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

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Abstract

OBJECTIVES—To evaluate the association of chronic kidney disease with 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 US clinical centers.

PARTICIPANTS—From 93,676 women ages 50–79 years 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 with a validated equation and categorized into three groups: 1) $eGFR_{cys\text{-}c}$ >90 mL/min/1.73 m^2; 2) $eGFR_{cys\text{-}c}$ 60–90 mL/min/1.73 m^2; or 3) $eGFR_{cys\text{-}c}$ <60 mL/min/1.73 m^2 indicating chronic kidney disease Stages 3–4.

RESULTS—The odds ratio (OR) for hip fracture was 2.50 (95% confidence interval (CI) 1.32–4.72) for $eGFR_{cys\text{-}c}$ <60 ml/min/1.73 m^2 compared to Stages 0–1 after adjustment for body mass, parental hip fracture, smoking, alcohol consumption and physical function. No association was observed for $eGFR_{cys\text{-}c}$ 60–90 mL/min/1.73 m^2 (OR=1.04; CI 0.66–1.64). These associations were

unaffected by additional adjustment for poor health status, hemoglobin, serum 25hydroxyvitamin D,

Elements of Financial/Personal Conflicts	Author 1 LaCroix, AZ		Author 2 Lee, JS		Author 3 Wu, L		Author 4 Cauley, JA	
	Yes	No	Yes	No	Yes	No	Yes	No
Employment or Affiliation		X		X		X		X
Grants/Funds		X		X		X	X	
Honoraria		X		X		X	X	
Speaker Forum		X		X		X	X	
Consultant		X		X		X	X	
Stocks		X		X		X		X
Royalties		X		X		X		X
Expert Testimony		X		X		X		X
Board Member		X		X		X		X
Patents		X		X		X		X
Personal Relationship		X		X		X		X

Elements of Financial/Personal Conflicts	Author 5 Shlipak, MG		Author 6 Ott, SM		Author 7 Robbins, J		Author 8 Curb, JD	
	Yes	No	Yes	No	Yes	No	Yes	No
Employment or Affiliation		X		X		X		X
Grants/Funds		X		X		X		X
Honoraria		X		X		X		X
Speaker Forum		X		X		X		X
Consultant		X		X		X		X
Stocks		X		X		X		X
Royalties		X		X		X		X
Expert Testimony		X		X		X		X
Board Member		X		X		X		X
Patents		X		X		X		X
Personal Relationship		X		X		X		X

Elements of Financial/Personal Conflicts	Author 9 Leboff, M		Author 10 Bauer, DC		Author 11 Jackson, R		Author 12 Kooperberg, C		Author 13 Cummings, SR	
	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
Employment or Affiliation		X		X		X		X		X
Grants/Funds	X		X			X		X		X
Honoraria		X	X			X		X		X
Speaker Forum		X		X		X		X		X
Consultant		X	X			X		X		X
Stocks	X			X		X		X		X
Royalties		X		X		X		X		X
Expert Testimony		X		X		X		X		X
Board Member		X		X		X		X		X
Patents		X		X		X		X		X
Personal Relationship		X		X		X		X		X

Explanations for “yes” answers in table here.

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or bone metabolism markers. Adjustment for plasma homocysteine reduced the OR for $eGFR_{cys-c}$ <60 mL/min/1.73 m^2 to 1.83 (CI 0.93–3.61).

CONCLUSION—Women with cystatin-C eGFR levels <60 have a substantially increased risk of hip fracture. This association may be partially mediated, or accompanied by, effects of renal function on homocysteine levels.

Keywords

chronic kidney disease; hip fracture; cystatin-C; renal function

INTRODUCTION

Severe kidney disease appears to increase the risk of hip fractures (1,2). Chronic kidney disease is common, but mild and moderate stages are frequently clinically unrecognized and therefore understudied. Two prior prospective studies support an association of moderate renal insufficiency as measured by serum creatinine estimated glomerular filtration rate (eGFR) or cystatin-C levels and increased hip fracture risk in older women. In both studies associations were diminished after full multivariate adjustment (3,4). However, 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.

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. We report here 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. Potential explanatory factors for an association of renal function with osteoporosis and/or hip fracture are explored.

METHODS

Study Group

The WHI-OS is a prospective cohort study that enrolled 93,676 women ages 50–79 years from 1994–1998 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 three 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. The study was reviewed and approved by Human Subjects Review Committees at each participating institution.

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 – 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. Hip fractures were verified by review of radiological, magnetic resonance imaging, or operative reports by trained physicians at each clinical center and then confirmed by blinded

central adjudicators (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 one year prior to enrollment, or currently taking androgens (anabolic steroids, DHEA, 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 by age at screening (+/− one year), race/ethnicity, and date of blood draw (+/− 120 days). Cystatin-C levels were obtained in 397 matched pairs.

Baseline Clinical Variables

All covariates were ascertained at baseline. Current use of prescription medications including thiazide diuretics and corticosteroids, was recorded by clinic interviewers at the first screening visit by direct inspection of 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 two 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/ethnicity, age at menopause, personal history of fracture after age 55, treated diabetes, 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 on the basis of 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 (MET score=kcal hours/week/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/vigorous activities, strength to lift, carry, stoop, or bend, stair climb, 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 centimeter using a wall-mounted stadiometer. Body mass index 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 using a particle-enhanced immunonepholometric assay at Medical Research Laboratories International in Highland Heights, Kentucky. The assay has a sensitivity of 0.02 mg/L and an inter-assay coefficient of variation of 5.7%. The measurement range is 0.25–7.9 mg/L with a reference range for ostensibly healthy people ages 1–78 of 0.53–0.93 mg/L.

Estimated glomerular filtration rate ($eGFR_{cys-c}$) was calculated using the formula 76.7 × cystatin-$C^{-1.18}$ which has been validated in large populations using urinary clearance of ^{125}I-iothalamate (51Cr-EDTA in Paris)(11).

Several laboratory biomarkers were investigated as potential mechanisms for an association of renal function with hip fracture. Serum total homocysteine is influenced by renal function and has been associated with increased risk of hip fracture (12–15). Levels were measured in fasting samples using a high-performance liquid chromatography assay (HPLC) at the same laboratory. The coefficient of variation was 7.3–7.6% with a range of 5–15 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 25hydroxyvitamin D was quantified by radioimmunoassay using reagents from Diasorin, (Stillwater, MN). C-terminal telopeptide of Type I collagen (CTX) and aminoterminal procollagen extension propeptide (PINP) were measured by immunoassay at Synarc (Lyon, France).

Statistical Methods

Baseline characteristics were compared between hip fracture cases and matched controls, 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/ethnicity, blood draw date). Associations were first examined without any additional adjustment and then with adjustment for body mass index (continuous), parental history of hip fracture, smoking, alcohol use, and Rand-36 physical function score (>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.

Cystatin-C levels were evaluated as a continuous variable and also across quartile categories defined based on the distribution in the control subjects. Using the $eGFR_{cys-c}$ formula, cystatin-C levels were categorized and analyzed per mL/min/1.73 m^2 in three groups: $eGFR_{cys-c}$ >90; $eGFR_{cys-c}$ 60–90; and $eGFR_{cys-c}$ <60 (16). Odds ratios and 95% confidence intervals were calculated from the conditional logistic regression models per standard deviation difference for continuous level of cystatin-C and in comparison to the best renal function group in the categorical models. To investigate mechanisms through which cystatin-C might be associated with hip fracture, we constructed a base model with adjustment for body mass index, parental history of hip fracture, smoking, and alcohol, and then added the following variables one at a time to determine their impact on the cystatin-C odds ratios: 1) markers for deteriorating health status (poor physical function, frailty score, number of chronic conditions, hemoglobin level); 2) homocysteine; 3) 25hydroxyvitamin D; and 4) bone turnover markers (CTX and PINP).

RESULTS

The mean age of cases and controls was 71 years and 95 percent were Caucasian (Table 1). Cases had lower body mass index 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 body mass index (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 function vs. only 16% of women in the highest quartile (r=−0.24). Frequent alcohol consumption was more common among women with lower cystatin-C levels. Levels of other fracture risk factors varied little across quartiles of cystatin-C.

The unadjusted odds ratio for incident hip fracture comparing highest vs. lowest quartiles of cystatin-C levels was 1.51 (95% CI 0.98–2.33). A stronger elevation in risk occurred after adjustment for body mass index (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, however 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 >90 mL/min/1.73 m^2, 517 with levels between 60–90 mL/min/1.73 m^2, and 144 women with levels below 60 mL/min/1.73 m^2. The latter group included 138 women with CKD Stage 3 ($eGFR_{cys\text{-}c}$ 30–59) and 6 with CKD Stage 4 ($eGFR_{cys\text{-}c}$ 15–29). No women had Stage 5 CKD ($eGFR_{cys\text{-}c}$ <15 ml/min/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 full multivariate models. The adjusted odds ratio relating $eGFR_{cys\text{-}c}$ <60 mL/min/1.73 m^2 to hip fracture was 2.50 (95% CI, 1.32–4.72), but no association was seen for $eGFR_{cys\text{-}c}$ 60–90 mL/min/1.73 m^2 (OR=1.04; 95% CI 0.66–1.64) compared to $eGFR_{cys\text{-}c}$ >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 odds ratio for $eGFR_{cys\text{-}c}$ < 60 mL/min/1.73 m^2 was elevated 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), however the numbers of case-control pairs by subtype were small, confidence intervals overlapped, and the difference in odds ratios was not statistically significant.

Odds ratios for $eGFR_{cys\text{-}c}$ 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). Odds ratios were also unaffected by adjustment for bone biomarkers (CTX, PINP). The observed correlation between cystatin-C and plasma homocysteine was 0.45. Adjustment for plasma homocysteine reduced the OR for $eGFR_{cys\text{-}c}$ < 60 mL/min/1.73 m^2 to 1.83 (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 $eGFR_{cys\text{-}c}$ < 60 mL/min/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 test for interaction between the two variables. Using conditional logistic regression retaining the matched design, we tested whether odds ratios differed for cystatin-C levels analyzed as a continuous variable and by $eGFR_{cys\text{-}c}$ categories in women with high and low BMI defined by the median cutpoint in controls (26.91 kg/m2). Odds ratios indicated increased risk of hip fracture in both BMI stratum with somewhat stronger associations in overweight women in both analyses. For cystatin-C as a continuous variable the odds ratio among overweight women was 1.64 per SD increase, 95% CI, 1.21–2.23) compared to an odds ratio of 1.12 in thinner women (95% CI, 0.90–1.39; p-value for interaction=0.03). Odds ratios comparing low (< 60 mL/min/1.73 m2) vs. high (> 60) $eGFR_{cys\text{-}c}$ 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=0.68).

DISCUSSION

This prospective, nested case-control investigation of cystatin-C levels shows a strong, independent association between $eGFR_{cys\text{-}c}$ levels<60 mL/min/1.73 m^2 and increased 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, body mass index, and physical function.

Studies relating renal function to risk of hip and other osteoporotic fractures are few in number. Among women requiring kidney dialysis, hip fracture rates were found to be 17 times higher than the general U.S. population (2). Cross-sectional studies have shown associations between chronic kidney disease defined using serum creatinine and self-reported history of hip and other fractures in the US and Germany, but these studies are unable to determine which condition occurred first (17–18). Two recent epidemiologic studies of older adults not selected on the basis of clinical kidney disease reported hazard ratios between serum creatinine eGFR levels <60 mL/min/1.73 m^2 and hip fracture ranging from 1.4–1.9 (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 support an association of impaired renal function with hip fracture. Both previous studies had less than half the number of hip fractures investigated in this report (<200 vs. 400). Cystatin-C levels were significantly associated with increased risk of hip fracture among women in one prior study (adjusted hazard ratio=1.7 for the 4^{th} vs. 1^{st} quartiles, 95% CI, 1.01–2.73) (4). Especially strong associations with trochanteric vs. 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. Chronic kidney disease could lead to an increased risk of fractures in association with secondary hyperparathyroidism, osteomalacia, iron or aluminum bone disease, adynamic bone disease, or osteoporosis. While clinical studies show that patients with severe kidney disease have decreased bone density especially at cortical sites, epidemiologic studies of renal function and bone density are inconsistent. While some cross-sectional studies have shown differences in bone density between people with chronic kidney disease and comparison subjects (19–21), another study did not find evidence of an independent association (22). Recent prospective analyses have either 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 decreased. Adjustment for markers of bone resorption and formation did not alter the odds ratios for cystatin-C $eGFR_{cys\text{-}c}$ categories, suggesting mechanisms independent of bone turnover, or more complicated pathways than could be detected with the present methods.

In addition to abnormal physiology which could directly impair bone quality, CKD is often associated with poor health status leading to frailty, falls, and ultimately fracture. However, odds ratios for CKD in this study did not diminish after adjustment for physical function, frailty, number of chronic conditions, or anemia. Adjustment for serum 25-hydroxyvitamin D levels also did not reduce the odds ratio for cystatin-C determined CKD stage here or in a previous study (3). Cystatin-C levels may predict hip fracture because the rate of renal function decline 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 by cystatin-C is an important determinant of homocysteine levels in older adults regardless of vitamin B12 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 increased 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 odds ratio for hip fracture from 2.6 in the base model to 1.8 after adding

homocysteine suggests that some portion of the association between cystatin-C and hip fracture may be mediated by effects of renal function on homocysteine. 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, decreased inter-individual variability, and independence from muscle mass and body weight (24), although some studies including ours show correlations between cystatin-C and body mass (25). We found some evidence of an interaction between cystatin-C and BMI, observing a stronger association between renal function and hip fracture risk among overweight and obese women. This may simply reflect the comparison to overweight women with normal renal function who have half the risk of hip fracture relative to thinner women with normal renal function. Cystatin-C levels maybe 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 $eGFR_{cys\text{-}c}$ levels over 60 mL/min/1.73 m^2.

The WHI-OS is a large, diverse cohort of postmenopausal women permitting us to conduct the largest investigation to date on this topic. 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 $eGFR_{cys\text{-}c}$ 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 Stages 4–5 CKD ($eGFR_{cys\text{-}c}$ < 30 mL/min/1.73 m^2) to estimate hip fracture risks associated with severe disease. As there was no gold standard measure of GFR, we cannot rule out the possibility of a direct mechanism linking cystatin-C with hip fracture risk, unrelated to GFR. Only 20 hip fractures occurred in minority women and, therefore, we are unable to determine whether differences exist between race/ethnicity groups.

We conclude that cystatin-C eGFR levels below 60 mL/min/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 Stages 1–3 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.

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Table 1

Baseline Characteristics Among Hip Fracture Cases and Controls

	Control		Case		
	N	**%**	**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/Pacific Islander	5	1.3	5	1.3	
Age group at screening, years					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 (BMI), kg/m2					0.001
<25	144	36.1	193	48.6	
25–30	150	37.6	127	32.0	
30+	105	26.3	77	19.4	
History of fracture on/after age 55	82	20.5	96	24.0	0.23
Parents broke hip	64	16.0	80	20.0	0.14
HRT usage status					0.80
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	0.007
RAND 36 – Physical Functioning > 90	117	30.1	84	21.8	0.009
General Health					0.06
Excellent/Very good	220	56.0	194	48.9	
Good	131	33.3	142	35.8	
Fair/Poor	42	10.7	61	15.4	
Treated diabetes (pills or shots)	19	4.8	24	6.0	0.43
Alcohol Use					0.61

	Control		Case		
	N	%	N	%	P-value
Non drinker	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					0.00
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 (MI)	14	3.5	22	5.5	0.17
History of stroke	8	2.0	18	4.5	0.05

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Table 2

Baseline Characteristics According to Quartiles of Cystatin-C in the Control Group*

	Quartile of serum cystatin-C								
	1		2		3		4		
	N	% Mean ± SD	N	% Mean ± SD	N	% Mean ± SD	N	% Mean ± SD	p-value
Ethnicity									
White	97	93.3	90	91.8	92	95.8	98	99.0	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/Pacific Islander	3	2.9	2	2.0	0	0.0	0	0.0	
Age group at screening, years									
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 (BMI), kg/m2									
18.5 – <25	53	51.5	43	43.9	29	30.2	19	19.2	<.001
25 – <30	33	32.0	38	38.8	36	37.5	42	42.4	
>=30	17	16.5	17	17.4	31	32.3	38	38.4	
Parents broke hip	16	15.4	18	18.4	14	14.6	15	15.2	0.89
HRT usage status									
Never used	81	77.9	71	72.5	72	75.0	76	76.8	0.82
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	0.28
General health (self report)									
Excellent/Very good	62	60.8	57	59.4	54	56.8	46	47.4	0.41
Good	28	27.5	32	33.3	30	31.6	40	41.2	
Fair/Poor	12	11.8	7	7.3	11	11.6	11	11.3	
Treated diabetes (pills or shots)	5	4.8	4	4.1	4	4.2	5	5.1	0.99
Alcohol use									
Non drinker	11	10.7	21	21.4	17	17.7	20	20.6	0.01

	Quartile of serum cystatin-C								
	1		2		3		4		
	N	% Mean ± SD	N	% Mean ± SD	N	% Mean ± SD	N	% Mean ± SD	p-value
Past drinker	19	18.5	13	13.3	26	27.1	20	20.6	
<7 drinks per week	53	51.5	52	53.1	48	50.0	52	53.6	
>=7 drinks per week	20	19.4	12	12.2	5	5.2	5	5.2	
RAND 36 physical functioning >90	48	47.1	33	34.4	21	22.8	15	15.6	<.001
Thiazides & Thiazide-like									
Diuretic Use	3	2.9	5	5.1	7	7.3	8	8.1	0.39
Smoking									
Never smoked	55	53.4	47	48.0	52	55.3	59	60.2	0.75
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	
Total Energy Expenditure from	10	17.5 ±		13.2 ±		12.5 ±		12.2 ±	
Physical Activity	1	18.7	97	13.1	94	12.5	97	15.7	0.05

*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 tests or Fisher exact test.

Table 3

Odds Ratio (95% Confidence Intervals) Relating Cystatin-C Levels to the Risk of Hip Fracture

Cystatin-C*	Unadjusted	Adjusted for body mass index	Multivariate- adjusted†
Per mg/L increase	2.08 (1.15, 3.77)	3.01 (1.54, 5.87)	2.92 (1.38, 6.21)
Per SD (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	p = 0.02	p = <0.001	p = 0.005
Number of missing pairs (total = 400)	6	10	32
Quartiles (mg/L cutpoints)			
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 fractures case and controls selection matched on age, ethnicity and blood draw date. Quartile cutponts defined based on the distribution in controls,

†Multivariate adjustment includes body mass index, parental history of hip fracture, smoking, alcohol use and RAND 36 physical functioning >90.

Table 4

Unadjusted and Adjusted Odds Ratios for Hip Fractures According to Baseline eGFR Categories as Defined by Serum Cystatin-C

		$eGFR_{cys-c}$ Category in mL/min/1.73 m^2			
	r[†]	**>90**	**60–<90**	**<60**	**P-trend**
		Odds Ratio (95 % confidence interval)			
Unadjusted		1.0	0.82 (0.56 – 1.20)	1.51 (0.92 – 2.47)	0.10
Adjusted for BMI	0.19	1.0	0.99 (0.66 – 1.48)	2.27 (1.31 – 3.94)	0.003
Base Analysis[*]		1.0	1.10 (0.70 – 1.71)	2.64 (1.41 – 4.97)	0.003
Base Analysis[*] + RAND 36 Physical Functioning >90	−0.24	1.0	1.04 (0.66 – 1.64)	2.50 (1.32 – 4.72)	0.005
Base Analysis [*]+ Frailty Score	0.19	1.0	1.05 (0.67 – 1.64)	2.52 (1.33 – 4.77)	0.005
Base Analysis[*] + number of chronic conditions	0.18	1.0	1.05 (0.67 – 1.65)	2.49 (1.32 – 4.70)	0.005
Base Analysis[*] + plasma homocysteine	0.45	1.0	0.96 (0.60 – 1.51)	1.83 (0.93 – 3.61)	0.105
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)	0.002
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)	0.005
Base Analysis[*] + aminoterminal procollagen extension propeptide	0.14	1.0	1.10 (0.71 – 1.73)	2.68 (1.41 – 5.08)	0.003

[*] Matched on age, ethnicity, blood draw date, controlled for BMI, parental history of hip fracture, smoking, alcohol use. Number of missing case-control pairs ranges from 32–39 in these analyses out of 397 total case-control pairs.

[†] Pearson Correlation Coefficient between Cystatin-C and the additional risk factor.