Cystatin-C, Renal Function, and Incidence of Hip Fracture in Postmenopausal Women

Andrea Z. LaCroix, PhD,[*] Jennifer S. Lee, MD,[†] LieLing Wu, MS,[*] Jane A. Cauley, DrPH,[‡] Michael G. Shlipak, MD,[§] Susan M. Ott, MD,[‖] John Robbins, MD,[#] J. David Curb, MD,[**] Meryl Leboff, MD,[††] Douglas C. Bauer, MD,[‡‡] Rebecca D. Jackson, MD,[§§] Charles L. Kooperberg, PhD,[*] and Steven R. Cummings, MD[‡‡]

OBJECTIVES: To evaluate the association between chronic kidney disease and incident hip fracture using serum cystatin-C as a biomarker of renal function calculated without reference to muscle mass.

DESIGN: Case-control study nested within a prospective study.

SETTING: The Women's Health Initiative Observational Study conducted at 40 U.S. clinical centers.

PARTICIPANTS: From 93,676 women aged 50 to 79 followed for an average of 7 years, 397 incident hip fracture cases and 397 matched controls were studied.

MEASUREMENTS: Cystatin-C levels were measured on baseline serum using a particle-enhanced immunonepholometric assay. Estimated glomerular filtration rates ($eGFR_{cys\text{-}c}$) were calculated using a validated equation and categorized into three groups ($\geq$90.0 mL/min per 1.73 m^2, 60.0–89.9 mL/min per 1.73 m^2, and $<$60.0 mL/min per 1.73 m^2 indicating chronic kidney disease Stages 3 to 4).

RESULTS: The odds ratio (OR) for hip fracture was 2.50 (95% confidence interval (CI) = 1.32–4.72) for $eGFR_{cys\text{-}c}$ less than 60 mL/min per 1.73 m^2 compared with Stages 0 to 1, after adjustment for body mass, parental hip fracture, smoking, alcohol consumption, and physical function. No association was observed for $eGFR_{cys\text{-}c}$ of 60 to 90 mL/min per 1.73 m^2 (OR = 1.04, 95% CI = 0.66–1.64). Additional adjustment for poor health status, hemoglobin, serum 25-hydroxy vitamin D, and bone metabolism markers did not affect these associations. Adjustment for plasma homocysteine reduced the OR for $eGFR_{cys\text{-}c}$ less than 60 mL/min per 1.73 m^2 to 1.83 (95% CI = 0.93–3.61).

CONCLUSION: Women with $eGFR_{cys\text{-}c}$ levels less than 60 mL/min per 1.73 m^2 have a substantially greater risk of hip fracture. Effects of renal function on homocysteine levels may partially mediate, or accompany, this association.
J Am Geriatr Soc 56:1434–1441, 2008.

Key words: chronic kidney disease; hip fracture; cystatin-C; renal function

From the [*]Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, Washington; [†]Department of Medicine, Division of Endocrinology, Clinical Nutrition, and Vascular Medicine, University of California at Davis, Sacramento, California; [‡]Department of Epidemiology, Graduate School of Pubic Health, University of Pittsburgh, Pittsburgh, Pennsylvania; [§]Veterans Affairs Medical Center and Department of Medicine, University of California at San Francisco, San Francisco, California; [‖]Department of Medicine, University of Washington, Seattle, Washington; [#]Center for Healthcare Policy and Research, University of California at Davis Medical Center, Sacramento, California; [**]Pacific Health Research Institute, Honolulu, Hawaii; [††]Division of Endocrinology, Brigham and Women's Hospital, Harvard University, Boston, Massachusetts; [‡‡]California Pacific Medical Center Research Institute and Departments of Medicine and Epidemiology, University of California at San Francisco, San Francisco, California; and [§§]Division of Endocrinology, Diabetes, and Metabolism, Ohio State University Medical Center, Columbus, Ohio.

This paper was presented at the 29th Annual Meeting of the American Society for Bone and Mineral Research, Honolulu, Hawaii, September 2007.

Address correspondence to Andrea Z. LaCroix, PhD, Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, 1100 Fairview Avenue North, P.O. Box 19024, Seattle, WA 98109-1024.
E-mail: alacroix@whi.org

DOI: 10.1111/j.1532-5415.2008.01807.x

Severe kidney disease appears to increase the risk of hip fracture.[1,2] Chronic kidney disease (CKD) is common, but mild and moderate stages are frequently clinically unrecognized and therefore understudied. Two prior prospective studies support an association between moderate renal insufficiency as measured using serum creatinine estimated glomerular filtration rates (eGFR) or cystatin-C levels and greater hip fracture risk in older women. In both studies, associations were lower after full multivariate adjustment,[3,4] although renal function biomarkers such as eGFR from serum creatinine are dependent on age and muscle mass, two major determinants of fracture risk. A method of estimating renal function that is not calculated using muscle mass or body size has potential to better define the risk of fractures due to emerging kidney disease.

0002-8614/08/$15.00

Cystatin-C is a 122-amino acid, 13-kDa protein originating broadly from nucleated cells that is filtered by the kidney and completely metabolized by the proximal tubule. Serum cystatin-C levels are reportedly independent of age and lean tissue mass and potentially superior to serum creatinine in detecting mild to moderate renal impairment. The results of a large case-control study nested within the prospective Women's Health Initiative Observational Study (WHI-OS) designed to determine whether cystatin-C levels are related to risk of hip fracture in postmenopausal women, independent of other hip fracture risk factors and in the absence of hormone therapy and osteoporosis medications, are reported here. Potential explanatory factors for an association between renal function and osteoporosis and hip fracture are explored.

METHODS

Study Group

The WHI-OS is a prospective cohort study that enrolled 93,676 women aged 50 to 79 from 1994 to 1998 at 40 clinical centers throughout the United States. Study methods have been described in detail elsewhere.[5] Briefly, women were eligible if they were postmenopausal, unlikely to move or die within 3 years, not enrolled in the WHI Clinical Trial, and not currently participating in any other clinical trial. At baseline, women completed screening and enrollment questionnaires by interview and self-report, a physical examination, and blood specimen collection. Human subjects review committees at each participating institution reviewed and approved the study.

Follow-Up and Outcome Ascertainment

Women were sent questionnaires annually to report the occurrence of any hospitalization and a wide variety of outcomes including clinical fractures of any type. Follow-up time ranged from 0.7 to 9.3 years per participant as of August 2004, with a median duration of 7.13 years. At that time, 3.7% of WHI OS participants had withdrawn or were lost to follow-up, and 5.3% had died. Trained physicians verified hip fractures by reviewing radiological, magnetic resonance imaging, or operative reports at each clinical center, and blinded central adjudicators confirmed.[6] Hip fractures with a possible or confirmed pathological cause (from malignancy, infection, or focal bone lesion) were excluded.

Nested Case-Control Study Design

The present study is a case-control study nested within the prospective design of the WHI-OS using incident hip fracture cases identified through August 2004. Participants were excluded if they had a prior history of hip fracture at baseline or were taking osteoporosis treatments (bisphosphonates, calcitonins, parathyroid hormone). Because endogenous hormone levels were also under investigation, women taking estrogen up to 1 year before enrollment or currently taking androgens (anabolic steroids, dehydroepiandrosterone, testosterone), selective estrogen receptor modulators, or antiestrogens were also excluded. Women without sufficient serum or with unknown ethnicity were also excluded, leaving a final study group of 39,795 eligible participants. From this group, 404 incident cases of hip fracture were identified. One control per case was selected with individual matching according to age at screening ($\pm$ 1 year), race or ethnicity, and date of blood draw ($\pm$ 120 days). Cystatin-C levels were obtained in 397 matched pairs.

Baseline Clinical Variables

All covariates were ascertained at baseline. Clinic interviewers recorded current use of prescription medications, including thiazide diuretics and corticosteroids, at the first screening visit by directly inspecting medicine containers. Prescription names were entered into the WHI database, which assigned drug codes using Medispan software.

Dietary supplements, including calcium preparations, taken at least twice weekly for the prior 2 weeks were entered directly from medicine containers as described above. Dietary intake of calcium was measured using a semi-quantitative food-frequency questionnaire.[7] Total calcium intake was defined as the sum of calcium from diet, supplements, and medications.

Baseline questionnaires ascertained information on race or ethnicity, age at menopause, personal history of fracture after age 55, treated diabetes mellitus, myocardial infarction, coronary revascularization or stroke, current and past smoking, parental history of hip fracture, and self-rated health status. Alcohol consumption was estimated using questionnaire items as servings per week. Physical activity was classified based on frequency and duration of four speeds of walking and mild, moderate, and strenuous activities in the prior week. Kilocalories of energy expended in a week on leisure-time activity was calculated (metabolic equivalent score = kcal h/wk per kg).[8] Physical function was measured using the 10-item Rand-36 Physical Function Scale, which includes items measuring whether health now limits physical function in moderate to vigorous activities; strength to lift, carry, stoop, or bend; ability to climb stairs; ability to walk various distances without difficulty; and self-care.[9] Frailty was defined as a score of 3 or more based on the sum of poor physical function (2 points), low physical activity, exhaustion, and weight loss as described previously using a measure validated in the WHI-OS.[10] Weight was measured to the nearest 0.1 kg on a balance beam scale with the participant dressed in indoor clothing without shoes. Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer. Body mass index (BMI) was calculated as weight (kg)/height (m^2).

Laboratory Procedures

Laboratory personnel were blinded to case-control status for all measurements. Serum cystatin-C levels were measured using the Dade Behring BN-II nephelometer and Dade Behring reagents (GMI Inc., Ramsey, MN) using a particle-enhanced immunonepholometric assay at Medical Research Laboratories International (Highland Heights, KY). The assay has a sensitivity of 0.02 mg/L and an inter-assay coefficient of variation of 5.7%. The measurement range is 0.25 to 7.9 mg/L, with a reference range for ostensibly healthy people aged 1 to 78 of 0.53 to 0.93 mg/L. Estimated glomerular filtration rate ($eGFR_{cys\text{-}c}$) was calculated using the formula $76.7 \times \text{cystatin-C}^{-1.18}$, which has

been validated in large populations using urinary clearance of ^{125}I-iothalamate (51Cr-ethylenediaminetetraacetic acid).[11]

Several laboratory biomarkers were investigated as potential mechanisms for an association between renal function and hip fracture. Renal function influences serum total homocysteine, which has been associated with risk of hip fracture.[12–15] Levels were measured in fasting samples using a high-performance liquid chromatography assay at the same laboratory. The coefficient of variation was 7.3% to 7.6%, with a range of 5 to 15 μmol/L. As part of the baseline screening process, hemoglobin levels were measured in local laboratories using standard clinical procedures for complete blood count. Serum 25-hydroxy vitamin D (25(OH)D) was quantified according to radioimmunoassay using reagents from Diasorin (Stillwater, MN). C-terminal telopeptide of Type I collagen (CTX) and aminoterminal procollagen extension propeptide (PINP) were measured according to immunoassay at Synarc (Lyon, France).

Statistical Methods

Baseline characteristics of hip fracture cases and matched controls were compared, with corresponding *P*-values calculated from chi-square tests for categorical variables and *t*-tests for continuous variables. To further assess the potential for confounding, baseline characteristics were compared across quartiles of cystatin-C levels in control participants. Associations between cystatin-C levels and incident hip fracture were assessed in conditional logistic regression models retaining the matched case–control design (age, race or ethnicity, blood draw date). Associations were first examined without any additional adjustment and then with adjustment for BMI (continuous), parental history of hip fracture, smoking, alcohol use, and RAND-36 physical function score (>90, ≤ 90). Covariates were selected for inclusion in the full multivariate model based on their association with incident hip fracture in the initial univariate analysis and their correlation with cystatin-C levels. Correlations between cystatin-C and other biomarker levels were assessed using Pearson correlation coefficients (r).

Cystatin-C levels were evaluated as a continuous variable and also across quartile categories defined based on distribution in the control subjects. Using the $eGFR_{cys\text{-}c}$ formula, cystatin-C levels were categorized and analyzed per mL/min per 1.73 m^2 in three groups (>90, 60–90, and <60).[16] Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated from the conditional logistic regression models per standard deviation difference for continuous level of cystatin-C and in comparison with the best renal function group in the categorical models. To investigate mechanisms through which cystatin-C might be associated with hip fracture, a base model was constructed with adjustment for BMI, parental history of hip fracture, smoking, and alcohol, and then the following variables were added one at a time to determine their effect on the cystatin-C ORs: markers for deteriorating health status (poor physical function, frailty score, number of chronic conditions, hemoglobin level), homocysteine, 25(OH)D, and bone turnover markers (CTX and PINP).

RESULTS

The mean age of cases and controls was 71, and 95% were Caucasian (Table 1). Cases had lower BMI and were more likely to be current smokers, use corticosteroids, have a history of stroke, exercise less, and have lower physical function scores.

Cystatin-C levels were weakly correlated with age ($r = 0.22$) and BMI ($r = 0.19$) (Table 2). There was an association between physical function and cystatin-C, with 47% of women in the lowest quartile reporting high

Table 1. Baseline Characteristics of Hip Fracture Cases and Controls

Characteristic	Control n (%)	Case n (%)	*P*-Value
Ethnicity			1.00
White	380 (95.0)	380 (95.0)	
Black	10 (2.5)	10 (2.5)	
Hispanic	2 (0.5)	2 (0.5)	
American Indian	3 (0.8)	3 (0.8)	
Asian or Pacific Islander	5 (1.3)	5 (1.3)	
Age at screening			1.00
50–59	25 (6.3)	25 (6.3)	
60–69	107 (26.8)	107 (26.8)	
70–79	268 (67.0)	268 (67.0)	
Body mass index, kg/m^2			.001
<25.0	144 (36.1)	193 (48.6)	
25.0–29.0	150 (37.6)	127 (32.0)	
≥ 30.0	105 (26.3)	77 (19.4)	
History of fracture on or after age 55	82 (20.5)	96 (24.0)	.23
Parent had broken hip	64 (16.0)	80 (20.0)	.14
Hormone replacement therapy usage			.80
Never used	302 (75.5)	305 (76.3)	
Past user	98 (24.5)	95 (23.8)	
Oral daily corticosteroid use	3 (0.8)	14 (3.5)	.007
RAND 36—Physical Functioning score >90	117 (30.1)	84 (21.8)	.009
General health			.06
Excellent to very good	220 (56.0)	194 (48.9)	
Good	131 (33.3)	142 (35.8)	
Fair to poor	42 (10.7)	61 (15.4)	
Treated diabetes mellitus (pills or injections)	19 (4.8)	24 (6.0)	.43
Alcohol use			.61
Nondrinker	70 (17.6)	58 (14.6)	
Past drinker	80 (20.2)	89 (22.4)	
<7 drinks per week	205 (51.6)	212 (53.3)	
≥ 7 drinks per week	42 (10.6)	39 (9.8)	
Smoking			.00
Never smoked	215 (54.3)	214 (54.3)	
Past smoker	171 (43.2)	144 (36.6)	
Current smoker	10 (2.5)	36 (9.1)	
History of myocardial infarction	14 (3.5)	22 (5.5)	.17
History of stroke	8 (2.0)	18 (4.5)	.05

Table 2. Baseline Characteristics According to Quartiles of Cystatin-C in the Control Group

	Quartile of Serum Cystatin-C*				
	1	2	3	4	
Characteristic	n (%)				*P*-Value
Ethnicity					
White	97 (93.3)	90 (91.8)	92 (95.8)	98 (99.0)	.008
Black	0 (0.0)	5 (5.1)	4 (4.2)	1 (1.0)	
Hispanic	1 (1.0)	1 (1.0)	0 (0.0)	0 (0.0)	
American Indian	3 (2.9)	0 (0.0)	0 (0.0)	0 (0.0)	
Asian or Pacific Islander	3 (2.9)	2 (2.0)	0 (0.0)	0 (0.0)	
Age at screening					
50–59	12 (11.5)	7 (7.1)	4 (4.2)	2 (2.0)	<.001
60–69	46 (44.2)	22 (22.5)	21 (21.9)	16 (16.2)	
70–79	46 (44.2)	69 (70.4)	71 (74.0)	81 (81.8)	
Body mass index, kg/m^2					
18.5–24.9	53 (51.5)	43 (43.9)	29 (30.2)	19 (19.2)	<.001
25.0–29.9	33 (32.0)	38 (38.8)	36 (37.5)	42 (42.4)	
≥30.0	17 (16.5)	17 (17.4)	31 (32.3)	38 (38.4)	
Parent had broken hip	16 (15.4)	18 (18.4)	14 (14.6)	15 (15.2)	.89
Hormone replacement therapy usage					
Never used	81 (77.9)	71 (72.5)	72 (75.0)	76 (76.8)	.82
Past user	23 (22.1)	27 (27.6)	24 (25.0)	23 (23.2)	
Oral daily corticosteroid use	0 (0.0)	0 (0.0)	2 (2.1)	1 (1.0)	.28
General health (self-report)					
Excellent to very good	62 (60.8)	57 (59.4)	54 (56.8)	46 (47.4)	.41
Good	28 (27.5)	32 (33.3)	30 (31.6)	40 (41.2)	
Fair to poor	12 (11.8)	7 (7.3)	11 (11.6)	11 (11.3)	
Treated diabetes mellitus (pills or injections)	5 (4.8)	4 (4.1)	4 (4.2)	5 (5.1)	.99
Alcohol use					
Nondrinker	11 (10.7)	21 (21.4)	17 (17.7)	20 (20.6)	.01
Past drinker	19 (18.5)	13 (13.3)	26 (27.1)	20 (20.6)	
<7 drinks/wk	53 (51.5)	52 (53.1)	48 (50.0)	52 (53.6)	
≥7 drinks/wk	20 (19.4)	12 (12.2)	5 (5.2)	5 (5.2)	
RAND 36 Physical Functioning score >90	48 (47.1)	33 (34.4)	21 (22.8)	15 (15.6)	<.001
Thiazides and thiazide-like diuretic use	3 (2.9)	5 (5.1)	7 (7.3)	8 (8.1)	.39
Smoking					
Never smoked	55 (53.4)	47 (48.0)	52 (55.3)	59 (60.2)	.75
Past smoker	46 (44.7)	48 (49.0)	39 (41.5)	37 (37.8)	
Current smoker	2 (1.9)	3 (3.1)	3 (3.2)	2 (2.0)	

* Quartiles defined based on the distribution of cystatin-C levels in the control group. Cases are omitted from the table. *P*-values from chi-square test or Fisher exact test.

function, versus 16% of women in the highest quartile ($r = -0.24$). Frequent alcohol consumption was more common in women with lower cystatin-C levels. Levels of other fracture risk factors varied little across quartiles of cystatin-C.

The unadjusted OR for incident hip fracture comparing the highest and lowest quartiles of cystatin-C levels was 1.51 (95% CI = 0.98–2.33). A greater increase in risk occurred after adjustment for BMI (OR = 2.14, 95% CI = 1.33–3.43), and this association persisted after additional adjustment for parental hip fracture, smoking, alcohol consumption, and physical function score. Highly significant linear trends between cystatin-C level considered as a continuous variable in the multivariate models and incident fracture were observed, although most of the risk appeared to be concentrated in the upper quartile (Table 3).

Conversion of cystatin-C levels into $eGFR_{cys\text{-}c}$ categories classified 133 women with levels greater than 90 mL/min per 1.73 m^2, 517 with levels between 60 and 90 mL/min per 1.73 m^2, and 144 women with levels less than 60 mL/min per 1.73 m^2. The latter group included 138 women with CKD Stage 3 ($eGFR_{cys\text{-}c}$ 30–59 mL/min per 1.73 m^2) and six with CKD Stage 4 ($eGFR_{cys\text{-}c}$ 15–29 mL/min per 1.73 m^2). No women had Stage 5 CKD ($eGFR_{cys\text{-}c}$ <15 mL/min per 1.73 m^2). Table 4 presents associations between these $eGFR_{cys\text{-}c}$ categories and risk of hip fracture without adjustment, adjusted for BMI alone, and then in

Table 3. Relationship Between Cystatin-C Levels and Risk of Hip Fracture

Cystatin-C*	Unadjusted	Adjusted for BMI	Multivariate Adjusted†
	Odds Ratio (95% Confidence Interval)		
Per mg/L increase	2.08 (1.15–3.77)	3.01 (1.54–5.87)	2.92 (1.38–6.21)
Per standard deviation (0.27 mg/L) increase	1.22 (1.04–1.43)	1.35 (1.12–1.61)	1.34 (1.09–1.64)
P for linear trend	.02	<.001	.005
Number of missing pairs (total = 400)	6	10	32
Quartile (mg/L cutpoint)			
1 (0.58–0.90)	1	1	1
2 (0.91–1.00)	0.91 (0.59–1.39)	1.08 (0.69–1.68)	1.07 (0.66–1.76)
3 (1.01–1.14)	1.14 (0.74–1.75)	1.41 (0.89–2.22)	1.42 (0.86–2.34)
4 (1.15–3.68)	1.51 (0.98–2.33)	2.14 (1.33–3.43)	2.07 (1.21–3.55)

* Hip fracture case and control selection matched on age, ethnicity, and blood draw date. Quartile cutponts defined based on distribution in controls.
† Multivariate adjustment included body mass index (BMI), parental history of hip fracture, smoking, alcohol use, and RAND 36 physical functioning score.

full multivariate models. The adjusted OR relating $eGFR_{cys-c}$ less than 60 mL/min per 1.73 m^2 to hip fracture was 2.50 (95% CI = 1.32–4.72), but no association was seen between the group with $eGFR_{cys-c}$ 60 to 90 mL/min per 1.73 m^2 (OR = 1.04, 95% CI = 0.66–1.64) and that with $eGFR_{cys-c}$ greater than 90 (Table 4). Additional adjustment for physical activity did not alter these results, nor did adjustment for thiazide diuretic use, loop diuretic use, years since menopause, or total calcium intake. In separate conditional logistic models for hip fracture subtypes, the OR for $eGFR_{cys-c}$ less than 60 mL/min per 1.73 m^2 was high for femoral neck fracture (OR = 3.89, 95% CI = 1.71–8.83; 227 matched pairs) but not for trochanteric fracture (OR = 0.98, 95% CI = 0.30–3.20, 135 matched pairs), although the numbers of case–control pairs according to subtype were small, CIs overlapped, and the difference in ORs was not statistically significant.

ORs for $eGFR_{cys-c}$ categories were similar regardless of which variable was used to account for overall poor health status (physical functioning, frailty score, number of chronic conditions, hemoglobin) (Table 4). In addition, adjustment for bone biomarkers (CTX, PINP) did not affect ORs. The observed correlation between cystatin-C and plasma homocysteine was 0.45. Adjustment for plasma homocysteine reduced the OR for $eGFR_{cys-c}$ less than 60 mL/min per 1.73 m^2 to 1.83 (95% CI = 0.93–3.61). In contrast, adjustment for serum 25(OH)D levels ($r = -0.10$ with cystatin-C) somewhat strengthened the association between $eGFR_{cys-c}$ less than 60 mL/min per 1.73 m^2 and hip fracture (OR = 2.95, 95% CI = 1.55–5.62) (Table 4).

Because associations between cystatin-C and hip fracture risk became stronger after adjustment for BMI, additional analyses were conducted to elucidate whether associations were consistent across BMI stratum and to

Table 4. Risk of Hip Fracture According to Baseline Estimated Glomerular Filtration Rate (eGFR) Categories as Defined According to Serum Cystatin-C

Variables Included in the Multivariate Model	r^*	$eGFR_{cys-c}$ Category in mL/min/1.73 m^2			*P*-Trend
		>90	60–<90	<60	
		Odds Ratio (95 % Confidence Interval)			
Unadjusted		1.0	0.82 (0.56–1.20)	1.51 (0.92–2.47)	.10
Adjusted for BMI	0.19	1.0	0.99 (0.66–1.48)	2.27 (1.31–3.94)	.003
Base analysis†		1.0	1.10 (0.70–1.71)	2.64 (1.41–4.97)	.003
Base analysis† + RAND 36 physical functioning >90	−0.24	1.0	1.04 (0.66–1.64)	2.50 (1.32–4.72)	.005
Base analysis† + frailty score	0.19	1.0	1.05 (0.67–1.64)	2.52 (1.33–4.77)	.005
Base analysis† + number of chronic conditions	0.18	1.0	1.05 (0.67–1.65)	2.49 (1.32–4.70)	.005
Base analysis† + plasma homocysteine	0.45	1.0	0.96 (0.60–1.51)	1.83 (0.93–3.61)	.105
Base analysis† + 25hydroxyvitamin D	−0.10	1.0	1.20 (0.76–1.90)	2.95 (1.55–5.62)	<.001
Base analysis† + hemoglobin	−0.11	1.0	1.08 (0.69–1.70)	2.74 (1.44–5.19)	.002
Base analysis† + C-terminal telopeptide of Type I collagen	0.20	1.0	1.11 (0.71–1.73)	2.51 (1.33–4.74)	.005
Base analysis† + aminoterminal procollagen extension propeptide	0.14	1.0	1.10 (0.71–1.73)	2.68 (1.41–5.08)	.003

* Pearson correlation coefficient between cystatin-C and the additional risk factor.
† Matched on age, ethnicity, blood draw date, controlled for body mass index (BMI), parental history of hip fracture, smoking, and alcohol use. Number of missing case–control pairs ranges from 32 to 39 in these analyses out of 397 total case–control pairs.

test for interaction between the two variables. Using conditional logistic regression retaining the matched design, whether ORs differed for cystatin-C levels analyzed as a continuous variable and by $eGFR_{cys-c}$ categories in women with high and low BMI defined by the median cutpoint in controls (26.91 kg/m^2) was tested. ORs indicated greater risk of hip fracture in both BMI strata, with somewhat stronger associations in overweight women in both analyses. For cystatin-C as a continuous variable, the OR in overweight women was 1.64 per standard deviation increase (95% CI = 1.21–2.23), compared with an OR of 1.12 in thinner women (95% CI = 0.90–1.39; *P*-value for interaction = .03). ORs comparing low (< 60 mL/min per 1.73 m^2) and high (> 60 mL/min per 1.73 m^2) $eGFR_{cys-c}$ categories were 1.99 for thinner women (95% CI = 0.98–4.03) and 2.41 (95% CI = 2.03–2.85) for overweight women (*P* value for interaction = .68).

DISCUSSION

This prospective, nested, case-control investigation of cystatin-C levels shows a strong, independent association between $eGFR_{cys-c}$ levels less than 60 mL/min per 1.73 m^2 and greater risk of hip fracture in postmenopausal women. Women with impaired renal function had 2.5 times the risk of hip fracture independent of well-established fracture risk factors including age, BMI, and physical function.

Studies relating renal function to risk of hip and other osteoporotic fractures are few in number. In women requiring kidney dialysis, hip fracture rates were found to be 17 times as high as in the general U.S. population.[2] Cross-sectional studies have shown associations between CKD defined using serum creatinine and self-reported history of hip and other fractures in the United States and Germany, but these studies were unable to determine which condition occurred first.[17,18] Two recent epidemiological studies of older adults not selected on the basis of clinical kidney disease reported hazard ratios between serum creatinine eGFR levels less than 60 mL/min per 1.73 m^2 and hip fracture ranging from 1.4 to 1.9.[3,4] These associations were not statistically significant after full multivariate adjustment in either study, including calcaneal bone density in one study,[3] but nonetheless supported an association between impaired renal function and hip fracture. Both previous studies had fewer than half the number of hip fractures investigated in this report (< 200 vs 400). Cystatin-C levels were significantly associated with greater risk of hip fracture in women in one prior study (adjusted hazard ratio = 1.7 for the fourth vs first quartiles, 95% CI = 1.01–2.73).[4] Especially strong associations with trochanteric versus femoral neck fractures were also seen in one study,[3] whereas the opposite pattern was observed in the present study. Measures of health status and frailty did not explain the divergent patterns of association in either study.

A major question to resolve is whether the association between renal function and hip fracture reflects abnormalities in bone metabolism associated with renal osteodystrophy. CKD could lead to a greater risk of fractures in association with secondary hyperparathyroidism, osteomalacia, iron or aluminum bone disease, adynamic bone disease, or osteoporosis. Although clinical studies show that patients with severe kidney disease have lower bone density, especially at cortical sites, epidemiological studies of renal function and bone density are inconsistent. Although some cross-sectional studies have shown differences in bone density between people with CKD and comparison subjects,[19–21] another study did not find evidence of an independent association.[22] Recent prospective analyses have shown no association with bone loss in women[22] or a significant association with serum creatinine and bone loss only if analyzed using the Cockcroft-Gault equation.[20] Bone quality could be compromised with renal insufficiency even if density is not low. Adjustment for markers of bone resorption and formation did not alter the ORs for cystatin-C $eGFR_{cys-c}$ categories, suggesting mechanisms independent of bone turnover or more-complicated pathways than could be detected with the present methods.

In addition to abnormal physiology that could directly impair bone quality, CKD is often associated with poor health status, leading to frailty, falls, and ultimately fracture. However, ORs for CKD in this study were not lower after adjustment for physical function, frailty, number of chronic conditions, or anemia. Adjustment for serum 25(OH)D levels also did not reduce the OR for cystatin-C–determined CKD stage here or in a previous study.[3] Cystatin-C levels may predict hip fracture, because the rate of decline in renal function is a strong indicator of biological aging independent of chronological age and clinically manifest disease. Biomarkers are lacking for exploring this hypothesis.

Recent experimental studies show that poor renal function as measured according to cystatin-C is an important determinant of homocysteine levels in older adults regardless of vitamin B_{12} and folate status, which likely explains the correlation of 0.45 between the two biomarkers in this study.[12] Previous studies have shown associations between homocysteine and hip fracture risk, perhaps explained by greater bone resorption, and it has been postulated that nutritional intervention with folate or B vitamins has potential to reduce fracture risk.[13–15] The reduction in OR for hip fracture from 2.6 in the base model to 1.8 after adding homocysteine suggests that effects of renal function on homocysteine may mediate some portion of the association between cystatin-C and hip fracture. Alternatively, homocysteine levels could simply rise as renal function declines without direct involvement in the physiological pathway leading to hip fracture.

Cystatin-C has been shown to correlate highly with direct measures of GFR such as $^{[-125]}$iothalamate clearance, even more so than creatinine-based eGFR.[23] Touted advantages of this biomarker include its precision, low interindividual variability, and independence from muscle mass and body weight,[24] although some studies, including the current one, show correlations between cystatin-C and body mass.[25] Some evidence was found of an interaction between cystatin-C and BMI, indicating a stronger association between renal function and hip fracture risk in overweight and obese women. This may simply reflect the comparison with overweight women with normal renal function who have half the risk of hip fracture of thinner women with normal renal function. Cystatin-C levels may be superior in measuring mild renal insufficiency. Although the present findings did not refute a linear association between cystatin-C levels and hip fracture, the categorical

data show no association for $eGFR_{cys-c}$ levels greater than 60 mL/min per 1.73 m^2.

The WHI-OS is a large, diverse cohort of postmenopausal women, allowing the largest investigation to date on this topic to be conducted. Strengths of this study include adjustment for numerous potential confounders, elimination of confounding by current hormone use, evaluation of cystatin-C as a continuous biomarker and in $eGFR_{cys-c}$ categories, and exploration of numerous potential underlying mechanisms. The present study was limited by having a single measurement of cystatin-C and no measurements of bone density, serum calcium, parathyroid hormone, bone-specific alkaline phosphatase, serum creatinine, inflammatory biomarkers, or proteinuria. Too few women had Stage 4 to 5 CKD ($eGFR_{cys-c} < 30$ mL/min per 1.73 m^2) to estimate hip fracture risks associated with severe disease. Because there was no criterion standard measure of GFR, the possibility of a direct mechanism linking cystatin-C with hip fracture risk, unrelated to GFR, cannot be excluded. Only 20 hip fractures occurred in minority women, and therefore it was not possible to determine whether differences exist between racial and ethnic groups.

Cystatin-C eGFR levels less than 60 mL/min per 1.73 m^2 are a strong, independent risk factor for hip fracture in postmenopausal women. Women with low bone density, normal parathyroid hormone and alkaline phosphatase, and Stage 1 to 3 CKD can reduce their fracture risk with treatment.[26,27] Postmenopausal women with CKD Stage 3 or higher should be considered at high risk and evaluated for bone disease.

ACKNOWLEDGMENTS

Program Office: (National Heart, Lung, and Blood Institute, Bethesda, Maryland) Elizabeth Nabel, Jacques Rossouw, Shari Ludlam, Linda Pottern, Joan McGowan, Leslie Ford, and Nancy Geller.

Clinical Coordinating Center: (Fred Hutchinson Cancer Research Center, Seattle, WA) Ross Prentice, Garnet Anderson, Andrea LaCroix, Charles L. Kooperberg, Ruth E. Patterson, Anne McTiernan; (Wake Forest University School of Medicine, Winston-Salem, NC) Sally Shumaker; (Medical Research Labs, Highland Heights, KY) Evan Stein; (University of California at San Francisco, San Francisco, CA) Steven Cummings.

Clinical Centers: (Albert Einstein College of Medicine, Bronx, NY) Sylvia Wassertheil-Smoller; (Baylor College of Medicine, Houston, TX) Jennifer Hays; (Brigham and Women's Hospital, Harvard Medical School, Boston, MA) JoAnn Manson; (Brown University, Providence, RI) Annlouise R. Assaf; (Emory University, Atlanta, GA) Lawrence Phillips; (Fred Hutchinson Cancer Research Center, Seattle, WA) Shirley Beresford; (George Washington University Medical Center, Washington, DC) Judith Hsia; (Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, Torrance, CA) Rowan Chlebowski; (Kaiser Permanente Center for Health Research, Portland, OR) Evelyn Whitlock; (Kaiser Permanente Division of Research, Oakland, CA) Bette Caan; (Medical College of Wisconsin, Milwaukee, WI) Jane Morley Kotchen; (MedStar Research Institute/Howard University, Washington, DC) Barbara V. Howard; (Northwestern University, Chicago/Evanston, IL) Linda Van Horn; (Rush Medical Center, Chicago, IL) Henry Black; (Stanford Prevention Research Center, Stanford, CA) Marcia L. Stefanick; (State University of New York at Stony Brook, Stony Brook, NY) Dorothy Lane; (The Ohio State University, Columbus, OH) Rebecca Jackson; (University of Alabama at Birmingham, Birmingham, AL) Cora E. Lewis; (University of Arizona, Tucson/Phoenix, AZ) Tamsen Bassford; (University at Buffalo, Buffalo, NY) Jean Wactawski-Wende; (University of California at Davis, Sacramento, CA) John Robbins; (University of California at Irvine, CA) F. Allan Hubbell; (University of California at Los Angeles, Los Angeles, CA) Howard Judd; (University of California at San Diego, La Jolla/Chula Vista, CA) Robert D. Langer; (University of Cincinnati, Cincinnati, OH) Margery Gass; (University of Florida, Gainesville/Jacksonville, FL) Marian Limacher; (University of Hawaii, Honolulu, HI) David Curb; (University of Iowa, Iowa City/Davenport, IA) Robert Wallace; (University of Massachusetts/Fallon Clinic, Worcester, MA) Judith Ockene; (University of Medicine and Dentistry of New Jersey, Newark, NJ) Norman Lasser; (University of Miami, Miami, FL) Mary Jo O'Sullivan; (University of Minnesota, Minneapolis, MN) Karen Margolis; (University of Nevada, Reno, NV) Robert Brunner; (University of North Carolina, Chapel Hill, NC) Gerardo Heiss; (University of Pittsburgh, Pittsburgh, PA) Lewis Kuller; (University of Tennessee, Memphis, TN) Karen C. Johnson; (University of Texas Health Science Center, San Antonio, TX) Robert Brzyski; (University of Wisconsin, Madison, WI) Gloria E. Sarto; (Wake Forest University School of Medicine, Winston-Salem, NC) Denise Bonds; (Wayne State University School of Medicine/Hutzel Hospital, Detroit, MI) Susan Hendrix.

Work was done when Jennifer S. Lee was at California Pacific Medical Center Research Institute and University of California at San Francisco.

Conflict of Interest: The editor in chief has reviewed the conflict of interest checklist provided by the authors and has determined that the authors have no financial or any other kind of personal conflicts with this manuscript. Dr. Cauley has received research support from Merck & Company, Eli Lilly & Company, Pfizer Pharmaceuticals, and Novartis Pharmaceuticals. She has also received consulting fees from Eli Lilly & Company and Novartis Pharmaceuticals. She is on the speakers' bureau for Merck & Co., Inc. Dr. Leboff has an Unrestricted Center of Excellence Educational Grant funded through Abbott. She also owns stock in Amgen but has no research activities with Amgen. In the last 5 years, Dr. Bauer has received research support from Amgen, Novartis, NPS, and P&G; received speaker honoraria from Merck and Novartis; and served as a consultant for Merck and Amgen.

This study was supported by a contract from the National Heart, Lung, and Blood Institute, U.S. Department of Health and Human Services, and National Institutes of Health Grants R01-AR048919 and AR053105 from the National Institute of Arthritis and Musculoskeletal and Skin Diseases.

Author Contributions: Drs. LaCroix, Lee, Cauley, Cummings, Shlipak, Kooperberg, Leboff, Bauer, Jackson, and Kooperberg were involved in study concept and design and acquisition of data. All authors were involved in

analysis and interpretation of the data and preparation and critical revision of the manuscript.

Sponsor's Role: The Women's Health Initiative program was funded by the National Heart, Lung, and Blood Institute of the National Institutes of Health, Department of Health and Human Services. The funding organization has representation on the Extension Study Executive Committee, which governs the design and conduct of the study, the interpretation of the data, and the preparation and approval of manuscripts. The National Heart, Lung, and Blood Institute Program Office has reviewed this manuscript.

REFERENCES

1. Alem AM, Sherrard DJ, Gillen DL et al. Increased risk of hip fracture among patients with end-stage renal disease. Kidney Int 2000;58:396–399.
2. Coco M, Ruch H. Increased incidence of hip fractures in dialysis patients with low serum parathyroid hormone. Am J Kidney Dis 2000;36:1115–1121.
3. Ensrud KE, Yi-Ying L, Taylor BC et al. for the Osteoporotic Fractures Research Group. Renal function and risk of hip and vertebral fractures in older women. Arch Intern Med 2007;167:133–139.
4. Fried LF, Biggs ML, Shlipak MG et al. Association of kidney function with incident hip fracture in older adults. J Am Soc Nephrol 2007;18:282–286.
5. Hays J, Hunt JR, Hubbell FA et al. The Women's Health Initiative recruitment methods and results. Ann Epidemiol 2003;13(9 Suppl):S18–S77.
6. Curb JD, McTiernan A, Heckbert SR et al. for the WHI Morbidity and Mortality Committee. Outcomes ascertainment and adjudication methods in the Women's Health Initiative. Ann Epidemiol 2003;13(9 Suppl):S122–S128.
7. Patterson RE, Kristal AR, Tinker LF et al. Measurement characteristics of the Women's Health Initiative food frequency questionnaire. Ann Epidemiol 1999;9:178–187.
8. Manson JE, Greenland P, LaCroix AZ et al. Walking compared with vigorous exercise for the prevention of cardiovascular events in women. N Engl J Med 2002;347:716–725.
9. Hays RD, Sherbourne CD, Mazel RM. The RAND 36-item Health Survey 1.0. Health Econ 1993;2:217–227.
10. Woods NF, LaCroix AZ, Gray SL et al. Frailty: Emergence and consequences in women aged 65 and older in the Women's Health Initiative Observational Study. J Am Geriatr Soc 2005;53:1321–1330.
11. Stevens L, Coresh J, Schmid CH et al. Estimating GFR using serum cystatin C alone and in combination with serum creatinine: A pooled analysis of 3418 individuals with chronic kidney disease. Am J Kidney Dis 2008;51:395–406.
12. Lewerin C, Llungman S, Nilsson-Ehle H. Glomerular filtration rate as measured by serum cystatin C is an important determinant of plasma homocysteine and serum methylmalonic acid in the elderly. J Intern Med 2006;261:65–73.
13. van Meurs JB, Dhonukshe-Rutten RA, Pluijm SM et al. Homocysteine levels and the risk of osteoporotic fracture. N Engl J Med 2004;350:2033–2041.
14. McLean RR, Jacques PF, Selhub J et al. Homocysteine as a predictive factor for hip fracture in older persons. N Engl J Med 2004;350:2042–2049.
15. Gjesda CG, Vollset SE, Ueland PM et al. Plasma homocysteine, folate and vitamin B12 and the risk of hip fracture: The Hordaland Homocysteine Study. J Bone Miner Res 2007;22:747–756.
16. National Kidney Foundation. K/DOQI clinical practice guidelines for chronic kidney disease: Evaluation, classification, and stratification. Am J Kidney Dis 2002;39(2 Suppl 1):S1–S266.
17. Nickolas TL, McMahon DJ, Shane E. Relationship between moderate to severe kidney disease and hip fracture in the United States. J Am Soc Nephrol 2006;17:3223–3232.
18. Dukas L, Schacht E, Stahelin HB. In elderly men and women treated for osteoporosis a low creatinine clearance of <65 mL/min is a risk factor for falls and fractures. Osteoporosis Int 2005;16:1683–1690.
19. Rix M, Andreassen H, Eskildsen P et al. Bone mineral density and biochemical markers of bone turnover in patients with predialysis chronic renal failure. Kidney Int 1999;56:1084–1093.
20. Jassal SK, von Muhlen D, Barrett-Connor E. Measures of renal function, BMD, bone loss, and osteoporotic fracture in older adults: The Rancho Bernardo Study. J Bone Miner Res 2007;22:203–210.
21. Klawansky S, Komaroff E, Cavanaugh PF Jr et al. Relationship between age, renal function and bone mineral density in the US population. Osteoporosis Int 2003;14:570–576.
22. Fried LF, Shlipak MG, Stehman-Breen C et al. Kidney function predicts the rate of bone loss in older individuals: The Cardiovascular Health Study. J Gerontol A Biol Sci Med Sci 2006;61A:743–748.
23. Hoek FJ, Kemperman FAW, Krediet RT. A comparison of cystatin C, plasma creatinine and the Cockcroft and Gault formula for the estimation of glomerular filtration rate. Nephrol Dial Transplant 2003;18:2024–2031.
24. Stevens LA, Coresh J, Greene T et al. Assessing kidney function-measured and estimated glomerular filtration rate. N Engl J Med 2006;354:2473–2483.
25. Knight EL, Verhave JC, Spiegelman D et al. Factors influencing serum cystatin C levels other than renal function and the impact on renal function measurement. Kidney Int 2005;67:777–778.
26. Jamal SA, Bauer DC, Ensrud KE et al. Alendronate treatment in women with normal to severely impaired renal function: An analysis of the Fracture Intervention Trial. J Bone Miner Res 2007;22:503–508.
27. Miller PD, Roux C, Boonen S et al. Safety and efficacy of risedronate in patients with age-related reduced renal function as estimated by the Cockcroft and Gault method: A pooled analysis of nine clinical trials. J Bone Miner Res 2005;20:2105–2115.