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Quantifying the balance between bone formation and resorption: An index of net bone formation.

A thesis submitted in partial satisfaction of the
requirements for the degree of Master of Science
in Clinical Research

by

Albert Shieh

2016

ABSTRACT OF THE THESIS

Quantifying the balance between bone formation and resorption: An index of net bone formation.

by

Albert Shieh

Master of Science in Clinical Research

University of California, Los Angeles, 2016

Professor Janet S. Sinsheimer, Chair

Context and Objective: Bone resorption and formation markers increase when turnover increases, whether there is net bone gain or net loss. The objective was to determine if resorption and formation markers can be combined to gauge net bone balance.

Design: Cohort followed across menopause transition; Study of Women's Health Across the Nation

Setting and Participants: Community-dwelling women, 42-52 years old, premenopausal or early perimenopausal at baseline.

Outcome Measures: 1) Bone balance index (BBI) created by estimating the in-balance relationship between resorption and formation markers in 685 women in stable bone balance (>5 years before the final menstrual period, FMP), and applying this relationship to measured turnover markers in 216 women beginning to lose bone (≤ 2 years from FMP). 2) Annualized percent declines over the following 3-4 years in lumbar spine (LS) and femoral neck (FN) bone mineral density (BMD).

Results: Adjusted for covariates, BBI was greater (more favorable) in women with greater body mass index ($p=0.03$), and lower (less favorable) in women closer to the FMP ($p=0.007$). Each standard deviation decrement in BBI was associated with 0.27%/year faster LS BMD decline ($p=0.04$) and 38% higher odds of faster-than-average loss of LS bone mass ($p=0.008$, c-statistic=0.76). BBI was not associated with decline in FN BMD. Urinary N-telopeptide (U-NTX) alone was not associated with either LS or FN BMD decline.

Conclusions: An index that quantifies net bone formation versus resorption can be created from turnover markers, and may help identify individuals at high risk for bone loss.

The thesis of Albert Shieh is approved.

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CHAPTER 1. MANUSCRIPT

ABSTRACT

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($p=0.04$) and 38% higher odds of faster-than-average loss of LS bone mass ($p=0.008$, c -statistic=0.76). BBI was not associated with decline in FN BMD. Urinary N-telopeptide (U-NTX) alone was not associated with either LS or FN BMD decline.

Conclusions: An index that quantifies net bone formation versus resorption can be created from turnover markers, and may help identify individuals at high risk for bone loss.

INTRODUCTION

Biochemical markers of bone resorption and formation (bone turnover markers, or BTMs) have been available for clinical and research purposes for many years (1-7). However, one reason that interpreting individual BTM values remains challenging is that bone resorption and formation are coupled. As such, markers of both bone resorption and formation increase in states of increased bone turnover regardless of whether there is net bone gain (e.g. after initiating regular exercise or teriparatide therapy) or loss (e.g. during the menopause transition) (3,5,8-13). This implies that in order to accurately assess bone metabolic balance, bone resorption and formation markers cannot be examined in isolation, but should ideally be combined to gauge the direction and magnitude of bone gain or loss (6,12,13).

In this proof-of-concept study, we aimed to develop and test a novel methodology for creating a bone balance index (BBI) that combines an individual's measured BTMs to gauge net bone balance. We hypothesized that 1) the quantitative relationship between bone resorption and formation markers in a state of bone balance (i.e. when bone resorption equals formation) can be estimated from a sample of healthy adults in stable bone balance; and 2) this estimate of the in-

balance relationship can be combined with an individual's measured BTMs (at a time point when that individual may not be in bone balance, such as during the menopause transition) to create a BBI that reflects the individual's net bone formation relative to resorption. Under this paradigm, a more positive BBI value indicates more favorable bone balance, and a more negative BBI value indicates less favorable bone balance.

Using data from the Study of Women's Health Across the Nation (SWAN), we used the above paradigm to create a BBI, and tested its ability to predict rate of bone loss. All participants in SWAN were pre-menopausal or early peri-menopausal at the baseline visit. Since baseline, almost the entire cohort has transitioned to postmenopause; and for many, we have determined the date of the final menstrual period (FMP). (Exact FMP date is not knowable in all women; for example, it is obscured in women who were taking hormone therapy). Thus, for participants with a dated FMP, we know how many years prior to their FMP they were at SWAN baseline. This allowed us to estimate the in-balance relationship between resorption and formation markers from baseline BTM data in women who were more than 5 years from their FMP (and therefore likely to be in stable bone balance). We then used this in-balance estimate to calculate BBI values in women who were 2 or fewer years from the FMP (and therefore had begun to lose bone mass). MT-related bone loss generally starts approximately 2 years before the FMP (14). In these women, we assessed the ability of BBI to predict longitudinal decline in bone mineral density (BMD). We hypothesized that more positive BBI values (reflecting more favorable bone balance) would be associated with slower declines in BMD.

MATERIALS AND METHODS

Study Samples

SWAN is a multi-center, longitudinal study of the MT in a community-based cohort. Participants at baseline were aged 42-52, premenopausal (menstruating 3 months prior to screening without change in menstrual regularity in the past year) or early perimenopausal (menstruating 3 months prior to screening with decreased regularity in the past year), had an intact uterus with 1 or 2 ovaries, were not pregnant, were not lactating, and were not taking exogenous sex steroid hormones or bone-active medications. Five of the seven SWAN study sites (Boston, Detroit, Los Angeles, Oakland, and Pittsburgh) measured BTMs and BMD. BTMs were measured at baseline; BMD was measured at baseline and on annual follow-up. Participants gave written informed consent, and sites obtained institutional review board approval. Detailed recruitment and participant information has been previously reported (14).

Of 2,413 SWAN Bone Cohort participants, the FMP date was known for 1,331 women. Major reasons for not being able to identify an FMP date were hysterectomy before the FMP and use of menopausal hormone therapy. Of these 1,331 women, 703 were more than 5 years before their FMP at the baseline visit. After excluding 18 women with missing BTM data, we were left with an In-Balance Sample (of women more than 5 years before the FMP) of size 685 for estimating the in-balance relationship between formation and resorption markers. Of the 1,331 women with known FMP date, 223 were 2 or fewer years before their FMP at the baseline visit; after excluding 7 women with missing BTM data, we had a Test Sample (of women no more than 2 years prior to their FMP) of size 216 to create and test BBI.

Measurements: Bone Turnover Markers

The bone formation marker, serum osteocalcin (OC), was measured from fasting blood collected before 10AM. The bone resorption marker, urinary NTX (U-NTX), was measured from a non-first voided urine collected before 10AM. If a woman could not provide a urine sample before 10AM, the time of collection was recorded. Because BTMs show diurnal variation, we adjusted for time of urine collection in sensitivity analyses described below (see Statistical Analyses). Specimens were stored at -20 to -80 degrees Celsius until centralized analysis (Medical Research Laboratories, Highland Heights, KY). OC was measured using the ELSA-OSTEO immunoradiometric assay (ng/mL; CisBio International, Bagnols/Seze, France; inter-assay CV <6%; intra-assay CV <6%). U-NTX was measured using the Osteomark competitive inhibition enzyme immunoassay (nM BCE; Osteomark, Ostex International Inc., Seattle WA; inter-assay CV <12%; intra-assay CV <8%). Urinary creatinine was measured using the Cobas Mira autoanalyzer (mM; Horiba ABX, Montpellier, France; inter-assay CV 4.1%; intra-assay CV 0.6%). U-NTX was normalized to urinary creatinine and expressed in nM BCE/mM Cr. U-NTX and OC measured at the SWAN baseline visit were used in this study to create BBI at SWAN baseline.

Measurements: Bone Mineral Density

Lumbar spine (LS) and femoral neck (FN) BMD were assessed by DXA (Hologic QDR 2000 at Pittsburgh and Oakland sites; Hologic QDR 4500A at Boston, Los Angeles, and Michigan sites). Calibration was completed across sites by using a single, circulating anthropomorphic spine calibration phantom initially and then local calibration to adjust for temporal variation.

Rates of Ongoing Change in BMD

BMD was measured at baseline and annually thereafter. In the Test Sample, we used BMD measured at baseline and at a follow up visit 3-4 years later to calculate the annualized percent change in BMD as the difference between BMD at the 2 visits divided by the starting BMD and the number of years between visits, and converted to a percentage.

Measurements: Covariates

Body mass index (BMI) was calculated from weight and height measurements [BMI = weight in kilograms/(height in meters)²]. FMP date for those who underwent natural menopause was defined as the last menstrual bleeding date reported during the visit immediately preceding the first visit at which participants were classified as postmenopausal (12 months of amenorrhea). Physical activity level was summarized by a score combining intensity with frequency of active living, home, and recreational physical activity from a modified Baecke interview (15).

Statistical Analysis

Creating the Bone Balance Index: A BBI that quantifies the net excess of bone formation over resorption was created in four steps. Step 1: The bone resorption marker [U-NTX normalized to urinary creatinine (nM BCE/mM Cr)] was regressed on the formation marker [OC (ng/mL)] in the In-Balance Sample. This provided a quantitative estimate of the in-balance relationship between resorption and formation markers, to use in constructing BBI in the Test Sample in the following 3 steps. Step 2: Regression coefficients from step 1 were combined with each individual's measured bone formation marker level(s) to create participant-specific predictions (P_NTXX) of the NTX level that would be expected when bone resorption equals formation. Step

3: A raw BBI value was generated for each participant as $P_NTX - NTX$, the difference between expected and measured values of the bone resorption marker. Step 4: BBI was standardized by dividing raw BBI values by the standard deviation (SD) of the raw BBI.

Testing the Bone Balance Index: In the Test Sample, we conducted three different types of construct validation. First, we examined the association between BBI and factors known to impact bone balance, namely physical activity, BMI, and time from FMP. Second, we assessed the ability of BBI to predict longitudinal decline in LS and FN BMD. Third, we assessed the ability of BBI to identify women who lost bone at a greater-than-average rate.

To examine the association of BBI with expected determinants of bone balance, we used multivariable linear regression to regress BBI on BMI, years from FMP, concurrently measured physical activity and relevant covariates (study site, race/ethnicity, and age).

We examined the ability of BBI to predict longitudinal bone loss and identify those who lost bone at a faster-than-average rate in a subset of 157 individuals from the Test Sample after excluding 59 participants who initiated bone-active medications between baseline and follow-up BMD measurements. Bone-active medications included osteoporosis medications (bisphosphonates, calcitonin, teriparatide), selective estrogen-receptor modulators, aromatase inhibitors, sex steroid hormones, GnRH agonists, glucocorticoids, anti-epileptics, and chemotherapeutic agents.

To examine the ability of BBI to predict rate of bone loss, we used multivariable linear regression to regress annualized percent change in BMD on BBI and relevant covariates (study site, race/ethnicity, BMI, age, and years from FMP). Rates of decline in LS and FN BMD were the dependent variables in separate analyses. We did not include starting BMD as a covariate to avoid bias due to measurement noise and regression to the mean (16).

To examine the ability of BBI to identify women whose bone loss was faster than average, we used multivariable logistic regression to regress faster-than-average bone loss (defined as annualized percent BMD decline rate exceeding the median value) on BBI and relevant covariates (study site, race/ethnicity, BMI, age, and years from FMP).

Sensitivity Analyses: Because not all women were able to provide urine samples before 10AM (N=10 in the In-Balance Sample, N=3 in the Test Sample), and BTMs have diurnal variation, we created an alternate BBI formula in which we adjusted for time of urine collection. In addition, because the race/ethnic distributions of the In-Balance and Tests Samples were different, we created a third BBI formula in which we adjusted for race/ethnicity. We then conducted construct validation for each alternate BBI.

RESULTS

Study Samples

Mean age in the In-Balance Sample (those for whom the FMP was more than 5 years away) was 44.6 years, and mean time before the FMP was 8.1 years. Nearly half of the In-Balance sample was Caucasian, 28.6% were African American, 11.4% were Chinese, and 13.8% Japanese (Table

1). The Test Sample (those who were within 2 years of their FMP) was older and closer to the FMP on average than the In-Balance Sample. Mean age here was 48.0 years and mean time before FMP was only 0.9 years. The Test Sample also had more African Americans and fewer Caucasians than the In-Balance Sample: 36.6% were African American and 37.0% were Caucasian (Table 1). Mean annualized percentage change in BMD from baseline to 3-4 years later was -1.7 % per year and -1.2 % per year at the LS and FN, respectively, but there was significant variability between women in the rate of change (Table 1).

BBI Creation

The quantitative estimate of the in-balance relationship between bone formation marker (OC) and bone resorption marker (U-NTX) was determined using measurements at the SWAN baseline visit from the In-Balance Sample. This was used to estimate P_NT_X, the participant-specific prediction of the NTX level expected if bone resorption equals formation. BBI at SWAN baseline was created for each participant in the Test Sample as P_NT_X – NT_X, and standardized to SD units. The resulting formula for BBI was: $[16.3 + 1.1 * OC - NTX] / 12.2$ where OC is measured in ng/mL, U-NTX in nM BCE / mM Cr, and the SD of the raw (unstandardized) BBI was 12.2 nM BCE /mM Cr.

Determinants of BBI

After adjusting for age, race/ethnicity, and study site, time from the FMP and BMI were independently associated with BBI, but physical activity was not. As expected, closer proximity to the FMP was associated with more negative (less favorable) BBI, such that every year closer to the FMP was associated with a 0.3 unit decrease in standardized BBI (p=0.007). Also as

expected, higher BMI was associated with more positive (more favorable) BBI, such that every 5 kg/m² increment in BMI was associated with a 0.1 unit increase in standardized BBI (p=0.03) (Table 2).

BBI as Predictor of Longitudinal Change in BMD

After adjusting for age, time from FMP, race/ethnicity, BMI, and study site, higher (more favorable) BBI was significantly associated with slower decline in LS BMD (p=0.03), but was not associated with rate of decline in FN BMD (p=0.5). Each unit decrement in standardized BBI was associated with a 0.27% faster annual decline in LS BMD change (p=0.03). In contrast to BBI, U-NTX was not significantly associated with rate of decline in either LS (p=0.1) or FN (p=0.5) BMD (Table 3).

BBI as Predictor of Faster-Than-Average Bone Loss

Median rate of decline in BMD from ~1 year before the FMP to 3-4 years after was 1.8 and 1.2% per year in the LS and FN, respectively. After adjusting for age, time from FMP, race/ethnicity, BMI, and study site, higher (more favorable) BBI was associated with lower odds of faster-than-median BMD decline at the LS (p=0.008), but not at the FN (p=0.9). Each unit increment in BBI was associated with 38% lower odds of LS BMD loss at a faster-than-median rate (p=0.008). The ability of BBI (in combination with the covariates listed) to identify the faster losers of LS BMD, as measured by the area under the receiver operating characteristic (ROC) curve, was 0.76 (Table 4). Recognizing that time from FMP cannot be readily discerned in real-time by pre- and early perimenopausal women before the FMP, and that study site is not relevant to clinical practice, we also tested the ability of BBI (in combination with age, race/ethnicity, and BMI) to

identify faster-than-average LS BMD loss. The area under the ROC curve for this model was 0.74. To test if BBI (measurable) is a good surrogate for years from FMP (not measurable), we tested the association between faster-than-average LS BMD loss with years from FMP age, adjusting for age, race/ethnicity, and BMI. The area under the ROC curve for this model was 0.72.

Sensitivity Analyses

Associations of the alternate BBIs (which included adjustment for time of urine collection or race/ethnicity) with 1) factors known to influence bone balance, 2) BMD decline rates, and 3) faster-than average bone loss were essentially the same (see Appendix).

DISCUSSION

This proof of concept study was designed to show that the balance between bone resorption and formation in an individual can be quantified by referencing currently measured BTMs against an estimate of the in-balance relationship between resorption and formation markers. Previous attempts to quantify net bone balance used the difference between Z-scores of bone formation and resorption markers. These were all developed and tested in study samples of fewer than 100 participants, and were based on the assumption that in a state of bone balance, each SD increment in bone resorption markers corresponds to a SD increment in bone formation markers (6,12,13,17-19). More recently, another group mathematically transformed BTM levels to generate graphic plots that provided visual indices of changes in the balance of resorption versus formation and in the rate of bone turnover of individual patients in relation to reference individuals (20). In contrast, our approach was to estimate the in-balance relationship between resorption and formation markers from a sample of healthy women in stable bone balance, and

combine this in-balance relationship with the BTMs from individuals potentially not in bone balance, to create an index of the excess of formation over resorption.

As a first step in construct validation of the new BBI, we confirmed that BBI did indeed increase (become more positive, indicating greater net formation relative to resorption) as BMI increased, consistent with our understanding that increased mechanical loading secondary to higher body weight stimulates bone formation (21,22). As hypothesized, BBI decreased (became more negative) as women approached the FMP concordant with increasing bone resorption relative to formation as the MT progresses (4,23-25).

For further construct validation of the BBI, we examined the ability of BBI to predict longitudinal change in BMD during the rapid phase of MT-related bone loss (14). As hypothesized, greater (more positive) BBI was associated with slower decline in BMD at the LS, but no association was evident with BMD decline at the FN. This is consistent with prior reports that bone resorption related to aging and menopause occurs primarily on trabecular surfaces, of which there are more in the LS than FN (26-28). It has also been reported previously that early in the MT, bone loss is greater at the spine than at the femoral neck (14). It should be noted that bone resorption marker U-NTX by itself did not predict bone loss at either the LS or FN. Finally, BBI was robust at identifying faster-than-average LS BMD loss, with an area under the ROC of 0.76 (29,30).

Our study has limitations that should be noted. First, we created the BBI using U-NTX and OC, BTMs that were measured in SWAN, when it was initiated in 1996. The International

Osteoporosis Foundation and the International Federation of Clinical Chemistry and Laboratory Medicine now recommend serum C-terminal cross-linking telopeptide of type I collagen (S-CTX) and serum procollagen type I N-terminal propeptide (S-PINP) as the bone resorption and formation markers of choice, respectively (31). Urinary measures of NTX are more variable than serum measures, and OC has been noted to be especially susceptible to sample deterioration (32). These sources of variability, however, would have biased our findings toward null. Future studies should recreate and test the BBI using referent BTMs, which may improve the ability of BBI to identify fast losers of bone. Second, we were unable to test the ability of BBI to predict fracture risk because of the small size of the Test Sample, despite the nearly 20 years of follow up in SWAN. Further studies are needed to assess the ability of BBI to predict fracture risk.

Despite these limitations, this proof-of-concept study demonstrated that a potentially useful index of net bone balance can be created by referencing currently measured BTMs against an estimate of the in-balance relationship between resorption and formation markers. An index, such as this, that reliably predicts future change in BMD could be used to identify individuals at the greatest risk for large declines in bone strength, making it possible to test the efficacy of interventions to prevent bone loss and maintain bone strength.

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Table 1Descriptive statistics^a for analytic samples; Study of Women's Health Across the Nation

	In-Balance Sample ^b N=683	Test Sample ^c N=216
Age (years)	44.6 (2.1)	48.0 (2.6)
Time before or after final menstrual period (years) ^d	-8.1 (2.1)	-0.9 (0.8)
Race/ethnicity		
Caucasian	316 (46.3)	80 (37.0)
African American	195 (28.6)	79 (36.6)
Chinese	78 (11.4)	28 (13.0)
Japanese	94 (13.8)	29 (13.4)
Body mass index (kg/m ²)	27.8 (7.2)	28.5 (8.1)
Physical activity score	7.8 (1.8)	7.7 (1.9)
Bone turnover markers		
N-telopeptide, urine (nM BCE/mM Cr)	32.6 (13.4)	38.1 (15.7)
Osteocalcin (ng/mL)	15.1 (5.1)	18.1 (6.6)
Bone balance index	0.0 (1.0)	-0.2 (1.1)
Annualized percent change in bone mineral density ^e		
Femoral neck (%/year)	N/A	-1.2 (1.8)
Lumbar spine (%/year)	N/A	-1.7 (1.8)

- a Count (percentage) for categorical variables; mean (standard deviation) for continuous variables. All variables (other than rate of change) were measured at baseline visit.
- b Sample of women who were ≥ 5 years from their final menstrual period at study baseline, in whom we estimated the in-balance relationship between bone resorption and formation to create the formula for calculating individual-specific bone balance index values
- c Sample of women who were ≤ 2 years from their final menstrual period at baseline, in whom we calculated individual-specific bone balance index values and tested their ability to predict longitudinal change in bone mineral density
- d Negative values indicate time prior to final menstrual period (the zero reference) and positive values indicate time after the final menstrual period
- e Based on measurements of bone mineral density from baseline visit and a follow up visit 3-4 years after the baseline visit

Table 2

Adjusted associations^a between characteristics known to affect bone balance and bone balance index

	β-coefficient (95% Confidence Interval)	p-value
Time from final menstrual period (per year)	-0.29 (-0.50, -0.08)	0.007
Body mass index (per 5 kg/m ²)	0.11 (0.01, 0.22)	0.04
Physical activity score (per unit score)	0.03 (-0.05, 0.11)	0.5

a Adjusted for study site, race/ethnicity, age

Table 3Adjusted associations^a between bone balance index (BBI) and rate of bone loss

	Annualized percent decline in bone mineral density (95% Confidence Interval) per unit decrement in standardized BBI	p value
Lumbar spine	0.27 (0.02, 0.51)	0.03
Femoral neck	-0.10 (-0.38, 0.17)	0.5

a Adjusted for study site, race/race/race/ethnicity, body mass index, age and years from the final menstrual period

Table 4

Adjusted associations^a between bone balance index (BBI) and odds of faster-than-average BMD loss

	OR (95% Confidence Interval)^b	c-statistic	p value
Lumbar spine	0.59 (0.45, 0.89)	0.76	0.004
Femoral neck	1.01 (0.74, 1.38)	0.62	0.9

a Adjusted for study site, race/race/race/ethnicity, body mass index, age and years from the final menstrual period

b Odds of faster-than-average BMD loss per unit increment in standardized BBI

CHAPTER 2. STATISTICAL APPENDIX

BONE BALANCE INDEX CREATION

Supplemental Analyses

The conceptual framework behind the BBI is that one can reference currently measured BTMs against an estimate of the in-balance relationship between resorption and formation markers to quantify an individual's net bone formation relative to resorption. To estimate the in-balance relationship between resorption and formation markers, we used linear regression to determine their average relationship in a population of women considered to be in stable bone balance. Prior to estimating this in-balance relationship, however, we first confirmed that the relationship between resorption and formation markers was indeed linear. To this end, we used the nonparametric Lowess smoothing method to generate a fitted curve through a plot of U-NTX on OC. After visually inspecting this curve for linearity (Figure 1), we proceeded to create the BBI as described in the text of the Chapter 1.

BONE BALANCE INDEX CONSTRUCT VALIDATION

Supplemental Figures

Our first approach to BBI construct validation was to examine its association with recognized determinants of bone balance. These included BMI and years from FMP. Below are scatterplots depicting the relationship of BBI with both BMI (Figure 2) and years from FMP (Figure 3).

Our second approach to BBI construct validation was to examine its ability to predict longitudinal LS and FN BMD loss. Below are scatterplots depicting the relationship of annualized percent change in LS (Figure 4) and FN (Figure 5) BMD loss with BBI.

Supplemental Analyses

Testing the need to account for time of urine collection during BBI creation and construct validation: Because BTMs show diurnal variation, we created an alternate BBI #1 formula in which we adjusted for time of urine collection, and completed construct validation for alternate BBI #1. Using an analogous methodology for BBI creation described in Chapter 1, the formula for alternate BBI #1 that accounted for urine collection time was: $9.2 + 1.1 * OC + 0.9 * (\text{urine collected after 10AM, yes}=1/\text{no}=0) - NTX/12.1$, where OC is measured in ng/mL, U-NTX in nM BCE/mM Cr, and the SD of the raw (unstandardized) BBI was 12.1 nM BCE /mM Cr. We then assessed the correlation between the original and alternate BBI #1, and found that the Spearman rank correlation was 1.0 ($p < 0.0001$) (Figure 6). After creating alternate BBI #1, we assessed its association with determinants of bone balance (Table 5) and tested its ability to predict longitudinal change in LS and FN BMD (Table 6) as well as faster-than-average LS and FN BMD loss (Table 7). All BBI construct validation models using alternate BBI #1 yielded nearly identical results as those using the original BBI.

Testing the need to account race/ethnicity during BBI creation and construct validation: Because the race/ethnicity composition of the In-Balance and Test samples were slightly different, we created an alternate BBI #2 formula in which we adjusted for race/ethnicity, and completed construct validation for alternate BBI #2. Using an analogous methodology for BBI creation

described in Chapter 1, the formula for alternate BBI #2 that accounted for urine collection time was: $20.1 + 0.9 \cdot OC - 0.9 \cdot \text{Ethnicity}$ (0=Caucasian, 1=African American, 2=Chinese, 3=Japanese) – NTX/14.6, where OC is measured in ng/mL, U-NTX in nM BCE/mM Cr, and the SD of the raw (unstandardized) BBI was 14.6 nM BCE /mM Cr. We then assessed the correlation between the original and alternate BBI #2, and found that the Spearman rank correlation was 0.99 ($p < 0.0001$) (Figure 7). After creating alternate BBI #2, we assessed its association with determinants of bone balance (Table 8) and tested its ability to predict longitudinal change in LS and FN BMD (Table 9) as well as faster-than-average LS and FN BMD loss (Table 10). All BBI construct validation models using alternate BBI #2 yielded nearly identical results as those using the original BBI.

Testing the association between BBI and concurrent BMD: In addition to the BBI construct validation approaches reported in Chapter 1, we considered testing the association between BBI and concurrently measured LS and FN BMD. With this approach, we hypothesized that more positive BBI (indicating more favorable bone balance) would be associated with higher concurrent BMD. We thus used multivariable linear regression with concurrent BMD (LS or FN) as the outcome variable; BBI as the primary predictor variable; and study site, race/ethnicity, BMI, age, and years from FMP as covariates. We found that each increment in standardized BBI was associated with 0.016 g/cm^2 ($p=0.07$) and 0.002 g/cm^2 ($p=0.7$) higher BMD at the LS and FN, respectively (Table 11).

In order for an association between BBI and concurrently measured BMD to have biologic meaning, however, we made the assumption that current BBI accurately reflects historical bone

balance (i.e. those that were breaking down bone faster in the past continue to break down bone faster now). To test this assumption, we used the Spearman rank correlation to assess the correlation between U-NTX measured at various time points [pre-/early perimenopause (U-NTX_{PRE/E-PERI}), late perimenopause (U-NTX_{L-PERI}), and early postmenopause (U-NTX_{E-POST})]. The Spearman's rank correlations between the three U-NTX measurements were: 0.43 (p<0.0001) between U-NTX_{PRE/E-PERI} and U-NTX_{L-PERI}, 0.48 (p<0.0001) between U-NTX_{L-PERI} and U-NTX_{E-POST}, and 0.36 (p<0.0001) between U-NTX_{PRE/E-PERI} and U-NTX_{E-POST}. Since the correlation between U-NTX measurements at different time points was only moderately strong, we concluded that testing the association between BBI and concurrently measured BMD not to be biologically meaningful.

Figure 1

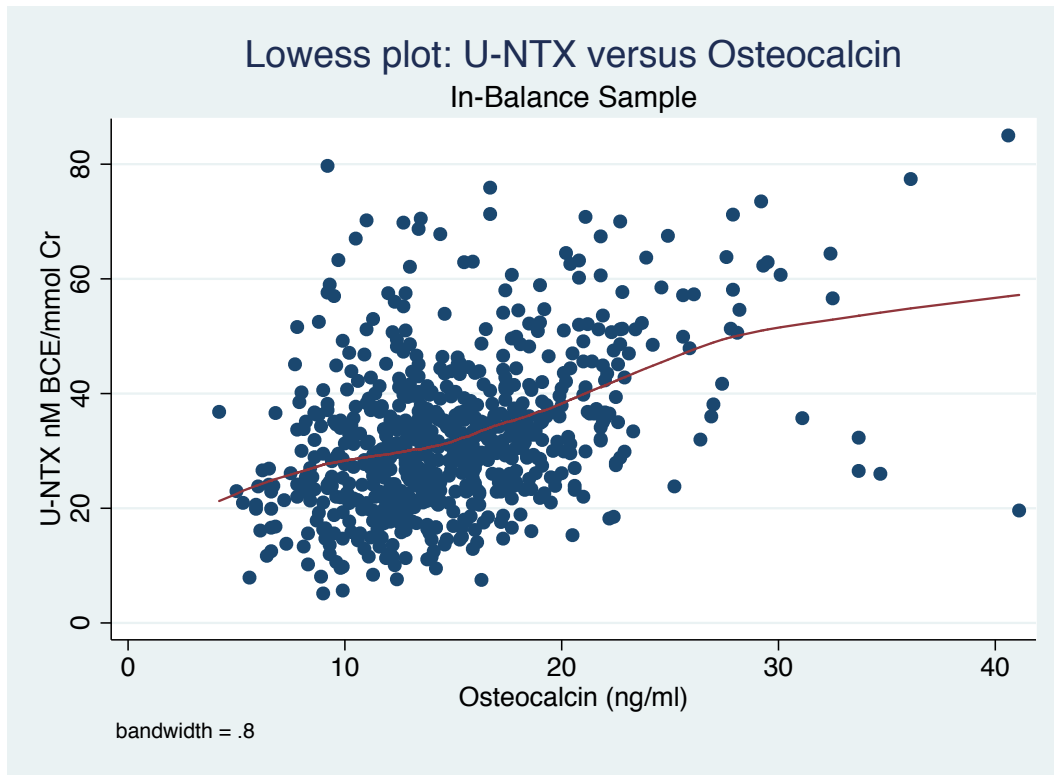


Figure 2

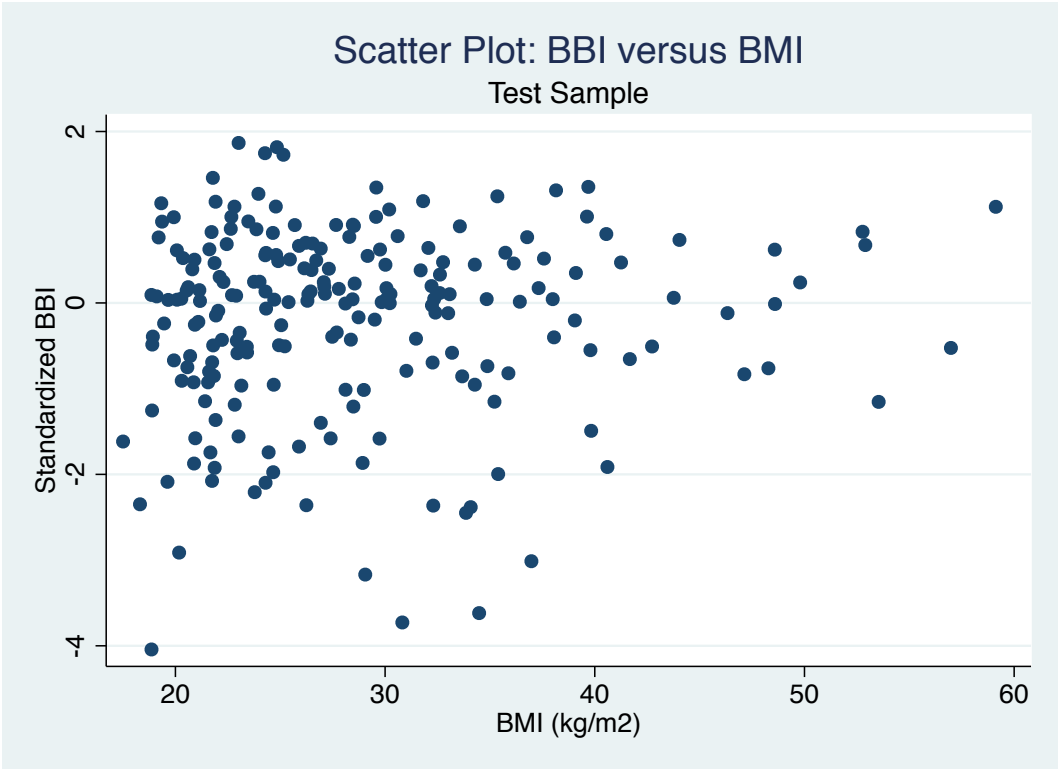


Figure 3

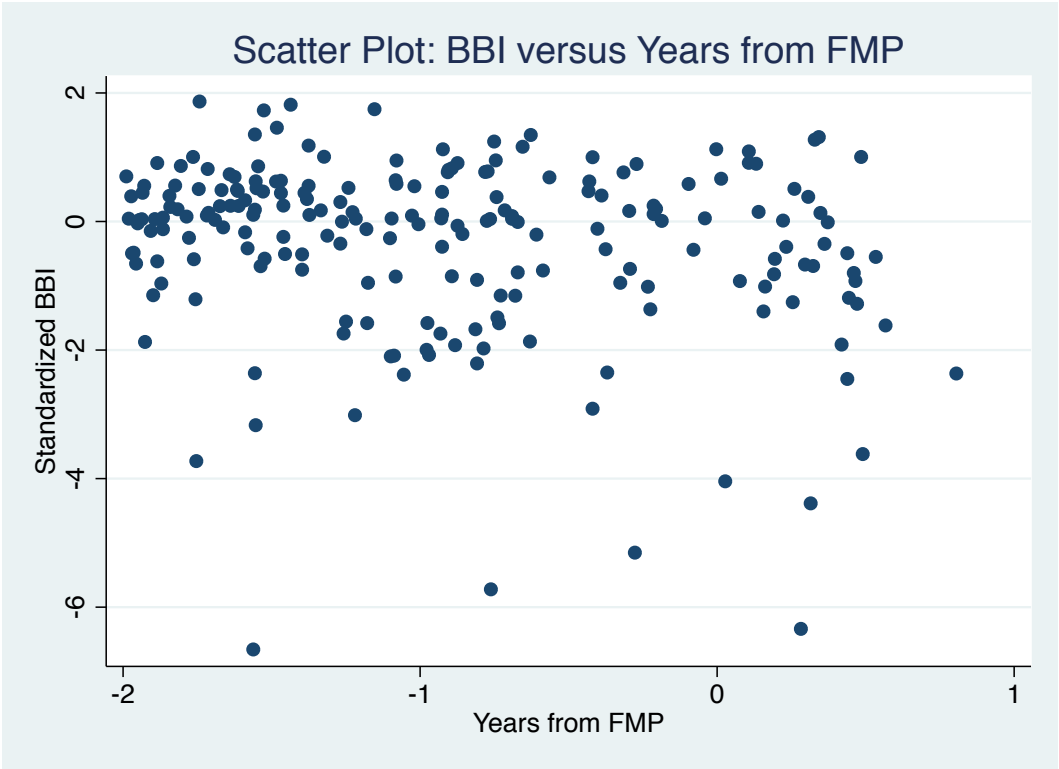


Figure 4

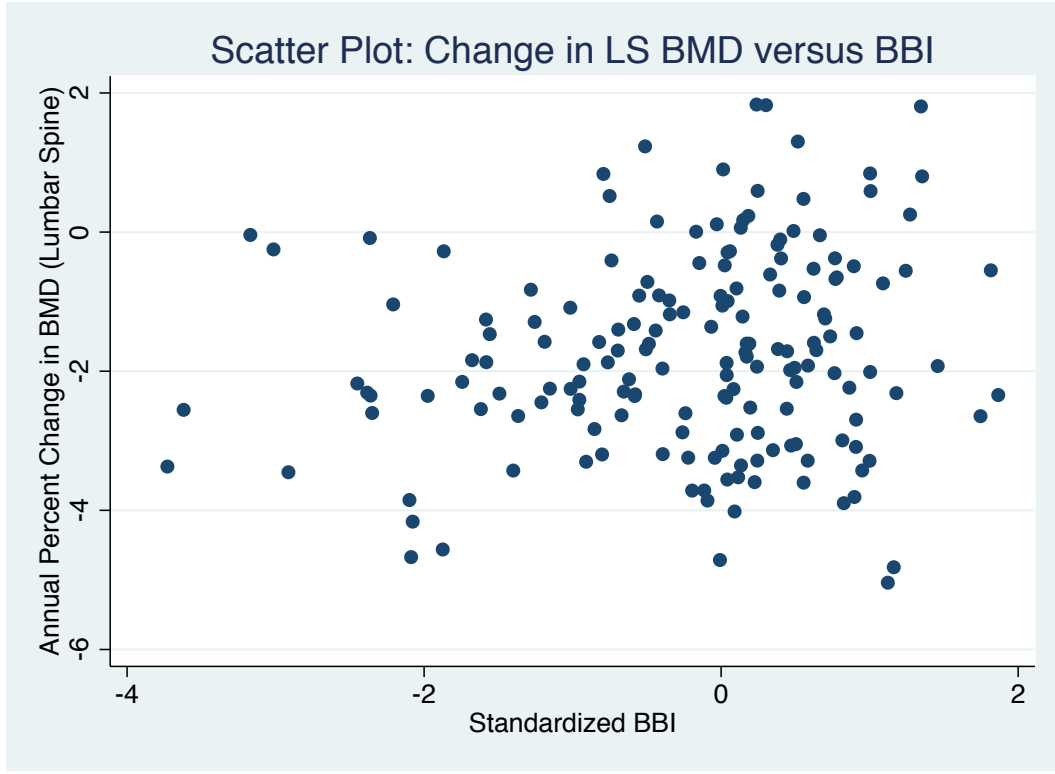


Figure 5

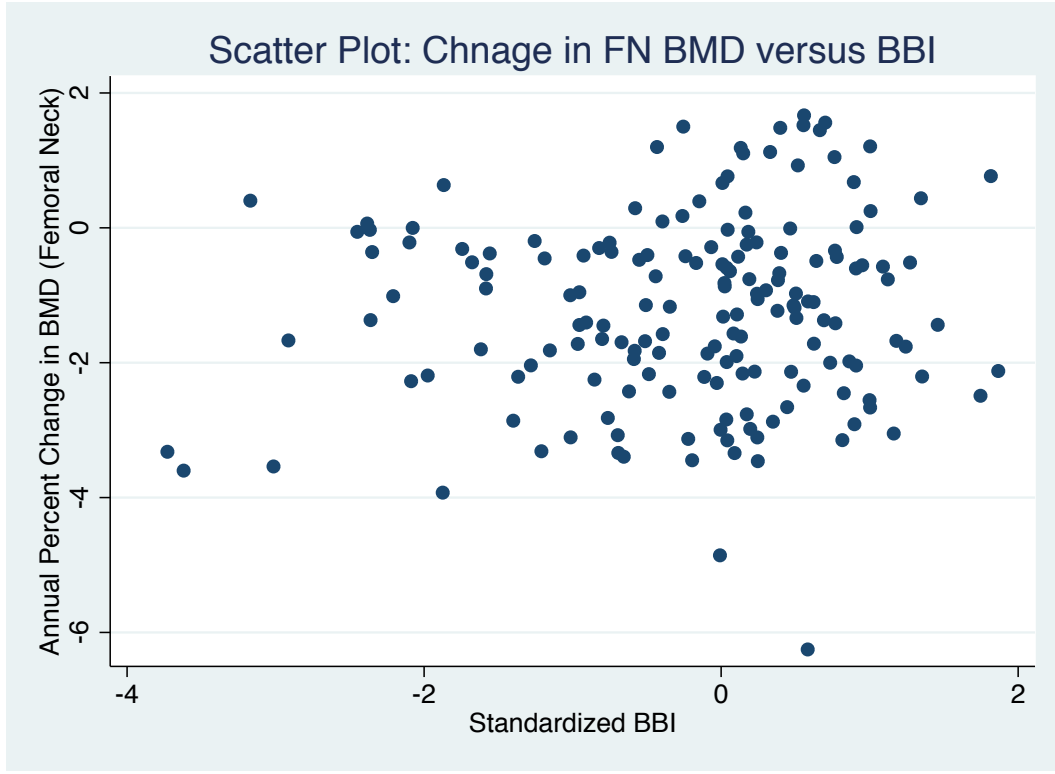


Figure 6

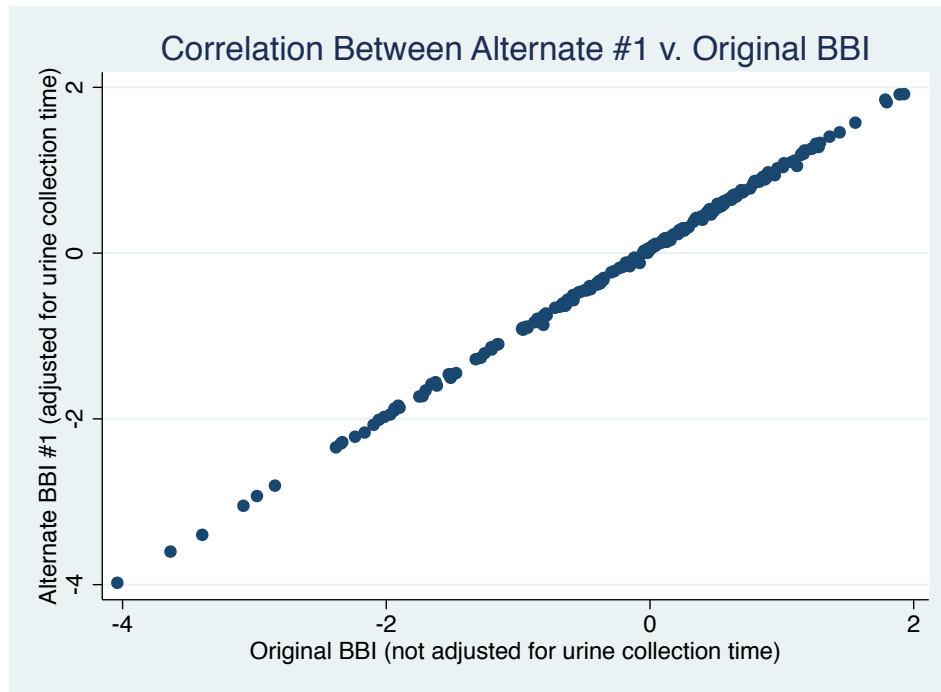


Figure 7

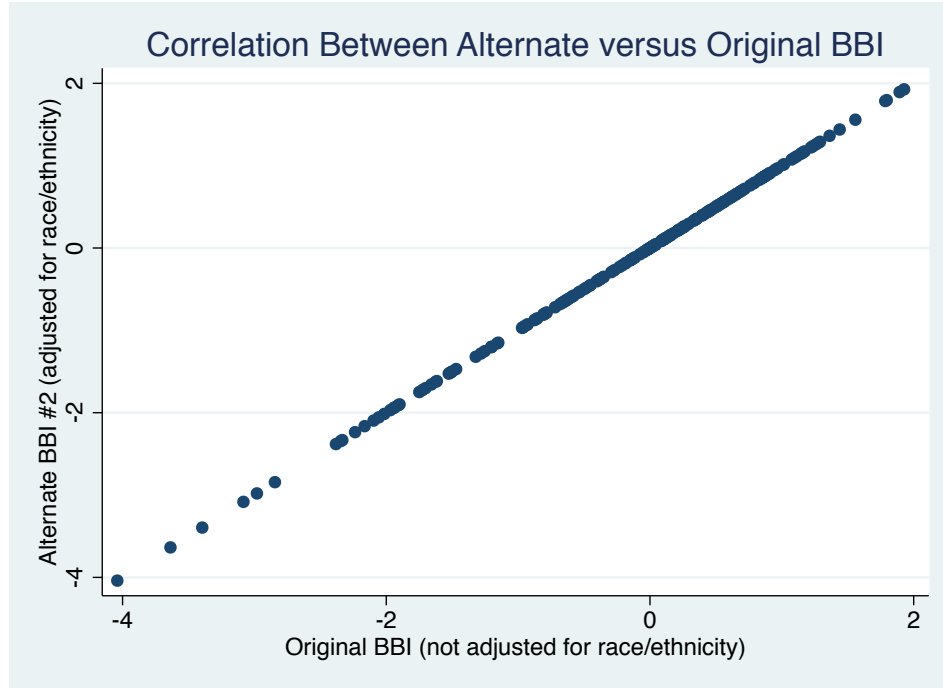


Table 5

Adjusted associations^a between characteristics known to affect bone balance and alternate^b bone balance index #1

	β-coefficient (95% Confidence Interval)	p-value
Time from final menstrual period (per year)	-0.31 (-0.53, -0.09)	0.007
Body mass index (per 5 kg/m ²)	0.12 (0.01, 0.23)	0.03
Physical activity score (per unit score)	0.03 (-0.05, 0.12)	0.4

a Adjusted for study site, race/ethnicity, age

b Adjusted for time of urine collection

Table 6

Adjusted associations^a between alternate^b bone balance index (BBI) #1 and rate of bone loss

	Annualized percent decline in bone mineral density (95% Confidence Interval) per unit decrement in standardized BBI	p value
Lumbar spine	0.25 (0.02, 0.49)	0.03
Femoral neck	-0.10 (-0.36, 0.16)	0.5

a Adjusted for study site, race/race/race/ethnicity, body mass index, age and years from the final menstrual period

b Adjusted for time of urine collection

Table 7

Adjusted associations^a between alternate^b bone balance index (BBI) #1 and odds of faster-than-average BMD loss

	OR (95% Confidence Interval)^c	c-statistic	p value
Lumbar spine	0.64 (0.45, 0.89)	0.76	0.008
Femoral neck	1.01 (0.76, 1.36)	0.62	0.9

a Adjusted for study site, race/race/race/ethnicity, body mass index, age and years from the final menstrual period

b Adjusted for time of urine collection

c Odds of faster-than-average BMD loss per unit increment in alternate standardized BBI

Table 8

Adjusted associations^a between characteristics known to affect bone balance and alternate^b bone balance index #2

	β-coefficient (95% Confidence Interval)	p-value
Time from final menstrual period (per year)	-0.27 (-0.48, -0.07)	0.01
Body mass index (per 5 kg/m ²)	0.10 (0.004, 0.20)	0.04
Physical activity score (per unit score)	0.03 (-0.05, 0.11)	0.4

a Adjusted for study site, race/ethnicity, age

b Adjusted for race/ethnicity

Table 9

Adjusted associations^a between alternate^b bone balance index (BBI) #2 and rate of bone loss

	Annualized percent decline in bone mineral density (95% Confidence Interval) per unit decrement in standardized BBI	p value
Lumbar spine	0.28 (0.02, 0.53)	0.03
Femoral neck	-0.10 (-0.39, 0.17)	0.5

a Adjusted for study site, race/race/race/ethnicity, body mass index, age and years from the final menstrual period

b Adjusted for race/ethnicity

Table 10

Adjusted associations^a between alternate^b bone balance index (BBI) #2 and odds of faster-than-average BMD loss

	OR (95% Confidence Interval)^c	c-statistic	p value
Lumbar spine	0.61 (0.43, 0.88)	0.76	0.008
Femoral neck	0.99 (0.72, 1.37)	0.63	0.9

a Adjusted for study site, race/race/race/ethnicity, body mass index, age and years from the final menstrual period

b Adjusted for race/ethnicity

c Odds of faster-than-average BMD loss per unit increment in alternate standardized BBI

Table 11

Adjusted associations^a between bone balance index (BBI) and concurrently measured bone mineral density (BMD)

	Change in BMD (95% Confidence Interval)^b per unit increment in standardized BBI	p value
Lumbar spine	0.015 (-0.002, 0.031)	0.03
Femoral neck	0.002 (-0.011, 0.015)	0.7

a Adjusted for study site, race/race/race/ethnicity, body mass index, age and years from the final menstrual period

b Reported as g/cm² change

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