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Biochemical markers of bone turnover and risk of incident hip fracture in older women: the Cardiovascular Health Study

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Abstract

Summary The relationships of osteocalcin (OC) and C-telopeptide of type I collagen (CTX) with long-term incidence of hip fracture were examined in 1680 post-menopausal women from a population-based study. CTX, but not OC, levels were associated with incident hip fracture in these participants, a relationship characterized by an inverted U-shape.

Introduction We sought to investigate the relationships of OC, a marker of bone formation, and CTX, a marker of bone resorption, with long-term incidence of hip fracture in older women.

Methods We included 1680 women from the population-based Cardiovascular Health Study (mean [SD] age 74.5 [5.0] years). The longitudinal association of both markers with incidence of hip fracture was examined using multivariable Cox models.

Results During a median follow-up of 12.3 years, 288 incident hip fractures occurred. Linear spline analysis did not demonstrate an association between OC levels and incident hip fracture. By contrast, increasing levels of CTX up to the middle-upper range were associated with a significantly greater risk of hip fracture (HR = 1.52 per SD increment, 95% CI = 1.10–2.09), while further increases were associated with a marginally non-significant lower risk (HR = 0.80 per SD increment, 95% CI = 0.63–1.01), after full adjustment for potential confounders. In analyses of quartiles, CTX exhibited a similar inverted U-shaped relationship with incident fracture after adjustment, with a significant association observed only for the comparison of quartile 3 to quartile 1 (HR = 1.63, 95% CI = 1.10–2.43). In a subset with available measures, both OC and CTX were inversely associated with bone mineral density of the hip.

Conclusion CTX, but not OC, levels were associated with incident hip fracture in post-menopausal women, a relationship characterized by an inverted U-shape. These findings highlight the complex relationship of bone turnover markers with hip fracture risk.

Keywords Bone turnover markers · Hip fracture risk · Osteoporosis · Postmenopausal women

Introduction

Fragility fractures are a major health problem in older adults, especially women [1]. Among such osteoporotic fractures, those involving the hip are of greatest consequence owing to their associated disability, mortality, and costs [2]. Although dual-energy X-ray absorptiometry (DXA) determination of bone mineral density (BMD) is

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widely used in clinical practice to stratify fracture risk [3], this approach is insensitive [4]. Measurement of circulating bone turnover markers (BTMs) allows evaluation of the underlying processes driving bone loss, namely, low formation, increased resorption, or both [5]. However, available studies examining the relationship of BTMs with incident fracture have yielded inconsistent results [6]. Hence, BTMs are currently used clinically to monitor treatment effects and adherence to antiresorptive and anabolic drugs, but they are not recommended clinically for prediction of fracture risk [7].

BTMs are subject to considerable pre-analytical variability, such as fasting or non-fasting specimen collection and assay preparation, for which most existing studies have not been designed to control [6]. This has led to calls for standardization of patient preparation and specimen handling in studies evaluating BTMs for fracture risk assessment [8]. Among BTMs, osteocalcin is a highly osteoblast-specific marker of bone formation, while C-terminal cross-linking telopeptide of type I collagen (CTX), though less specific for bone, has been identified by expert panels as the most promising measure of bone resorption [9, 10].

The relationships of osteocalcin and CTX with fracture incidence among women have been evaluated in multiple longitudinal studies [11–22]. Earlier studies have reported both positive and null associations with fracture risk, but they have involved varying fracture types and locations and, importantly, most have not included morning fasting specimens or complete adjustment for confounding factors. A meta-analysis of such earlier studies reported a positive association for the bone formation marker procollagen type I N-terminal propeptide (PINP) and for CTX with all fractures in women, but not for hip fractures alone [23]. In a more recent study of post-menopausal women that overcame limitations of specimen preparation and insufficient adjustment for clinical covariates, neither PINP nor CTX was associated with incident hip fracture, but confidence bounds were broad [24].

We undertook measurement of OC and CTX among female participants of the population-based Cardiovascular Health Study (CHS) in a project designed to investigate the independent associations of these BTMs with geriatric outcomes—incident hip fracture, diabetes, and clinically significant aortic stenosis. Such evaluations called for careful adjustment for a range of factors apt to influence bone metabolism [25] and potentially confound the associations of interest. Here we focus on the relationships of OC and CTX with incident hip fracture, our primary outcome, and with hip BMD in the subset of participants who underwent DXA scanning as our secondary outcome. We tested the hypothesis that high levels of CTX, reflecting higher bone resorption, and, reactively, OC [25] would be associated with increased hip fracture risk.

Materials and methods

Study population

CHS is a population-based longitudinal study of community-dwelling, ambulatory adults 65 to 100 years old. Eligible participants were sampled from Medicare-eligibility lists at four US field centers, as previously described [26]. Briefly, participant eligibility required age ≥ 65 years, expectation to live in the area for 3 years after recruitment, absence of active cancer treatment, and the ability to provide consent. The rate of participation refusal was 38.6%. An original cohort of 5201 mostly white participants was recruited in 1989–1990, followed by a supplemental cohort of 687 predominantly African-American individuals in 1992–1993. In-person examinations were performed annually through 1998–1999 and, in a subset, again in 2005–2006. Telephone interviews were conducted semiannually from 1989 to 1999 and biannually thereafter. Participants underwent standardized health assessments for demographic data, medical history, lifestyle habits, medication inventory, and diagnostic and laboratory testing [27, 28]. All field centers and the CHS Coordinating Center received institutional review board approval for the study and participants gave informed consent.

Our ancillary study included 1760 women who completed the 1992–1993 exam and had never-thawed fasting serum available that was drawn during this exam. For the present analysis, we excluded 15 participants with prevalent hip fracture and 65 on oral glucocorticoids or vitamin K antagonists, leaving 1680 participants.

Bone turnover markers

Serum specimens were collected after an 8-h overnight fast during a morning visit and stored at − 80 °C until measurement in 2017 at the CHS Core Laboratory (Laboratory for Clinical Biochemistry Research, University of Vermont) [29]. Total OC was measured using electrochemiluminescent immunoanalysis with validated automated methods (Roche Diagnostics, Indianapolis, IN). The interassay coefficient of variation (CV) at the CHS Core Laboratory ranged between 5.0 and 7.6%. CTX was measured using a Roche beta-CrossLaps assay (Roche Diagnostics, Indianapolis, IN). The interassay (CV) ranged between 4.2 and 6.75%.

Hip fractures

The primary outcome of this study was time to first hospitalization for hip fracture, identified using International Classification of Diseases, Ninth Revision (ICD-9) codes from hospitalization records that were prospectively gathered from all CHS participants every 6 months. Hip fracture was defined as inpatient ICD-9 code 820.xx at any position.

Follow-up for incident hip fracture was complete and extended from the 1992–1993 visit until incident hip fracture, death, or June 30, 2013, whichever was earliest. By the end of follow-up, 1374 participants died and 306 were alive.

Bone mineral density

During the 1994–1995 examination, participants at two of the four field centers underwent DXA using array beam mode QDR 2000 or 2000+ bone densitometers (Hologic, Inc., Bedford, MA) according to a standardized protocol [30]. All scans were interpreted blindly and monitored for quality control by a core laboratory, as previously described [30]. The secondary outcome measures of interest were BMD of the total hip and femoral neck, each with a CV < 0.75%, which were available in 425 participants (mean age 73.8 [4.3] years) included in the current study sample.

Covariates

All covariates were collected at the 1992–1993 exam, unless otherwise noted. Estimated glomerular filtration rate (eGFR) was calculated based on serum cystatin C using a validated formula [31]. Physical activity was measured in kcal/week using published methods [27]. Prevalent diabetes mellitus was defined by fasting glucose ≥ 126 mg/dL, non-fasting glucose ≥ 200 mg/dL, or treatment with hypoglycemic medication. Excessive alcohol use was defined as > 7 drinks/week. Prevalent cardiovascular disease (CVD) included coronary heart disease (CHD), congestive heart failure (CHF), claudication, atrial fibrillation, and stroke or transient ischemic attack (TIA), ascertained at enrollment for the original (1989–1990) and supplemental (1992–1993) cohorts, and the intervening period for the original cohort, as previously described [27, 32, 33]. Activities of daily living (ADLs), history of falls, and mobility impairment were ascertained by questionnaire [34]. Limitation in ADLs was defined as difficulty in ≥ 1 of the following ADLs: bathing, dressing, eating, getting in and out of bed or chair, walking around home, and using the toilet [35]. Mobility impairment was defined as difficulty in walking 1/2 mile or walking up 10 steps. Cognitive impairment was defined as a Modified Mini-Mental State Examination (3MSE) score < 80 [36]. Frailty was defined as a clinical syndrome in which three or more of the following were present: unintentional weight loss (≥ 10 lb in past year), self-reported exhaustion, weakness (grip strength), slow walking speed, and low physical activity [34].

Statistical analysis

Distributions of OC and CTX levels were assessed by visual inspection and were deemed to be approximately normal. The shape of the relationship between each biomarker and the primary outcome was evaluated in Cox models with penalized cubic splines. To eliminate the influence of extreme values in these spline analyses, such values were winsorized at the 99th percentile of the distribution of both biomarkers. Owing to departure from linearity in the relationship of CTX, and to a lesser extent, OC, with incident hip fracture, we modeled the association for each marker using two linear splines with a knot chosen at the point of change in the hazard ratio. These associations are presented for continuous levels (per SD increment) of the BTMs for values below and above the inflection point identified by penalized cubic splines after full adjustment. In addition, we evaluated the associations of OC and CTX with the primary outcome by quartiles of each BTM. Confounders were a priori selected for adjustment in sequential models based on prior associations or known biological mechanisms. Model 1 included demographic variables along with location and timing of examination, namely, age, race, field center, and season. In model 2, we additionally included social and lifestyle factors, specifically education, body mass index (BMI), physical activity, smoking status, alcohol consumption, and estrogen replacement therapy. In model 3, we added cardiovascular and metabolic risk factors, and prevalent CVD: systolic blood pressure, treatment with anti-hypertensive medications, diabetes, calcium supplementation, low-density lipoprotein cholesterol (LDLc), high-density lipoprotein cholesterol (HDLc), triglycerides, eGFR, C-reactive protein (CRP), and prevalent coronary CHD, CHF, claudication, atrial fibrillation, stroke, and TIA. In the fully adjusted model (model 4), we added measures of functional and cognitive decline, including limitation in ADLs, fall history, and cognitive impairment. Owing to substantial missing data, we examined the impact of further adjusting for 25-hydroxyvitamin D, calcium, phosphate, and parathyroid hormone (PTH) levels in a separate exploratory model. Adjustment for BMD was not performed because this measure was only available in a small subset of the study sample. In separate sensitivity analyses, we excluded participants taking vitamin D, calcitriol, and bisphosphonates; African-American participants [37]; and those taking thiazide diuretics. In our Cox models, the proportionality assumption was tested using Schoenfeld residuals, which revealed no violations. In a secondary analysis, we assessed the quasi-cross-sectional associations of BTMs with BMD measured 2 years later. These relationships were modeled using multiple linear regression in the subgroup with available BMD measurements and adjusted for the same potential confounders as above. All analyses were performed using R Statistical Software, version 3.5.1. (Vienna, Austria). A two-tailed $p < 0.05$ was used to define statistical significance.

Table 1 Main demographic, behavioral, and clinical characteristics at baseline by quartiles of bone turnover markers

Quartiles	OC					CTX				
	Quartile 1 (n = 421)	Quartile 2 (n = 420)	Quartile 3 (n = 419)	Quartile 4 (n = 420)	p	Quartile 1 (n = 422)	Quartile 2 (n = 420)	Quartile 3 (n = 416)	Quartile 4 (n = 422)	p
BTM range (ng/mL)	≤17.0	17.1–23.0	23.1–30.0	>30.0		≤0.23	0.24–0.37	0.38–0.52	>0.52	
Demographic/timing										
Age (years)	73.4±4.4	74.4±4.9	74.3±5.0	76.0±5.5	<0.001	73.2±4.4	74.7±4.9	74.5±4.9	75.7±5.5	<0.001
Black, n (%)	79 (18.8)	68 (16.2)	69 (16.5)	41 (9.8)	0.002	70 (16.6)	63 (15.0)	62 (14.9)	62 (14.7)	0.864
Season, n (%)					0.061					0.305
Summer	79 (18.8)	88 (21.0)	77 (18.4)	83 (19.8)		70 (16.6)	77 (18.3)	81 (19.5)	99 (23.5)	
Fall	100 (23.8)	116 (27.6)	102 (24.3)	81 (19.3)		95 (23.5)	104 (24.8)	101 (24.3)	99 (23.5)	
Winter	112 (22.6)	103 (24.5)	106 (25.3)	97 (23.1)		121 (28.7)	98 (23.3)	99 (23.8)	100 (23.7)	
Spring	130 (30.9)	113 (26.9)	134 (32.0)	159 (37.9)		136 (32.2)	141 (33.6)	135 (32.5)	124 (29.4)	
Social/lifestyle										
Education ≤ grade 11, n (%)	121 (28.7)	119 (28.5)	121 (29.0)	121 (28.9)	0.356	118 (28.0)	120 (28.8)	101 (24.3)	143 (34.0)	0.104
BMI (kg/m²)	27.8±5.1	27.3±5.0	26.7±5.0	25.5±4.8	<0.001	27.6±4.9	27.2±5.0	26.7±5.2	25.7±4.9	<0.001
Physical activity (kcal/week)	810 (293–1888)	803 (270–1755)	767 (240–1658)	697 (223–1474)	0.264	808 (270–1890)	769 (270–1739)	783 (266–1665)	718 (158–1470)	0.221
Smoking, n (%)					0.023					0.090
Never	214 (51.9)	240 (57.8)	242 (59.5)	258 (63.1)		220 (52.8)	246 (60.3)	236 (58.1)	252 (61.2)	
Former	148 (35.9)	144 (34.7)	128 (31.4)	113 (27.6)		149 (35.7)	134 (32.8)	131 (32.3)	119 (48.3)	
Current	50 (12.1)	31 (7.5)	37 (9.1)	38 (9.3)		48 (11.5)	28 (6.9)	39 (9.6)	41 (10.0)	
Alcohol > 7 drinks/week	34 (8.1)	24 (5.7)	25 (6.0)	22 (5.2)	0.330	27 (6.4)	29 (6.9)	23 (5.5)	26 (6.2)	0.874
ERT, n (%)	134 (31.8)	45 (10.7)	35 (8.4)	13 (3.1)	<0.001	148 (35.1)	47 (11.2)	23 (5.5)	9 (2.1)	<0.001
Cardiovascular/metabolic										
Systolic BP (mmHg)	136±21	137±22	137±23	139±22	0.142	136±20	137±21	137±23	139±23	0.038
Anti-hypertensive Rx, n (%)	224 (53.2)	233 (55.5)	191 (45.6)	206 (49.0)	0.020	230 (54.5)	221 (52.6)	185 (44.5)	218 (51.7)	0.021
Thiazides, n (%)	124 (29.5)	92 (21.9)	58 (13.8)	65 (15.5)	<0.001	128 (30.3)	90 (21.4)	68 (16.3)	53 (12.6)	<0.001
Calcium Rx, n (%)	130 (30.9)	131 (31.2)	101 (24.1)	75 (17.9)	<0.001	139 (32.9)	120 (28.6)	106 (25.5)	72 (17.1)	<0.001
Diabetes, n (%)	98 (23.3)	63 (15.0)	43 (10.3)	35 (8.3)	<0.001	90 (21.3)	65 (15.5)	36 (8.7)	48 (11.4)	<0.001
LDLc (mg/dL)	125±35	134±32	134±32	135±35	<0.001	126±34	134±33	135±32	132±35	0.041
HDLc (mg/dL)	60±17	56±14	57±14	56±14	0.003	60±16	57±15	57±14	55±13	<0.001
Triglycerides (mg/dL)	134 (102–198)	128 (87–179)	120 (92–164)	126 (93–166)	<0.001	139 (102–198)	134 (93–190)	118 (89–165)	123 (85–161)	<0.001
eGFR (mL/min/1.73m²)	80.4±18.1	77.0±18.1	75.3±17.6	69.2±19.6	<0.001	80.1±18.7	76.1±17.7	75.3±17.9	70.4±19.5	<0.001
CRP (mg/L)	4.3 (1.8–8.8)	3.2 (1.4–6.0)	2.2 (1.1–5.2)	1.7 (0.8–4.0)	0.018	4.3 (1.8–8.6)	2.7 (1.2–5.8)	2.5 (1.1–5.5)	1.8 (1.0–4.5)	<0.001
Prevalent CHD, n (%)	59 (14.0)	81 (19.3)	81 (19.3)	94 (22.4)	0.002	65 (15.4)	73 (17.4)	68 (16.3)	109 (25.9)	<0.001
Prevalent CHF, n (%)	11 (2.6)	12 (2.9)	20 (4.8)	31 (7.4)	0.002	8 (1.9)	20 (4.8)	16 (3.8)	30 (7.1)	0.003
Prevalent stroke/TIA, n (%)	18 (4.3)	20 (4.8)	23 (5.5)	24 (5.7)	0.765	13 (3.1)	21 (5.0)	23 (5.5)	28 (6.6)	0.121

Prevalent claudication, 26 (1.5%); prevalent AF, 25 (1.5%)

AF atrial fibrillation, *BMI* body mass index, *BP* blood pressure, *BTM* bone turnover marker, *CRP* C-reactive protein, *eGFR* estimated glomerular filtration rate based on cystatin C (CKD-EPI), *ERT* estrogen replacement therapy, *HDLc* high-density lipoprotein cholesterol, *LDLc* low-density lipoprotein cholesterol, *TIA* transient ischemic attack, *Rx* treatment

Results

Characteristics of the study cohort and relations to BTM levels

The mean (SD) age of the study sample was 74.5 (5.0) years. Fifteen percent were African-American. There was a strong correlation between serum OC and CTX (Pearson's $r = 0.80$, $p < 0.001$). The distributions of the main sociodemographic, clinical, and laboratory characteristics at baseline are shown in Table 1 according to quartiles of circulating BTM levels. Participants with higher concentrations of serum OC and CTX were older, had higher systolic blood pressure and LDLc levels, and were more likely to have a history of CHD, CHF, and stroke/TIA. Those with higher concentrations of the BTMs were also less likely to be African-American or to have ever smoked; had lower BMI, less glucose dysregulation, lower HDLc, triglycerides, CRP, and eGFR; reported less physical activity; and were

less likely to report estrogen replacement therapy, calcium supplementation, and thiazide diuretic use. Blood samples with higher concentrations of OC were more likely to have been collected in the spring, while those with higher levels of CTX were more likely to have been collected in the summer.

Table 2 gives the distributions by BTM quartiles of mineral metabolism, physical function, and cognitive measures at baseline, and of DXA-determined BMD. Frequency of ADL impairment and proportion of participants with history of fall increased, while 3MSE score declined with increasing quartiles of serum OC and CTX. Furthermore, 25-hydroxyvitamin D levels and BMD at the total hip and femoral neck declined, while PTH increased, with rising quartiles of both BTMs.

BTMs and incident fracture risk

During median follow-up of 12.3 (IQR 7.3–18.5, maximum 22.1) years, a total of 288 participants suffered a hip fracture

Table 2 Measures of physical function, cognition, mineral metabolism, and bone mineral density by BTM quartiles

Quartiles	OC					CTX				
	Quartile 1 (n = 421)	Quartile 2 (n = 420)	Quartile 3 (n = 419)	Quartile 4 (n = 420)	p	Quartile 1 (n = 422)	Quartile 2 (n = 420)	Quartile 3 (n = 416)	Quartile 4 (n = 422)	p
BTM range (ng/mL)	≤ 17.0	17.1–23.0	23.1–30.0	> 30.0		≤ 0.23	0.24–0.37	0.38–0.52	> 0.52	
Functional/cognitive										
ADL impairment, n (%)	39 (9.3)	33 (7.9)	47 (11.2)	52 (12.4)	0.131	37 (8.8)	26 (6.2)	45 (10.8)	63 (14.9)	< 0.001
Mobility impairment, n (%)	136 (32.9)	124 (30.0)	115 (28.3)	119 (29.4)	0.523	129 (30.9)	115 (28.0)	114 (28.2)	136 (33.3)	0.312
Fall in previous year, n (%)	64 (15.2)	78 (18.6)	71 (16.9)	95 (22.6)	0.035	53 (12.6)	75 (17.9)	73 (17.5)	107 (25.4)	< 0.001
Frailty score	1 (0–2)	1 (0–2)	1 (0–2)	1 (0–2)	0.052	1 (0–2)	1 (0–2)	1 (0–2)	1 (0–2)	0.002
3MSE score < 80, n (%)	25 (5.9)	38 (9.0)	37 (8.8)	45 (10.7)	0.096	24 (5.7)	31 (7.4)	37 (8.9)	53 (12.6)	0.003
Mineral metabolism										
Calcium (mg/dL)*	9.5 ± 0.4	9.5 ± 0.3	9.5 ± 0.4	9.6 ± 0.4	0.007	9.5 ± 0.4	9.5 ± 0.4	9.5 ± 0.3	9.5 ± 0.4	0.349
Phosphate (mg/dL)*	3.6 ± 0.5	3.7 ± 0.4	3.7 ± 0.4	3.8 ± 0.5	< 0.001	3.6 ± 0.5	3.7 ± 0.4	3.7 ± 0.4	3.9 ± 0.5	< 0.001
PTH (mg/dL)*	45 (34–59)	52 (40–65)	54 (39–70)	58 (45–81)	< 0.001	47 (35–62)	52 (40–65)	53 (43–70)	57 (41–78)	< 0.001
Vitamin D (mg/dL)*	26.4 ± 16.3	24.4 ± 10.4	23.0 ± 8.7	22.9 ± 9.6	< 0.001	27.0 ± 16.6	23.7 ± 9.9	22.8 ± 9.5	23.0 ± 8.8	< 0.001
DXA outcome measures										
BMD total hip (g/cm²)†	0.81 ± 0.16	0.76 ± 0.13	0.73 ± 0.13	0.70 ± 0.13	< 0.001	0.80 ± 0.16	0.76 ± 0.13	0.72 ± 0.13	0.71 ± 0.13	< 0.001
BMD femoral neck (g/cm²)†	0.69 ± 0.15	0.65 ± 0.11	0.63 ± 0.11	0.60 ± 0.12	< 0.001	0.68 ± 0.14	0.66 ± 0.13	0.62 ± 0.11	0.61 ± 0.11	< 0.001

BMD bone mineral density, *BTM* bone turnover marker, *PTH* parathyroid hormone

*Missing in at least $n = 467$ ($n = 474$ for vitamin D)

† Available in $n = 425$ participants

Table 3 Incidence and adjusted hazard ratios for hip fracture by levels of serum OC and CTX

	Incident fracture (n)	Unadjusted incidence per 1000 PY (95% CI)	Model 1 HR (95% CI)	Model 2 HR (95% CI)	Model 3 HR (95% CI)	Model 4 HR (95% CI)
Osteocalcin						
Quartile 1 (≤ 17.0 ng/mL)	62	10.99 (8.57–14.09)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
Quartile 2 (> 17.0–23.0 ng/mL)	78	14.94 (11.97–18.65)	1.27 (0.91–1.77)	1.32 (0.91–1.90)	1.34 (0.91–1.98)	1.36 (0.92–2.02)
Quartile 3 (> 23.0–30.0 ng/mL)	72	13.50 (10.72–17.01)	1.12 (0.80–1.58)	1.16 (0.80–1.68)	1.23 (0.82–1.85)	1.23 (0.82–1.84)
Quartile 4 (> 30.0 ng/mL)	76	16.02 (12.79–20.05)	1.20 (0.85–1.69)	1.16 (0.79–1.70)	1.30 (0.86–1.97)	1.30 (0.86–1.98)
Per SD* increment (< 19.3 ng/mL)			1.54 (0.89–2.66)	1.54 (0.84–2.85)	1.70 (0.90–3.21)	1.72 (0.91–3.25)
Per SD* increment (≥ 19.3 ng/mL)			0.96 (0.82–1.12)	0.94 (0.80–1.11)	0.98 (0.82–1.16)	0.97 (0.82–1.15)
CTX (ng/mL)						
Quartile 1 (≤ 0.23 ng/mL)	60	10.61 (8.24–13.67)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
Quartile 2 (> 0.23–0.37 ng/mL)	75	13.97 (11.14–17.52)	1.16 (0.83–1.64)	1.22 (0.84–1.76)	1.33 (0.90–1.97)	1.39 (0.93–2.06)
Quartile 3 (> 0.37–0.52 ng/mL)	82	16.12 (12.99–20.02)	1.42 (1.01–1.98)	1.43 (0.99–2.07)	1.64 (1.11–2.43)	1.63 (1.10–2.43)
Quartile 4 (> 0.52 ng/mL)	71	14.69 (11.64–18.54)	1.18 (0.83–1.68)	1.11 (0.75–1.64)	1.33 (0.87–2.02)	1.33 (0.87–2.04)
Per SD† increment (< 0.43 ng/mL)			1.34 (1.03–1.76)	1.36 (1.01–1.83)	1.53 (1.11–2.10)	1.52 (1.10–2.09)
Per SD† increment (≥ 0.43 ng/mL)			0.86 (0.69–1.06)	0.79 (0.63–0.99)	0.81 (0.64–1.03)	0.80 (0.63–1.01)

Model 1: adjusted for age, race, field center, and season. Model 2 additionally adjusted for BMI, education, smoking status, alcohol consumption, physical activity, and estrogen replacement therapy. Model 3 additionally adjusted for systolic blood pressure, anti-hypertensive therapy, diabetes, calcium supplementation, LDLc, HDLc, triglycerides, prevalent CHD, prevalent CHF, prevalent claudication, prevalent AF, prevalent stroke/TIA, eGFR, and C-reactive protein. Model 4 additionally adjusted for ADL impairment, cognitive impairment, and fall history

CI confidence interval, *PY* person-years

*SD = 10.75 ng/mL

† SD = 0.23 ng/mL

(13.75 cases per 1000 person-years). Table 3 shows the unadjusted incidence of hip fracture by quartiles of OC and CTX. The lowest fracture rates were observed for the lowest quartiles of serum OC and CTX compared with their upper three quartiles.

For both biomarkers, the penalized cubic spline plots revealed departures from a monotonic linear relationship in their adjusted relationships with hip fracture (Fig. 1a, b). The relationship with incident hip fracture for OC appeared to show an increasing slope at the lower end of its concentration before leveling off early within the second quartile and seeming to show a decrease at the upper end of its concentration. In turn, CTX displayed an inverted U-shape relationship with hip fracture, with risk increasing from its lowest concentration and peaking within the third quartile, before decreasing with rising concentrations to the upper end of its distribution. Accordingly, the associations were modeled using linear splines below and above the inflection points on the corresponding cubic spline curves for each BTM (Fig. 1c, d), as well as by comparison of increasing quartiles to the lowest quartile (Table 3).

For OC, there were no significant associations with incident hip fracture at any level of adjustment in analyses of linear splines below or above the inflection point of 19.3 ng/mL (corresponding to the 35th percentile of OC's distribution) or when modeled by quartiles, as shown in Table 3. For CTX, there was a significant positive association with incident hip fracture up to the

inflection point of 0.43 ng/mL (corresponding to the 62nd percentile of CTX distribution) that became slightly stronger at with greater adjustment (Table 3). In the fully adjusted model (model 4), every SD increment in CTX concentration up to the inflection point (among n = 1035 participants) was associated with a 52% (10 to 109%) greater hazard of hip fracture (p = 0.011). At CTX concentrations beyond this inflection point (involving n = 645 participants), there was a significant inverse association with hip fracture after adjustment for demographic and behavioral risk factors (model 2). Additional adjustment for clinical variables rendered the association marginally non-significant, such that every SD increment in CTX was associated with a 20% (− 1 to 37%) lower hazard of hip fracture (p = 0.060). As also detailed in Table 3, comparison of quartiles showed a significantly increased risk of hip fracture for the third, but not the second or fourth, quartile as compared to the first quartile. Specifically, comparison of quartile 3 versus quartile 1 showed a 63% (10 to 143%) greater hazard for the primary outcome in the fully adjusted model (p = 0.016), a risk estimate that was modestly stronger than in the minimally adjusted model.

In sensitivity analyses that separately excluded women taking vitamin D, calcitriol, and bisphosphonates; thiazide diuretics; and African-Americans, the relationships between OC and CTX and incident hip fracture were not materially different (data not shown).
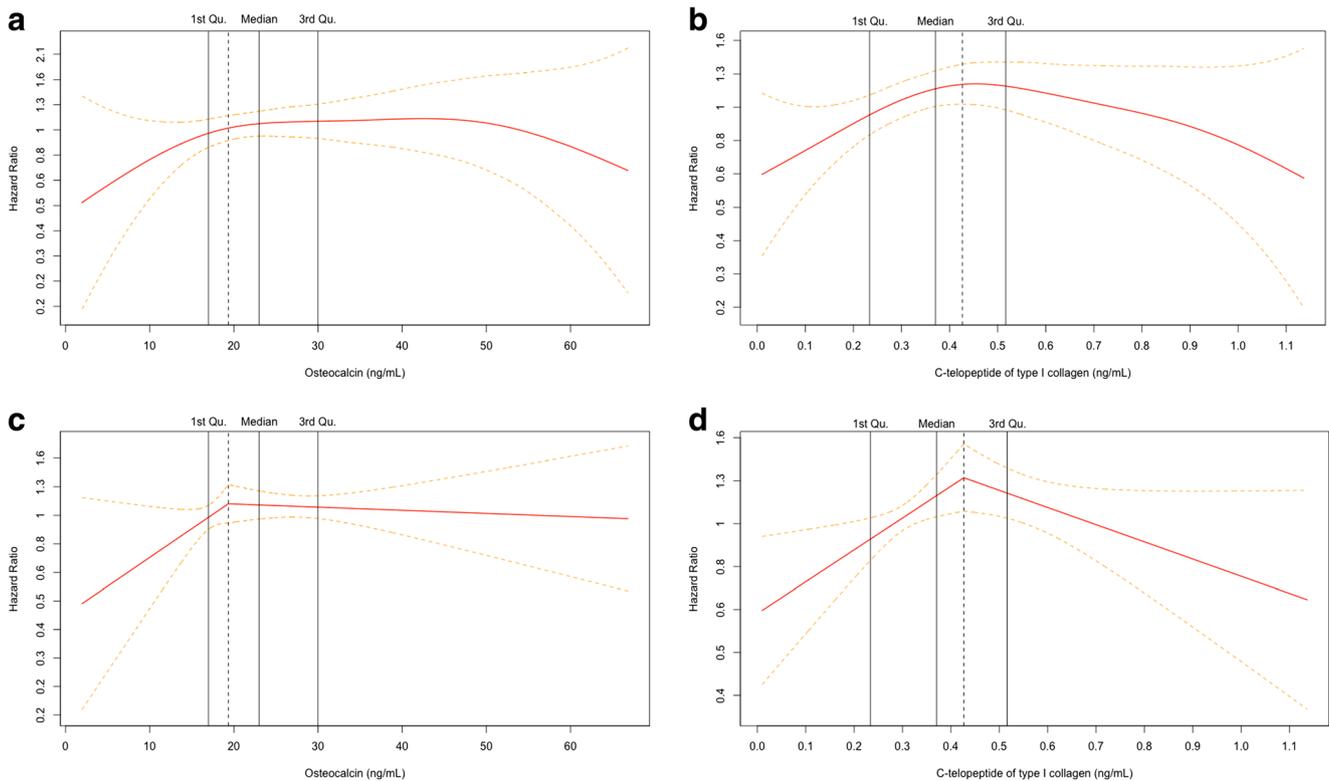
Fig. 1 Penalized cubic spline plot for OC (**a**) and CTX (**b**) winsorized at the 99% percentile. Linear spline plot for OC (**c**) and CTX (**d**) winsorized at the 99% percentile. Models are adjusted for covariates in model 4

BTM and BMD

Assessment of the relationships of serum OC and CTX with BMD showed no apparent departures from linearity (plots not shown). As presented in Table 4, there were significant inverse associations for both OC and CTX with BMD at the total hip and femoral neck after minimal adjustment. These persisted after full adjustment for behavioral and clinical covariates, such that each SD increment in OC was associated with 0.021 g/m² lower total hip BMD and 0.017 g/m² lower femoral neck BMD, and each SD increment in CTX was associated with 0.19 g/m² lower total hip BMD and 0.015 g/m² lower femoral neck BMD.

Discussion

Main results

The present study examined the relationship of two important serum biomarkers of bone turnover with incident hip fracture in a large sample of older postmenopausal women. We found no association between OC and incident hip fracture but did observe an inverted U-shaped association for CTX. Rising concentrations of CTX to the high-middle range of its distribution were associated with a significant and moderately increased risk of hip fracture, while further increments in concentration were associated with a more modest lower risk of this outcome that was marginally non-significant. Sensitivity analyses restricting the sample to white women and excluding participants on bone-influencing medications did not meaningfully alter these associations. By contrast, both OC and CTX showed significant but modest inverse associations with hip BMD after similar adjustment in a subset of participants who underwent DXA.

Previous studies

A host of longitudinal studies has examined BTMs in relation to future fracture risk in men and women [11–22, 24, 38–41]. While several have reported significant associations of BTMs with incident fracture, even independent of BMD [12–15, 19, 38, 40], many have failed to detect such associations [16, 20, 21]. These inconsistent results are difficult to interpret on account of differences in age and sex of study participants, the diverse fracture types considered, the varying and frequently incomplete levels of adjustment for potential confounders, and for a large majority, lack of appropriate sample collection procedures. Among available BTMs, international expert panels

Table 4 Relationship of serum OC and CTX with BMD at the total hip and femoral neck

	Total hip			Femoral neck		
	Beta per SD increment	95% CI	p	Beta per SD increment	95% CI	p
OC						
Model 1	− 0.025	− 0.038, − 0.012	< 0.001	− 0.022	− 0.034, − 0.011	< 0.001
Model 2	− 0.018	− 0.030, − 0.006	0.003	− 0.016	− 0.026, − 0.005	0.004
Model 3	− 0.020	− 0.033, − 0.008	0.002	− 0.016	− 0.027, − 0.005	0.006
Model 4	− 0.021	− 0.033, − 0.008	0.002	− 0.017	− 0.028, − 0.005	0.004
CTX						
Model 1	− 0.025	− 0.037, − 0.014	< 0.001	− 0.021	− 0.032, − 0.011	< 0.001
Model 2	− 0.018	− 0.029, − 0.007	0.001	− 0.015	− 0.025, − 0.005	0.003
Model 3	− 0.019	− 0.031, − 0.007	0.001	− 0.014	− 0.024, − 0.004	0.009
Model 4	− 0.019	− 0.031, − 0.007	0.002	− 0.015	− 0.025, − 0.004	0.006

Model 1: adjusted for age, race, field center, and season. Model 2: additionally adjusted for BMI, education, smoking status, alcohol consumption, physical activity, and estrogen replacement therapy. Model 3: additionally adjusted for systolic blood pressure, anti-hypertensive therapy, diabetes, calcium supplementation, LDLc, HDLc, triglycerides, prevalent CHD, prevalent CHF, prevalent claudication, prevalent AF, prevalent stroke/TIA, eGFR, and C-reactive protein. Model 4: additionally adjusted for ADL impairment, cognitive impairment, and fall history

CI confidence interval

have identified PINP and CTX as the most promising measures of bone formation and resorption, respectively, recommending them as reference markers for observational and interventional studies [9, 10]. But consensus has emerged that proper assessment of the role of BTMs for fracture prediction must control for factors contributing to major preanalytical variability of such biomarkers, with particular attention to morning collection of fasting blood samples [8].

Here we chose osteocalcin as our marker of bone formation, reflecting our study's focus on bone health and glucose dysregulation [29], while selecting CTX as the optimal marker for bone resorption. Several longitudinal studies have assessed the relationship of osteocalcin specifically with incident hip fracture in women [18–20]. None found a significant association, but specimen collection was either non-fasting [19, 20] or unspecified [18], and the numbers of events were small to moderate (*n* = 33 to 120 hip fractures). A more recent study of older adults that did undertake fasting blood collection also failed to detect an association for osteocalcin in post-menopausal women, but there were only 55 incident hip fracture cases among female participants [22].

As relates to CTX, a meta-analysis of earlier studies reported a significant association with any fracture, but not hip fracture, in women [23]. Such studies did not perform fasting

blood collection and undertook limited adjustment for covariates [15, 19, 20]. Two more recent prospective studies evaluating the relationship between serum CTX, measured in morning fasting specimens, and hip fracture in postmenopausal women also failed to detect a significant association for CTX [22, 24]. The first [22] was underpowered, however, while the second [24]—a nested study with 400 cases and 400 controls—had substantial missingness for covariates in the fully adjusted model (*n* = 608 cases and controls examined). The latter study reported fully adjusted risk estimates for interquartile comparisons that had wide confidence intervals (quartile 3 vs. 1: OR = 1.53, 95% CI = 0.82, 2.85; quartile 4 vs. 1: OR = 1.25, 95% CI = 0.68–2.30).

Our findings for osteocalcin are consistent with previous results [16, 18–20] and accord with prior scientific statements designating PINP over osteocalcin as the preferred biomarker of bone formation [9, 10]. The results for CTX stand out because they reveal a non-linear relationship with incident hip fracture. This inverted U-shaped association has not been previously reported although, as noted, a majority of previous studies have lacked power to investigate the functional form of CTX's relationship with hip fracture. Interestingly, in the aforementioned recent nested case-control study [24], the reported analysis of CTX quartiles showed a higher risk estimate for hip fracture for quartile 3 than for quartile 4. The same study also assessed

for, and did not find, non-linear associations using generalized additive models, but corresponding plots were not presented.

Interpretation of study findings

As CTX reflects osteoclastic digestion of the major protein in bone and collagen and correlates with histomorphometric indices of bone resorption, we anticipated fracture risk to be greatest in those with the highest CTX. The reasons why this was not the case are unclear, but several possibilities merit consideration. As in previous studies [42], both CTX and OC were linearly inversely related to hip BMD in the subset of participants who underwent DXA. It is well documented, however, that fall risk is a more important determinant of hip fracture incidence than bone metabolism per se [43]. But both ADL difficulty and history of previous fall, like cognitive impairment, were positively associated with CTX (as with OC) and highest in its upper quartile, and adjustment for these factors did not alter the CTX-hip fracture association. Cardiovascular and kidney disease were likewise most prevalent in the upper quartile of the two BTMs. Such comorbidities have also been linked to an increased risk of fracture [44], yet adjustment for such factors modestly increased the risk estimates for both quartile 3 and quartile 4. Hence, one possibility for the declining hip fracture risk as concentrations of CTX rose to their highest levels is that lower physical activity by individuals with the comorbidities enriched in the upper quartile of CTX may have outweighed their otherwise higher susceptibility for fracture. Indeed, participants in the highest quartile of CTX had the lowest level of physical activity, yet residual or unmeasured confounding could explain the lower risk at these upper CTX concentrations.

Furthermore, BTMs are dynamic and influenced by recent events including fractures, immobility, and short-term changes in exercise, diet, weight, and some medications [7]. Thus, it is possible that the measured level of resorption represents such recent events rather than the chronic status of skeletal health in some of our participants. Such factors could have also contributed to the failure to detect a dose-dependent relationship between CTX and hip fracture. We assessed the robustness of the relationship by excluding participants taking key medications affecting mineral metabolism, but such sensitivity analyses did not meaningfully alter the association between CTX and risk of hip fracture.

Additionally, participants with the highest bone resorption and lowest BMD may have compensatory mechanisms that offset hip fracture risk by positively affecting other bone qualities that maintain bone strength, such as bone size and geometry. In older adults, there is evidence to suggest periosteal bone expansion counterbalances the high bone resorption and endocortical expansion that characterizes age-related bone loss [45], a mechanism that may be relevant in other age groups as well [46]. In one study,

adolescents with a genetic predisposition to higher bone resorption had greater bone size despite lower BMD, suggesting higher bone resorption is offset by greater periosteal expansion [46]. Information on bone geometry is lacking in our cohort.

Implications

The present investigation is the largest to date to assess both OC and CTX in relation to incident hip fracture in postmenopausal women. Strengths of the study include its sample size and power, which allowed assessment of the functional form and provide insights into the non-linear relationship observed here; the use of fasting blood specimens; and extensive adjustment for potential confounders. Laboratory analysis of bone turnover markers for this ancillary study was performed on never-previously-thawed serum specimens stored at −80 °C after collection under standardized conditions, thereby keeping biomarker degradation to a minimum. As such, the current findings yield important insights regarding the associations of these two major BTMs. In regard to total OC, the current study's failure to detect a significant association with incident hip fracture, notwithstanding OC's close correlation with CTX, adds support to the view that this marker of bone formation is of limited value for hip fracture prediction. As relates to CTX, the reverse U-shaped association reflects the multiple factors that influence this foremost bone resorption marker's relationship with hip fracture risk, highlighting the challenge of using CTX and other BTMs for prediction of this outcome. While it remains to be determined whether the documented association of CTX with hip fracture is independent of BMD, the modest inverse relationship observed between CTX and BMD does suggest that the marker may be of value for hip fracture prediction. This will require further testing in appropriately powered studies, but our findings make clear that careful consideration of clinical and functional risk factors will be necessary for adequate characterization of this relationship and corresponding evaluation of risk prediction models.

Limitations

Our study has several limitations. Measures that could have provided further information on bone metabolism such as BMD and biochemical calciotropic factors (vitamin D, PTH, etc.) were only available in a small subset of participants and we therefore could not include them in our final adjusted models. Moreover, BMD measures were available 2 years after BTM determination. Nonetheless, we did observe the anticipated relationships of BTMs with vitamin D, PTH, and, notwithstanding the later time point, BMD in these subsets. Measurements of PINP, the recommended marker of bone

formation, were not available. Furthermore, we only have information regarding the occurrence of hip fracture and not vertebral or other types of non-vertebral fractures. It is possible that we may have found stronger or monotonic relationships between BTMs and fracture risk with inclusion of other types of fracture or with a focus on fracture at more metabolically active skeletal sites. Moreover, fracture cases were identified by ICD-9 codes and were not adjudicated, which could have led to misclassification. We also do not have any information regarding history of low trauma or fragility fracture. Since fracture risk is higher among those with a prior fragility fracture, this too could have influenced our results.

Conclusions

Among older women, CTX exhibited an inverted U-shaped association with incident hip fracture after adjustment for potential confounders, with risk peaking at the mid-upper range of the marker's distribution, but no significant association was demonstrated for osteocalcin. Further work is necessary to understand this non-linear association, whether it is independent of BMD, and if CTX can be harnessed to improve prediction of hip and other fracture types.

Compliance with ethical standards

All field centers and the CHS Coordinating Center received institutional review board approval for the study and participants gave informed consent.

Conflicts of interest Jorge R. Kizer reports stock ownership in Amgen, Bristol-Myers Squibb, Gilead Sciences, Merck, Johnson and Johnson, and Pfizer. Daniele Massera, Shuo Xu, Marcella D. Walker, Rodrigo J. Valderrábano, Kenneth J. Mukamal, Joachim H. Ix, David S. Siscovick, Russell P. Tracy, John A. Robbins, Mary L. Biggs, and Xiaonan Xue declare that they have no conflict of interest.

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