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Research Article

An index of net bone formation relative to resorption is associated with incident fracture during the menopause transition and postmenopause

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Abstract

We previously created a bone balance index (BBI) that combines bone resorption and formation markers (CTX and PINP, respectively) to estimate bone balance. The bone balance index quantifies net bone formation relative to resorption; greater values indicate a more favorable balance. This study examined the association of the BBI with incident fracture across the menopause transition and tested each individual bone turnover marker (BTM) (either CTX or PINP) as a separate predictor of fracture. We included 451 participants from the Study of Women's Health Across the Nation (SWAN) cohort study, with 2667 longitudinal observations from pre-, early peri-, late peri-, or post-menopause. Participants did not report fractures or the use of bone-active medications before their BTM assessments. Using repeated measures Cox proportional hazards regression, we examined the associations of the BBI, CTX, or PINP with 2 different fracture outcomes: incident fracture at any site or incident fracture at a major osteoporotic fracture (MOF) site (spine, hip, radius, or humerus). Women were censored at the first use of bone-beneficial medications during follow-up. Covariates were age, BMI, race/ethnicity, family history of hip fracture, cigarette use, diabetes mellitus, bone-detrimental medication use, menopause transition stage, FN BMD, and SWAN study site. The mean age across the BBI, CTX, and PINP assessments was 52 yr. Seventy-eight women sustained an incident fracture over a 15.1 yr of follow-up. Adjusted for covariates, each SD increment in the BBI was associated with a 20% smaller hazard for fracture at any site (hazard ratio [HR] [95% CI]: 0.80 [0.67, 0.97], $p = .02$) or a 25% lesser hazard for fracture at a MOF site (HR [95% CI]: 0.75 [0.61, 0.85]), $p = .01$). In contrast, when considered singly in adjusted analyses, neither CTX ($p > .2$) nor PINP ($p > .1$) was related to either fracture outcome. We conclude that BBI predicts fracture, independent of clinical and densitometric fracture risk factors.

Keywords bone turnover, bone turnover markers, menopause, fractures, epidemiology, cohort, longitudinal

Lay Summary

We previously created a bone balance index that estimates the relative amounts of bone an individual breaks down versus builds. In this analysis, we found that this bone balance index predicts future bone fractures, even after accounting for other fracture risk factors, including BMD.

Introduction

Biochemical markers of bone resorption and formation (bone turnover markers, BTMs) are non-invasive indices of total-body osteoclast or osteoblast activity, respectively.[1] The International Osteoporosis Foundation and International Federation of Clinical Chemistry and Laboratory Medicine recommend using serum CTX (resorption) and serum PINP (formation) as reference BTMs for research and clinical practice.[2]

One potential application of BTMs is fracture prediction, with recent research focusing on whether CTX or PINP should be included in clinical fracture prediction algorithms, eg, the FRAX

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Fracture Risk Assessment Tool.[1–3] Several prospective analyses found that greater CTX or PINP (as a separate predictor) relates to greater fracture risk.[4–11] Despite these findings, BTMs have not been adopted for use in fracture prediction tools, in part because CTX and PINP levels were not associated with fractures in all studies,[10,12,13] and when they were, the association was not always independent of BMD.[9–11]

We hypothesize that we can improve BTM prediction of fractures by combining bone resorption and formation markers into a composite index of total-body bone balance. Bone strength is determined by the balance between osteoclast and osteoblast activity, which mediates 2 competing processes: bone loss and skeletal compensation for that loss. Bone loss results from excess resorption relative to formation and leads to lower bone mass, impaired bone microarchitecture, and diminished bone strength.[14–21] To counter the deleterious effects of bone loss on bone strength, osteoblasts repair microcracks in the trabecular compartment and add new bone to the outer, periosteal surface (periosteal expansion), augmenting bone size and strength.[22,23] Within this physiologic framework, a composite exposure that indexes both osteoclast and osteoblast function may better predict fractures than does a bone resorption or formation marker alone.

To estimate total-body bone balance, we combined CTX and PINP to create a bone balance index (BBI).[15] In brief, the BBI quantifies balance by referencing paired CTX and PINP measurements against their average *in-balance* relationship (when resorption approximates formation). We designed the BBI to estimate net bone formation relative to resorption; thus, more positive values reflect a more favorable balance. For BBI construct validation, we showed that more positive BBI levels related to lower rates of subsequent BMD decline.[15] Whether BBI predicts fracture is unknown.

This study examined whether the BBI predicts fracture, independent of clinical and densitometric fracture risk factors among pre-, peri-, and post-menopausal women. To examine how the BBI vs its component BTMs relates to fracture, we also tested the associations of CTX or PINP (as separate exposures) with fracture. This analysis was conducted in the Study of Women Health Across the Nation (SWAN), a longitudinal study of the menopause transition that provides longitudinal assessments of BTMs, clinical characteristics, BMD, and fracture occurrence.

Materials and methods

The Study of Women Health Across the Nation is an ongoing, multicenter, longitudinal cohort study of the menopause transition and aging in a racially/ethnically diverse cohort of community-dwelling women. At the SWAN baseline visit, participants were 42-52 yr old, had an intact uterus and >1 ovary, and were in premenopause (no change from usual menses) or in early perimenopause (less predictable menses, but at least once every 3 mo). Women were recruited from 7 clinical sites, with each site recruiting White women, along with women from one other racial/ethnic group: Black (Boston, Chicago, Detroit, Pittsburgh), Chinese (Oakland), non-White Hispanic (Newark), and Japanese (Los Angeles).

The full SWAN cohort includes 3301 participants. The SWAN Bone Cohort is a subset of 2365 women from 5 sites (Chicago and Newark excluded). In total, 1 baseline and 16 follow-up visits were available for analysis. The median (p25, p75) time between visits was 1.1 (1.0, 1.4) yr. Sixty-three percent of surviving women ($N = 2067$) participated in the 16th follow-up visit (conducted between March 2015 and March 2018), which was the last follow-up at which fracture outcomes were available for this analysis. The mean (SD) age at the 16th follow-up visit was 66.6 (2.7) yr, with a range of 61.6-73.5 yr; all women were postmenopausal by this visit. Participants provided written informed consent, and each site obtained institutional review board approval.

Study sample

CTX and PINP were assayed in 2021 and 2022 at the University of Sheffield, using banked repository serum samples.[24] In brief, we measured CTX and PINP using sera collected at multiple time points across the menopause transition from 541 women who: (1) had a natural menopause; (2) had a known final menstrual period (FMP) date; (3) did not use a bone-active medication prior to the BTM assessment; and (4) had banked samples available from >2 (and up to 10) study visits. Bone-active medications were those that are beneficial (hormone therapy, calcitonin, calcitriol, bisphosphonates, denosumab, or parathyroid hormone) or detrimental (oral or injectable glucocorticoids, aromatase inhibitors, gonadotropin-releasing hormone agonists, or antiepileptics) to bone. Among the 541 women meeting the above criteria, we obtained 3340 paired, serial assessments of CTX and PINP. Sera for BTM measurements were collected over an interval spanning 10 yr before to 13 yr after the FMP. The median number of CTX and PINP assessments per participant was 7 (range 2-10), and median length of time between BTM measurements was 1.9 yr (range 0.8-5.1 yr). Banked sera from the SWAN baseline visit are reserved and were therefore not available for the current project. We refer to the first instance at which we assayed CTX and PINP as the initial analytic visit.

This study tested the BBI, CTX, or PINP as the predictors of time to the first fracture. To be included in the analysis sample, participants were required to: have at least 1 study visit with concurrent measures of CTX and PINP; have no fracture before the BTM assessment; and have at least 1 study visit after the BTM measurements to permit observation for fracture. A total of 451 women with 2667 observations met these criteria (Figure 1).

Outcomes

The outcome in these analyses was the time to the first fracture after the BTM predictor assessment (BBI, CTX or PINP). We conducted 2 sets of analyses, each using a different fracture outcome. The first outcome was a fracture at any non-craniofacial and non-digital site, and the second was a fracture at a major osteoporosis fracture (MOF) site (spine, hip, radius, or humerus).[3] Fracture occurrence and site were determined using standardized questionnaires. Fractures that occurred before SWAN enrollment were recorded at the baseline visit. The Study of Women Health Across the Nation initiated the adjudication of on-study fractures at the seventh follow-up visit; for fractures reported at the first 6 follow-up visits, the fracture date was imputed as the midpoint between the participant's prior and current visits. For instance, if a participant reported a new fracture at follow-up visit 5, the fracture date was imputed as the midpoint between the dates of follow-up visit 4 and follow-up visit 5. Since inauguration of adjudication,

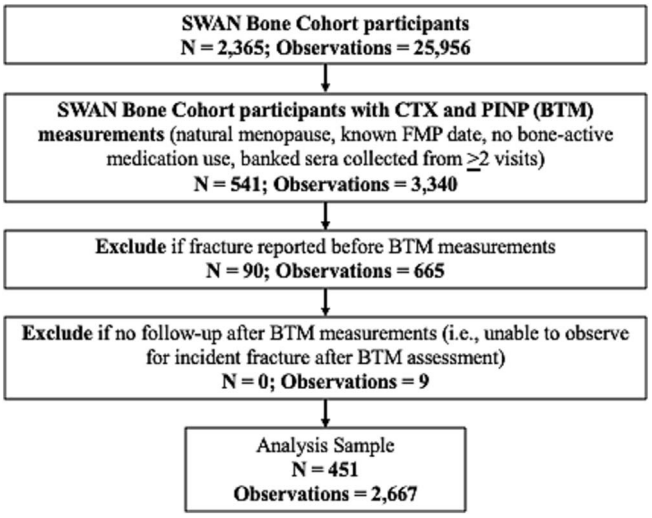

Figure 1 Study sample derivation. Bone-active medications were agents that benefit (hormone therapy, calcitonin, calcitriol, bisphosphonates, denosumab, and parathyroid hormone) or are detrimental (oral or injectable glucocorticoids, aromatase inhibitors, gonadotropin-releasing hormone agonists, or anti-epileptics) to bone. Abbreviation: FMP, final menstrual period.

95% of reported fractures were confirmed. This analysis included both low- and high-trauma fractures, as both fracture types are associated with low BMD are risk factors for future fractures.[25–27] Fractures were categorized as low-trauma if they were sustained after a fall from a height of <6 inches, and did not occur during a motor vehicle accident, rapid movement, playing sports, or from an impact with heavy or fast-moving projectiles.

Primary predictors

Analyses used the BBI (methodology detailed in next paragraph), CTX, or PINP as primary predictors in separate models. CTX and total PINP were quantified from stored sera acquired at fasting blood draws conducted before 10:00 AM.[2,28–30] Specimens were stored at a central repository at −80° C and did not undergo freeze-thaw cycles before BTM assay performance. The Bone Biochemistry Laboratory at the University of Sheffield performed all CTX and PINP assays using electrochemiluminescence (Cobas e411 analyzer, Roche Diagnostics, Germany). Assays for the same individual were performed in a single batch. The intra-assay coefficients of variation (CV) were 3.4% (CTX) and 3.5% (PINP). The inter-assay CVs were 5.1% for CTX and 3.3% for PINP.

Conceptually, the BBI quantifies bone balance by referencing paired CTX and PINP measurements against an estimate of their average relation when resorption approximates formation. More negative BBI values reflect a less favorable balance, and more positive values reflect a more favorable balance. The BBI was computed using 2 steps.[15]

1) Step 1. We employed linear regression to quantify the *in-balance* relationship between CTX (as the dependent variable) and PINP (as the independent variable) in women in whom resorption approximated formation (ie, when they were premenopausal, and BMD was stable). This step was previously completed using CTX and PINP data from 388 premenopausal SWAN participants.[15]
2) Step 2. We calculated individual-specific BBI values, by referencing paired CTX and PINP measurements (obtained in women in the current analysis sample) against their in-balance relationship. Specifically, we applied the regression coefficients from Step 1 to each PINP measurement to calculate P_CTX (the predicted value of CTX corresponding to each PINP level if resorption and formation were in balance). The regression equation for P_CTX was $4.567 * \text{PINP} + 86.32$. We then calculated the raw BBI value as P_CTX − CTX (the difference between the *expected* and *measured* CTX values) to estimate net bone formation relative to resorption. To standardize the BBI values, we divided each raw BBI value by the SD of all raw BBI values; the BBI, therefore, has no units. The final BBI formula was $\text{BBI} = (4.567 * \text{PINP} + 86.32 - \text{CTX})/154.2$.

Covariates

To determine whether BBI, CTX, or PINP was associated with fracture, independent of clinical risk factors and BMD, models were adjusted for the following variables: age, BMI, race/ethnicity, family history of hip fracture (yes/no), cigarette use (yes/no), diabetes mellitus (yes/no), use of bone-detrimental medications during the observation period for fractures, clinical menopause transition stage (pre-, early peri-, or late-perimenopause), SWAN study site, and FN BMD. We selected covariates based on their inclusion in the FRAX Fracture Risk Assessment Tool (age, BMI, race/ethnicity, family history of hip fracture, cigarette use, and FN BMD[3]) or their previously published relation to fracture in SWAN or other cohorts (diabetes mellitus,[31–33] use of bone-detrimental medications,[19,24,34] and clinical menopause transition stage[34]). For all SWAN analyses, we include study site as a covariate, to account for within-site correlations.

The Study of Women Health Across the Nation measured body weight and height using calibrated scales and stadiometers and calculated BMI as weight in kilograms divided by the square of height in meters. Participants were considered to have diabetes mellitus if their fasting blood glucose was >126 mg/dL (as per American Diabetes Association guidelines)[35] or if they reported using a diabetes medication (metformin, sulfonylurea, meglitinide, thiazolidinedione, DPP-IV inhibitor, GLP-agonist, and insulin). Clinical menopause transition stage was determined using menstrual bleeding patterns, with premenopause defined as no change from the usual bleeding pattern, early perimenopause as less predictable menses at least once every 3 mo, late perimenopause as less predictable bleeding once every 3-12 mo, and postmenopause as 12 or more months of amenorrhea. We modeled exposure to bone-detrimental medications during follow-up as the proportion of visits starting from the BMT predictor assessment until the time of censoring that use of a bone-detrimental medication was reported. We did not adjust for exposure to bone-beneficial medications during follow-up; rather, participants were censored at the first reported use (see "Data Analysis" for details).

BMD (g/cm^2) was measured using Hologic instruments (Hologic, Inc., Waltham, MA, USA). Boston, Detroit, and Los Angeles sites began SWAN with Hologic 4500A models and subsequently upgraded to Hologic Discovery A instruments. Davis and Pittsburgh started SWAN with Hologic 2000 models and later upgraded to Hologic 4500A machines. When a site upgraded its

hardware, it scanned ~40 women on its old and new machines to develop cross-calibration regression equations. The Study of Women Health Across the Nation circulated an anthropomorphic spine phantom for cross-site calibration. A standard quality control program included daily phantom measurements and a local site review of all scans.

Data analysis

We generated descriptive statistics for all variables and described participant characteristics using means (SD) for normally distributed continuous variables, medians (p25, p75) for continuous variables with skewed distributions, and counts (percentages) for categorical variables. CTX and PINP values were not normally distributed; we used base-2 log-transformed values for the analyses.

We conducted 2 sets of primary analyses. The first examined the associations of BBI, CTX, or PINP with incident fracture at any non-craniofacial and non-digital site, and the second modeled BBI, CTX, or PINP as the predictors of fractures at a MOF site. For each set of analyses, we employed repeated measures Cox proportional hazards regression with BBI, CTX, or PINP as separate primary predictors and time to first fracture as the dependent variable (with fracture clock starting at the time of the BBI, CTX, or PINP assessment). To account for within-woman correlations in repeated observations, cluster robust standard errors were used. If a woman started a bone-beneficial medication during follow-up, we treated observations after treatment initiation as missing. We handled such observations as missing (instead of adjusting for the use of bone-beneficial medication) because treatments for osteoporosis are usually taken for prolonged time periods and substantially impact fracture risk.[36] Women who did not fracture, and did not use bone-beneficial medications were censored at their last observation. Covariates in all models were age, BMI, race/ethnicity, family history of hip fracture (yes/no), cigarette use (yes/no), use of bone-detrimental medications during the observation period for fractures, FN BMD, clinical menopause transition stage (pre-, early peri-, late-perimenopause, or postmenopause), and SWAN study site. Values for age, BMI, cigarette use, FN BMD, and clinical menopause transition stage were obtained from the exposure visit.

We also conducted 2 sets of sensitivity analyses. In the first, we used Cox proportional hazards regression to quantify the associations of BBI, CTX, or PINP with incident *low-trauma* fractures (at any site or at a MOF site). In the second, we employed Fine-Gray competing risk regression to model the associations of BBI, CTX, or PINP with incident low- or high-trauma fractures at any site or at a MOF site, with death as a competing risk.[37,38] For all sensitivity analyses, we generated separate models for each predictor-fracture outcome pair, and the models were adjusted for the same covariates as those in our primary analyses.

Results

Participant characteristics

Table 1 summarizes the relevant characteristics, measured at the SWAN baseline visit, of the parent SWAN Bone Cohort ($N = 2365$) and our current analysis sample ($N = 451$); the characteristics of these samples were similar. The 451 women in the analysis sample afforded 1795 longitudinal observations. The median (p25, p75) observations per participant was 4 (3, 5). The mean (SD) follow-up time was 12.58 (4.23) yr. The racial/ethnic composition of the sample was 23% Black ($N = 103$), 15% Chinese ($N = 67$), 15% Japanese (N = 68), and 47% White ($N = 213$) (Table 1).

Bone balance index values were normally distributed, but CTX and PINP values were not. At the initial analysis visit (first visit with CTX and PINP measurements), the median CTX and PINP levels were 272 ng/L and 42.8 ug/L, respectively; the BBI averaged −0.046. At the analysis outset, participants were, on average, 47.76 yr of age (range 42.87-54.61 yr). Thirty percent of the women were in premenopause, 68% were in early perimenopause, and 2% were in late perimenopause. The mean BMI was 26.73 kg/m^2, 8% of the participants had diabetes, and 13% reported using cigarettes. The average FN BMD level was 0.829 g/cm^2 (Table 2).

At the last BTM measurement visit (the last follow-up at which with CTX and PINP measures were obtained), the mean participant age was 57.59 yr (range 44.86-67.7 yr), and the BMI averaged 27.54 kg/m^2. Also at this time point, 79% of the women were in late perimenopause, and 21% were in postmenopause. The median CTX and PINP values were 431 ng/L and 56.7 ug/L, respectively, and the mean BBI level was −0.581 (Table 2). More negative BBI values indicate less favorable balance; thus, during the period between the initial and last visits at which BBI was measured, the balance between resorption versus formation increasingly favored resorption.

Also summarized in Table 2 are the characteristics of the participants at the final analysis visit (SWAN follow-up visit 16), which was the last follow-up visit at which fracture outcomes were available for this analysis. At this time, the mean age was 65.88 yr.

BBI, CTX, or PINP as the predictors of time to first fracture at any site

Of the 451 participants in the analysis sample, 78 sustained a first non-craniofacial or non-digital fracture during 15.1 yr of follow-up (51% of these fractures were categorized as low-trauma). The most common fractures sites were the wrist (19%), upper arm (17%), ankle (17%), and hands or feet (16%) (Table 3).

Adjusted for clinical covariates (age, BMI, race/ethnicity, family history of hip fracture, use of bone-detrimental medications, cigarette use, diabetes, clinical menopause stage, and SWAN site) and FN BMD, a greater BBI was associated with a lower rate of future fractures at any site: the adjusted hazard ratio (HR) per SD increment in BBI level was 0.80 (95% CI: 0.67, 0.97; $p = .02$). In contrast, adjusted for the same covariates, neither CTX ($p = .5$) nor PINP ($p = .1$) alone was associated with fracture hazard (Table 4).

BBI, CTX, or PINP as the predictors of incident fracture at a MOF site

Thirty of the 78 fractures sustained during follow-up occurred at a MOF site (50% were low-trauma). In multiply adjusted models, each SD increment in BBI was related to a 25% smaller hazard for fracture at a MOF site (HR [95% CI]: 0.75 [0.61, 0.95]; $p = .01$). In contrast, CTX ($p = .2$) or PINP ($p = .4$) was not significantly associated with fracture in multiply adjusted models (Table 5).

Table 1 Descriptives[a] for the SWAN Bone Cohort and analysis sample.

	SWAN Bone Cohort ***N*** **= 2365**	**Analysis sample** ***N*** **= 451**
Time-fixed variables		
Race/ethnicity	658 (28)	103 (23)
Black	245 (11)	67 (15)
Chinese	266 (11)	68 (15)
Japanese	1166 (50)	213 (47)
White		
Family history of hip fracture (yes)	209 (13)	44 (11)
Characteristics at the SWAN baseline visit		
Age (yr)	46.34 (2.67)	46.01 (2.48)
BMI (kg/m^2)	27.75 (6.98)	26.73 (6.55)
Cigarette use (yes)	403 (17)	59 (13)
Diabetes (yes)	197 (8)	34 (7)
FN BMD (g/cm^2)	0.823 (0.137)	0.817 (0.131)

[a] Mean (SD) for continuous, normally distributed variables (age, BMI, and FN BMD); count (%) for categorical variables.

Table 2 Descriptives[a] for the analysis sample at initial study visit[b], last BMD measurement visit[c], and final analysis visit[d].

	Analysis sample ***N*** **= 451** **Observations = 2667**		
	Initial analysis visit	**Last BTM measurement visit**	**Final analysis visit**
Menstrually-based MT stage			
Premenopause	134 (30)	0 (0)	0 (0)
Early perimenopause	305 (68)	0 (0)	0 (0)
Late perimenopause	12 (2)	357 (79)	0 (0)
Postmenopause	0 (0)	94 (21)	451 (100)
Age (yr)	47.76 (2.68)	57.59 (5.10)	65.88 (3.29)
BMI (kg/m[b])	26.73 (6.53)	27.54 (6.71)	28.65 (6.81)
FN BMD (g/cm[b])	0.829 (0.131)	0.758 (0.135)	0.726 (0.125)
Cigarette use (yes)	59 (13)	41 (9)	43 (10)
Diabetes (yes)	9 (2)	20 (4)	23 (5)
CTX (ng/L)	272 (195, 367)	431 (304, 560)	N/A
PINP (ug/L)	42.8 (33.1, 55.6)	56.7 (43.9, 71.0)	N/A
BBI	−0.046 (0.759)	−0.581 (0.995)	N/A

[a] Mean (SD) for continuous, normally distributed variables (age, BMI, FN BMD, BBI); median (p25, p75) for continuous, non-normally distributed variables (CTX, PINP); count (%) for categorical variables. [b] "Initial analysis visit" defined as the first SWAN follow-up visit at which participants had banked serum sample to assay CTX and PINP. As banked serum samples from the SWAN baseline visit were not available, we do not have CTX and PINP measurements from this time point. [c] "Last BTM measurement visit" defined as the last follow-up visit at which participants had concurrent CTX, PINP, and BBI assessments. [d] "Final analysis visit" defined as the last follow-up visit at which fracture outcome data were available. Abbreviations: BBI, bone balance index; CTX, collagen type I C-telopeptide; MT, menopause transition; PINP, procollagen type I N-terminal propeptide.

Sensitivity analyses

In our first set of sensitivity analyses, we additionally assessed the associations of BBI, CTX, or PINP with incident *low-trauma* fractures at any site or at a MOF site. After adjusting for the same clinical covariates above, the HR (95% CI) per SD increment in BBI was 0.79 (0.66, 0.98) for fracture at any site ($p = .03$) or 0.70 (0.50, 0.97) for fracture at a MOF site ($p = .03$). As in the primary analyses, neither CTX ($p > .3$) nor PINP ($p > .8$) was significantly associated with incident low-trauma fracture in multiply adjusted models.

In the second set of sensitivity analyses, we used Fine-Gray competing risk regression to quantify the associations of BBI, CTX, or PINP with incident low- or high-trauma fractures (at any site or at a MOF site) with death as a competing risk. The proportion of participants with a fracture was 17.3% and that for death was 2.0%. Adjusted for the same covariates above, the subdistribution HRs for fracture per SD increment in BBI, log2CTX, or log2PINP were similar to the HRs generated by our primary (non-competing risk) approach (data not shown).

Table 3 Incident (first) fractures[a] during the study observation period.

Fracture site	Number of fractures (%)
Wrist	15 (19)
Hip	2 (3)
Spine	4 (5)
Ribs	6 (8)
Upper arm or shoulder	14 (17)
Leg above ankle	10 (12)
Ankle	14 (17)
Hands or feet (non-digital)	13 (16)
Total	78 (100)

[a] Includes low-trauma ($N = 40$) and high-trauma ($N = 38$) fractures. See "Materials and methods" for definition of low- vs high-trauma.

Table 4 Adjusted associations[a] of BBI, CTX, or PINP with incident fracture (at any non-craniofacial or non-digital site) in midlife women.

	Hazard ratio (95% CI) for fracture per SD increment in log_2CTX, log2PINP, or BBI	*p*-value
BBI	0.80 (0.67, 0.97)	.02
CTX	1.03 (0.82, 1.30)	.5
PINP	0.86 (0.72, 1.04)	.1

[a] Hazard ratios estimated using repeated measures Cox proportional hazards regression models with BBI, log_2CTX, or log_2PINP as predictors in separate models and time to first fracture as outcome, adjusted for age, BMI, race/ethnicity, family history of hip fracture, use of bone-detrimental medications, cigarette use, diabetes, menopause transition stage (pre-, early peri-, late-perimenopause, or postmenopause), FN BMD, and SWAN study site. Values for time-varying covariates were contemporaneous with predictor assessments. We used cluster robust standard errors to account for within-woman correlations in repeated measures. Abbreviations: BBI, bone balance index; CTX, collagen type I C-telopeptide; PINP, procollagen type I N-terminal propeptide.

Table 5 Adjusted associations[a] of BBI, CTX, or PINP with incident fracture (at a major osteoporotic fracture site) in midlife women.

	Hazard ratio (95% CI) for fracture per SD increment in log2CTX, log2PINP, or BBI	*p*-value
BBI	0.75 (0.61, 0.95)	.01
CTX	1.19 (0.88, 1.62)	.2
PINP	0.90 (0.69, 1.17)	.4

[a] Hazard ratios estimated using repeated measures Cox proportional hazards regression models with BBI, log_2CTX, or log_2PINP as predictors in separate models and time to first fracture at a major osteoporotic fracture site as outcome, adjusted for age, BMI, race/ethnicity, family history of hip fracture, use of bone-detrimental medications, cigarette use, diabetes, menopause transition stage (pre-, early peri-, late-perimenopause, or postmenopause), FN BMD, and SWAN study site. Values for time-varying covariates were contemporaneous with predictor assessments. We used cluster robust standard errors to account for within-woman correlations in repeated measures. Abbreviations: BBI, bone balance index; CTX, collagen type I C-telopeptide; PINP, procollagen type I N-terminal propeptide.

Discussion

This study examined the association of a novel BBI with incident fractures among pre-, peri-, and post-menopausal women with an age range of 42-72 yr over the course of the observation period. We report that greater BBI (indicating a more favorable bone balance) related to lower fracture hazard. Specifically, each SD increment in BBI related to a 20% lesser rate of fracture at any site and a 25% lower incidence of fracture at a MOF site. These associations were independent of clinical risk factors and FN BMD. Contrary to BBI, neither CTX nor PINP was significantly associated with future fractures in this analysis sample.

An ongoing topic of investigation among BTM researchers is whether CTX or PINP can aid clinical fracture prediction. In particular, recent studies focus on whether individual BTMs should be included in clinical fracture prediction algorithms (eg, the FRAX Fracture Risk Assessment Tool).[1–3] Neither CTX nor PINP has been adopted in such tools, in part, because their levels do not consistently relate to fracture risk, and when they do, the relations are not always independent of BMD.[1,9–13] For instance, although some prior analyses reported that higher CTX levels were associated with a greater risk of fractures at any site,[39] vertebral fractures,[10] non-vertebral fracture,[4] or hip fractures,[5,12] findings were not uniform across studies, and the associations were not always significant after controlling for BMD.[4,10,12,13] Like CTX, the PINP level was also inconsistently associated with risk of fracture.[4,8,13,39,40]

Our current findings support the hypothesis that combining bone resorption and formation markers in a composite estimate of bone balance advances our ability to forecast fractures, among women with characteristics like those of our analysis sample. Indeed, in this analysis, BBI was associated with the incident fracture hazard, when CTX or PINP was not. Why is BBI more strongly associated with incident fractures than its component BTMs? By accounting for the balance between osteoclast and osteoblast activity across the skeleton, BBI provides an aggregated signal of the opposing effects of bone resorption and formation on fracture resistance. Plausibly, the CTX component of BBI indexes the degree of bone loss and bone microarchitectural damage, while PINP captures the extent to which bone formation compensates for these losses (via its beneficial effects on bone remodeling balance and periosteal expansion).

Our results showing that BBI predicts fractures, independent of clinical risk factors and BMD, indicates that, among pre-, peri-, and post-menopausal women who range in age from 42 to 72 yr, BBI may overcome a key hurdle that impedes fracture prediction using CTX or PINP. Future research will quantify the degree to which BBI improves fracture prediction beyond that afforded by clinical and densitometric variables. Whether our results are generalizable to older women and men adults is uncertain. This question should be addressed by additional research, as most fractures occur in later life. Practically, BBI would be well-suited for inclusion in prediction tools. Because BBI is computationally complex, individual BBI levels are cumbersome to calculate manually. However, it could be readily programed into prediction algorithms, such that users would simply input a pair of CTX and PINP values to obtain BBI estimates.

This study has several methodological strengths. First, is its longitudinal study design and repeated measures modeling strategy. Second, sera for BTM assessments were collected under optimal

conditions (fasting, before 10:00 AM) that minimize variability in CTX measurements.[1] Third, CTX and PINP were quantified using widely available, automated electrochemiluminescent assays that offer superior precision and accuracy relative to older radioimmunoassays or ELISA.[1]

We also consider several limitations. The first relates to our fracture outcomes. To maximize the number of fracture events, we included both low- and high-trauma fractures in composite outcomes. We justify this approach because both fracture types are associated with lower BMD and are risk factors for future events.[26,27] Indeed, some clinical trials of osteoporosis therapies include pooled low- and high-trauma fractures as study outcomes.[41,42] Moreover, in sensitivity analyses, the associations of BBI with incident *low-trauma* fractures were like those of BBI with incident *low- or high-trauma* fractures. Because our study sample consisted of younger women, a small number of fractures occurred at MOF sites, and we could not test the ability of BBI to predict vertebral and hip fractures. Before BBI can be considered for clinical use, more research should optimally be conducted to characterize its association, in both older postmenopausal women and men, with various fracture outcomes (vertebral, non-vertebral, hip, and MOF) over specified follow-up periods. Second, we measured CTX and PINP using serum samples because plasma was unavailable from the repository. Some investigators suggest that CTX may be more stable in plasma than in serum.[1] In addition, although CTX is known to be stable in samples stored at −80° C for at least 3 yr,[1,43] we are unaware of any published studies assessing CTX stability when stored for longer durations. SWAN has not examined the stability of CTX in blood specimens stored up to 24 yr (the longest duration of storage for samples used in this study). To optimize analyte stability, we promptly centrifuged blood specimens (within 2 h of sample collection), stored sera at −80° C, and did not thaw samples until assay performance. Finally, we did not exclude women with chronic kidney disease, a condition that affects CTX and PINP levels if glomerular filtration rates are below 60 mL/min.[1] This limitation was unavoidable, as SWAN did not obtain serum creatinine measurements in participants. Nonetheless, this limitation does not alter our conclusions because including women with chronic kidney disease would have caused BTM measurements to be less accurate indices of bone resorption and formation and reduced our ability to discern an association of BBI with incident fractures.

Among middle-aged pre-, peri-, and post-menopausal women, a novel BBI that quantifies total-body bone balance from paired CTX and PINP measurements predict fractures, independent of clinical and densitometric risk factors. Unlike BBI, neither the CTX nor the PINP level was associated with incident fractures in our analysis sample. We conclude that the BBI advances our ability to use BTMs to predict fractures.

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Author contributions

Albert Shieh contributed to study design, statistical analysis, and primary manuscript drafting. Arun S. Karlamangla contributed to statistical analysis and critical review of manuscript. Fatma Gossiel and Richard Eastell contributed to BTM assay performance, results interpretation and critical review of manuscript. Sherri-Ann Burnett-Bowie contributed to study design and critical review of manuscript. Gail A. Greendale contributed to participant recruitment for the parent SWAN study, study design, and primary manuscript drafting.

Albert Shieh (Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Writing—original draft, Writing—review & editing), Arun S. Karlamangla (Conceptualization, Formal analysis, Writing—review & editing), Fatma Gossiel (Data curation, Investigation, Methodology, Writing—review & editing), Richard Eastell (Data curation, Investigation, Methodology, Writing—review & editing), Sherri-Ann Burnett-Bowie (Data curation, Investigation, Writing—review & editing), and Gail A. Greendale (Conceptualization, Formal analysis, Investigation, Supervision, Writing—review & editing)

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Conflicts of interest

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Data availability

Some or all datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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