture risk (7–9). In this study of postmenopausal women enrolled in the WHI-OS, every SD increase in serum homocysteine levels was associated with a significant 36% elevated risk of hip fracture. The quartile analysis revealed that most of this association was concentrated in the upper quartile. Our results remained unchanged after additional adjustment for total intake of folate and vitamins B6 and B12. Adjustment for cystatin-C, however, diminished the homocysteine association; women in the highest quartile of both homocysteine and cystatin-C had a much larger, 2.8-fold risk of suffering hip fracture compared with women without elevations in either biomarker. These data suggest that high homocysteine

TABLE 4. Adjusted ORs for hip fractures, according to quartiles of homocysteine (μmol/liter) and cystatin-C (mg/liter)

	Homocysteine quartiles[c]			
Cystatin-C quartiles	**1st (<8.4)**	**2nd (8.4 to <10.0)**	**3rd (10.0 to <12.5)**	**4th (≥12.5)**
4th (≥1.15)		1.10 (0.68, 1.77)[a]		2.80 (1.61, 4.87)[a,b]
3rd (1.01 to <1.15)				0.86 (0.53, 1.39)[a]
2nd (0.91 to <1.01)		1.00 (ref)		
1st (<0.91)				

[a] Matched on age, ethnicity, blood draw date, and adjusted for BMI, parental history of hip fracture, treated diabetes, smoking, alcohol use, history of stroke, total calcium intake, folate, vitamin B6, and vitamin B12.

[b] $P < 0.001$.

[c] P for interaction between 4th quartile *vs.* not of cystatin-C and 4th quartile *vs.* not of homocysteine = 0.026.

levels are associated with an increased risk of hip fracture, especially in women with poor renal function as assessed by cystatin-C levels rather than dietary intakes of vitamins B6 and B12 or folate.

Moderately elevated homocysteine levels increase with age and occur in about 5–7% of the population (21, 22). Data from two different cohort studies show that high homocysteine levels are associated with risk for fractures (7–9). In the Framingham Study, hip fractures doubled in women (n = 146 hip fractures) who had homocysteine levels in the highest quartile (8). Of note, absolute homocysteine levels in the highest quartile of the women in that study were higher than those from our study. Nonfasting plasma samples for the Framingham study were collected between 1979 and 1982, and women were followed for incident hip fractures through 1998. In the WHI-OS, blood samples were collected in a fasting state between 1993 and 1998 (34% of blood drawn before 1996). Because foods were fortified with folic acid in the United States starting in 1996 (to reduce neural tube defects), the high homocysteine levels in the Framingham Study may be attributable, in part, to the different intakes of folic acid; fortification of foods may result in a reduction in high fasting homocysteine levels from 18.7 to 9.8% (10, 11). In the Rotterdam and Longitudinal Aging Study Amsterdam studies, fractures doubled in women with homocysteine levels in the highest quartile (7). However, a recent population-based study in Sweden was conducted to examine the association between homocysteine and bone turnover, bone mineral density, and fracture risk. The authors reported that high homocysteine levels were associated with higher bone turnover and poor physical performance, but not total fracture risk (23).

In addition, the mechanisms through which high homocysteine levels affect bone have not been fully elucidated. Studies *in vivo* in animal models have shown that hyperhomocysteinemia leads to a disruption of collagen cross-links that may adversely affect bone formation and skeletal strength (24–26). Other studies indicate that high homocysteine levels are associated with increased markers of bone resorption, with a net increase in bone breakdown relative to formation (23, 27). End-stage renal disease is associated with an increased risk of fractures in association with several metabolic bone disorders (28). Although bone density testing does not distinguish the type of renal osteodystrophy, the National Health and Nutrition Examination Survey III analyses indicate that 85% of women with glomerular rates less than 60 cc/min have osteoporosis according to bone density criteria (29). New data also show that mild to moderate kidney dysfunction, as assessed by a novel marker, cystatin-C, is associated with an increased risk for hip fracture (10). Recently, Lewerin *et al.* (14) reported that renal function as assessed by cystatin-C is also a determinant of plasma homocysteine levels. Our new data indicate the involvement of renal function in the relationship between homocysteine and hip fracture risk. It is important to note that previous studies reporting the relationship between homocysteine and fracture risk did not include cystatin-C in their analyses. Recent data from the WHI-OS that specifically examined the association of cystatin-C concentrations, levels of renal impairment, and hip fracture showed that hip fracture risk was elevated in women whose estimated glomerular filtration rates were less than 60 mg/ml per 1.73 m^2 (30, 31).

Cystatin-C is a low molecular weight protein produced by nucleated cells. It is filtered by the kidney and undergoes metabolism after tubular resorption. Unlike creatinine, cystatin-C is not affected by muscle mass and age. Thus, serum creatinine may not serve as the appropriate biomarker to precisely measure mild to moderate renal insufficiency (14, 32, 33). Our study confirms an association between homocysteine levels and hip fracture risk, but it indicates that the risk for hip fracture is no longer significant when these data are adjusted for cystatin-C level, an index of renal function. Hence, our findings indicate that homocysteine is associated with hip fracture only when renal function is impaired, and that homocysteine may be a mediator of the association between renal insufficiency and hip fracture or share a causal pathway. However, the present study was not designed to determine whether the two biomarkers share a common causal pathway or how each biomarker relates to the pathophysiology of osteoporosis and hip fracture.

As expected, our data show a negative linear correlation between homocysteine levels and intake of folate ($r = -0.25$); intakes of folate and B vitamins were similar in hip fracture cases and controls. Previous studies by Selhub *et al.* (11) reported an inverse relation between homocysteine and plasma folate. In another study by Ubbink *et al.* (34), there were significantly lower plasma concentrations of vitamin B6, B12, and folate in men with hyperhomocysteinemia.

Vitamin B12 status has also been linked to reduced bone mass in elderly women. Investigators for the Hordaland study recently reported that plasma folate, but not vitamin B12, was an independent predictor of hip fracture among women (31). Although the association between B vitamins/folate and homocysteine has

been reported, there currently are not conclusive data to prove the relationship between folate and B vitamins and risk of fractures (35). One study reported that low serum folate and not homocysteine is responsible for the association between homocysteine and risk of osteoporotic fracture in elderly persons (36). Among 111 women and men with hip fractures, low levels of B vitamins were a risk factor for hip fractures, but the association of homocysteine and hip fractures was independent of these levels. More recently, Sato *et al.* (37) showed that in patients with strokes, supplementation with folate and B vitamins reduced hip fracture risk. Randomized, controlled studies are being conducted to understand better the effects of B vitamins and folate and reductions in homocysteine levels on fracture risk.

Our study includes a population of postmenopausal women from clinical centers all over the United States, thus enhancing the generalizability of the results. Strengths of our study are the inclusion of 1) the large number of adjudicated hip fractures (n = 400) and carefully matched controls (n = 400); and 2) dietary information and lifestyle variables, which enabled us to evaluate for intakes of folate and B vitamins and other confounding factors. A limitation of our study is that we did not measure serum levels of folate and B vitamins. Although there are advantages of using cystatin-C as a marker of renal function, a limitation is that we did not also measure creatinine levels. Another limitation is that there is a possibility of residual confounding by the variables that we cannot account for (38).

High homocysteine levels are associated with an increased risk of hip fracture and could be accounted for by poor renal function. These novel biomarkers may lead to a new understanding about the pathophysiology of hip fracture and how it can be prevented. Intervention trials targeting homocysteine levels should carefully evaluate the role of renal function when assessing the efficacy of nutritional supplementation.

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Trial Registration: The name of trial registry, registration number, and URL of the registry will be forthcoming.

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M.S.L., A.L., and L.W. had full access to all the data in this study and take responsibility for the integrity of the data and, to the best of their knowledge, the accuracy of the data and the data analysis.

Disclosure Summary: R.N., A.L., L.W., R.J., J.L., D.C.B., C.K., C.L., A.M.T., and S.C. have nothing to declare. J.C. currently is working on projects involving research grants from Merck, Eli Lilly, Pfizer Pharmaceuticals, and Novartis Pharmaceuticals. M.S.L. works on active research projects that are supported by the National Institutes of Health. J.C. has received honorariums from Merck, Eli Lilly, and Novartis Pharmaceuticals. M.S.L. has an unrestricted Center of Excellence education grant (2005–2006) from Abbott. At the time of this study, J.C. was on the Speaker's Bureau for Merck.

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