Research Article
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Bone turnover markers in old vs early postmenopausal women

Abstract

Background and objectives: Osteoporosis has two distinct varieties described–post-menopausal and senile. We hypothesize that bone turnover markers may help distinguish between these two pathogeneses.

Design and participants: A retrospective review of 976 fasting metabolic bone studies (FMBS) performed in an outpatient clinic identified 55 patients who met inclusion criteria. They were divided into the postmenopausal (age 50-65) and old-old (age 75 and above) groups.

Measurements: We compared bone resorption (urinary N-Telopeptide/Creatinine (NTx/Cr)) and formation (Alkaline Phosphatase (ALP) and Procollagen type 1 N-terminal propeptide (P1NP) in the two groups using independent sample t-tests.

Results: P1NP was significantly lower in the OO group (73.9 vs 41.6, p=0.037). There was no difference in ALP (88.7 vs 78.3, p=0.127) and NTx/Cr (40.0 vs 42.8, p=0.554).

Conclusion: This study suggests that in PM osteoporosis bone formation is preserved with increased resorption. In senile osteoporosis there is reduced formation combined with high resorption suggesting uncoupling. This supports the hypothesis of senile vs postmenopausal osteoporosis being different in pathogenesis. This may be important in choice of treatments. P1NP is a good marker of formation, but ALP is not. Bone ALP may need study. NTx/Cr may be influenced by other physiological and bone factors.

Volume 4 Issue 1 - 2019

Charles Inderjeeth,[1,2] Preeti Nair A,[1] Kien Chan,[1] Warren Raymond,[1] EE Mun Lim[2,3]
[1]Area Rehabilitation and Aged Care, North Metropolitan Health Service, Australia
[2]University of Western Australia, Australia
[3]Area Rehabilitation and Aged Care, North Metropolitan Health Service, Path West Laboratory Perth WA, Australia

Correspondence: Charles A Inderjeeth, Clinical Professor North Metropolitan Health Service and University of Western Australia QE 2 Medical Centre, Hospital Avenue Nedlands WA6009, Tel 0864573333, Fax 086457281 1, Email charles.inrjeeth@health.wa.gov.au

Received: December 18, 2018 | **Published:** January 10, 2019

Introduction

Osteoporosis increases with age affecting 1 in 2 women and 1 in 3 men. Osteoporosis is systemic bone loss that increases fracture risk.[1] An osteoporotic state occurs when bone resorption is greater than bone formation. This imbalance in bone turnover results in reduced bone density, and microarchitectural deterioration of bone tissue, leading to increased fragility and as a consequence increase in fracture risk.[1]

It is increasingly recognised that osteoporosis is not a homogenous disease. Postmenopausal osteoporosis tends to present differently to osteoporosis in the old-old, with more trabecular bone loss and associated vertebral fractures, as compared to cortical bone loss and associated hip fracture in the elderly as described by Riggs et al.[2] This concept is examined in our analysis by comparing the bone turn over markers (BTMs) in these two groups of people.

Wider availability has increased the utility of BTMs as a disease marker and diagnostic tool for the assessment and management of osteoporosis. Commonly used BTMs have established reliability, rapidity, non-invasiveness, patient satisfaction, cost-effectiveness, and improved diagnostic ability (sensitivity and specificity) of the assays.[1]

Bone biopsy is the gold standard, but invasive, in assessing bone quality changes across the age spectrum. It is neither practical nor readily available. Bone Density, whilst useful to assess osteoporosis, does not distinguish between problems with formation or resorption. Hence this study looks at the biochemical markers of bone formation and resorption as potential disease markers of the underlying pathogenesis of these 2 types of osteoporosis.

The skeletal system is metabolically active. Over a person's lifetime the skeleton is continually being repaired and remodeled in response to multifaceted stresses. Osseous tissue is replaced annually at a rate of approximately 10-20%, varying by site and type.[3] Bone remodelling is an active and highly regulated process that includes resorption (osteoclasts) and formation (osteoblasts).[4]

BTMs provide a potential non-invasive window into the balance between formation and resorption. It presents a metabolic snapshot of the skeletal health, supplementary, to the non invasive gold standard BMD diagnostic imaging of bone mass (density), structure, or geometry, for the purposes of determining fracture risk in postmenopausal women and older men[5] and has been extensively investigated. It is recognised as one of the strongest independent risk factor for bone loss and fracture (9). There are a number of limitations with BTM measurements that limit their diagnostic utility and have to be interpreted in the context of the individual patient risk factors. However the main utility may be in those with significant depression or elevation of markers and in individuals across time spans to assess trends as an early warning regarding risk.

Bone formation markers are either by-products of active osteoblasts expressed during the various phases of their development or osteoblastic enzymes. Measured in serum or plasma, they include: bone specific alkaline phosphatase (BSAP), osteocalcin and the carboxy- and amino-terminal propeptides of type 1 collagen (P1CP, P1NP). P1NP has several functional advantages and comes recommended by the Bone Marker Standards Working Group; due to low inter-individual variability,[6] and is relatively stable at room temperature.[7] Whilst total serum alkaline phosphatase levels are included in fasting metabolic bone study panels, in the presence of liver disease the specificity is improved by measuring the bone specific alkaline phosphatase iso-enzyme level.[8]

Serum and plasma have become the preferred means of measuring resorption. C-terminal telopeptide of type 1 collagen CTX is the preferred resorption marker.[9] A major disadvantage of CTX are the

large circadian variations necessitating a morning fasted sample for accurate interpretation.[10] Urine N-terminal telopeptide (NTX) may be the preferred marker in the clinic setting. Urine NTX, unlike plasma CTX, is not as sensitive to circadian changes, is not influenced by food intake, it avoids venepuncture, and might be a patient preference.[11]

The main limitation of BTM measurements are the pre-analytical (biological), analytical and Inter- laboratory variability.[5,12] Notwithstanding this BTM has clinical utility in appropriate clinical settings to assist in risk assessment, diagnosis, monitoring and treatment of osteoporosis and fracture risk especially with significant elevation or reduction to suggest elevated resorption or decreased formation respectively.[13]

Our aim was to compare bone turnover markers between postmenopausal women and the old-old. We hypothesize that the difference in their profiles would reflect the differences in underlying mechanisms of osteoporosis – increased resorption in the postmenopausal group versus decreased formation and possibly resorption (stasis) in the old.

Methodology

Retrospective audit of all patients who required a fasting bone turnover marker studies (fasting metabolic bone studies [FMBS]) performed by a single accredited laboratory (author EML) and requested by a single clinician (author C.I) in the outpatient clinic as part of their osteoporosis risk review over ten years during the period 2002-2012. All included patients' case notes and clinic letters were reviewed for exclusion criteria.

Classification of patient categories:

In the screening process the patients were broadly identified as belonging to one of the four categories listed below.

1. Those who have a definite diagnosis of osteoporosis listed or mentioned, regardless of whether BMD noted or not.
2. Those that have definitely no diagnosis of osteoporosis. The letter specifically mentioned negative assessment for osteoporosis, e.g. "BMD normal", "not osteoporotic", etc.
3. Those that have a probable diagnosis of osteoporosis. Letters and test results available where we could assume, but not conclude, that the patient is not osteoporotic.
4. Those where the status remains unclear or unknown.

Inclusion criteria:

i. Female gender

ii. Group 1: Postmenopausal (PM) if age 50-65 years

iii. Group 2: Age 75 years and above at date of FMBS assessment were classified as old-old women (OO).

iv. FMBS completed by a single onsite laboratory.

Where multiple FMBS were performed on the same patient, we included only the earliest record in the analysis .

Exclusion criteria (strictly applied)

i. Age <50 or age 66-74

ii. Male gender

iii. Prior use of anti-resorptive medication (excluding calcium and Vitamin D)

iv. Patients with current or prior use of long term steroids

v. Any fracture within the preceding 6 months

vi. Bone disorder that may affect bone turnover markers including Paget's disease, ankylosing spondylitis, radiation induced osteoporosis, long term paraplegia, bone malignancy, bone metastases, primary hyperparathyroidism

vii. FMBS performed by a different laboratory

viii. Where insufficient information was available to verify criteria

Data methodology

Statistical analysis was performed using the IBM SPSS Statistics 22 package. To present the baseline characteristics normally distributed anthropometric and biochemical variables were assessed with independent t-test between the postmenopausal and old-old groups. Those variables that violated normality assumptions were assessed with the Mann-Whitney U-test (e.g. weight). A univariate analysis of the mean bone turnover markers between postmenopausal and old-old groups was conducted and adjusted for age, BMI or weight and urine creatinine levels. A multiple linear regression was utilised to determine the associations between bone turnover markers and selected predictor variables. Statistical significance was set at the 5% level, α=0.05.

Results

976 FMBS were performed by a single laboratory and requested by a single clinician between January 2002 and March 2012. Of these, 55 met the strict inclusion criteria after review of the available notes and letters and were included in the final analyses. A total of 26 were included in the post-menopausal group and 27 were included in the old-old group.

Table 1 demonstrates the clinical categories and Table 2 the patient characteristics of the study. The post- menopausal group had a mean age of 59.12±4.36 years, and the old-old group had a mean age of 80.27±3.48 years. The mean difference in age groups was confirmed with an independent t-test, $p<0.001$. The post- menopausal group was taller with a mean height of 160.26±5.99cm compared to the old-old group with a mean height of 153.29±6.67, which was statistically significant, $p=0.05$. The older group was lighter numerically but not statistically different, $p=0.063$. The post-menopausal group had a mean weight of 76.09±19.40kg compared to old-old group with a mean weight of 63.97±17.48. Furthermore, corrected for height, Body Mass Index (BMI) was similar across both groups. The post-menopausal group had a BMI of 27.42±10.29 compared to the old-old group having a BMI of 27.18±8.06. The mean difference in BMI was not statistically significant, $p=0.864$.

Table 3 demonstrates the fasting basic serum and urine biochemistry and formation and resorption markers conducted on the study sample. The following basic fasting serum biochemical markers had similar distributions across postmenopausal women and old-old women: corrected serum calcium–ionized ($p=0.48$), corrected plasma calcium–adjusted ($p=0.19$), plasma phosphate ($p=0.19$), plasma creatinine ($p=0.41$), parathyroid hormone ($p=0.38$), and vitamin D ($p=0.20$). Plasma albumin was significantly different between

the groups (PM 44.35±2.64 vs OO 42.63±2.27g/L) at the 5% level of significance, p=0.01. The following fasting urine biochemical markers had similar distributions across postmenopausal women and old-old women: calcium (p=0.47), creatinine (p=0.95), phosphate (p=0.78), N-telopeptide (p=0.71), urine calcium/creatinine ratio (p=0.10), and, urine calcium excretion (p=0.08), renal phosphate threshold (p=0.10). Bone formation markers were assessed. The mean distribution of plasma alkaline phosphatase (ALP) was similar between postmenopausal and old-old women (p=0.11). The mean distribution of P1NP was different between post-menopausal (89.38±29.60ug/L) and old-old women (78.35±18.48ug/L). This was statistically significant at the 5% level, p=0.02. The only bone resorption marker measured was the N-telopeptide/creatinine ratio. There was no significant difference between the 2 groups, p=0.84.

Table 1 Osteoporosis status at baseline assessment

Label	Post- menopausal (n=26)	Old-old (n=27)
Osteoporosis	9	16
Probable diagnosis of OP	3	1
Not osteoporotic	7	3
Unknown status of OP	7	7

Table 2 Patient characteristics

Values	Postmenopausal l women (n=26) 50 – 65 years	Old-Old (n=27) 75+ years	Difference in means
	Mean±SD	Mean ± SD	P-value
Age (years)**	59.12±4.36	80.27±3.48	<0.001*
Height (cm)**	160.26 ±5.99	153.29 ±6.67	0.050*
Weight (kg)***	76.09±19.40	63.97±17.48	0.063
BMI (kg/m2)*	27.42±10.29	27.18±8.06	0.864

*p<0.05
**Normally distributed variable compared with independent t-test
***Mann-Whitney U Test for non-normal variables.

Table 4 compares the mean levels of BTMs across the post-menopausal and old-old women groups after adjusting for BMI or weight, and serum creatinine. The univariate regression model adjusting the mean bone formation marker units for BMI determined that P1NP was significantly higher in the postmenopausal group compared to the old-old group. The postmenopausal group had a larger mean P1NP marker by 20.54ug/L (95%CI 4.88, 36.21), than the old-old group. There was no statistically significant difference in either the total ALP marker or the NTx/Cr marker after adjustment for BMI and serum creatinine, respectively.

Table 3 Fasting metabolic bone study

Values (reference range)	Postmenopausal women (n=26)	Old Old (n=27)	Significance
	Mean ± SD	Mean±SD	p-value
Fasting Serum Biochemistry			
P1NP ug/L (<90ug/L)	60.62±18.20	41.61±12.09	0.02*
Plasma Alkaline Phosphatase U/L (35-135)	89.38±29.60	78.35±18.48	0.11
Plasma Albumin g/L (35-50)	44.35±2.64	42.63±2.27	0.01*
Plasma Creatinine umol/L (45-90)	69.23±10.21	72.04±14.18	0.41
Vitamin D nmol/L (>50)	67.65±23.42	60.19±32.05	
Parathyroid Hormone pmol/L (0.70-7.00)	6.71±2.51	8.24±5.42	0.38**
Serum Calcium - Ionised (adjusted) mmol/L (1.12-1.32)	1.23±0.04	1.23±0.04	0.48
Plasma Calcium Adjusted mmol/L (2.15-2.55)	2.29±0.07	2.32±0.09	0.19**
Plasma Phosphate (P04) mmol/L (0.80-1.50)	1.19±0.16	1.13±0.15	0.19
Fasting Urine Biochemistry			
N-Telopeptide/Creatinine nmol BCE/mmol (<50)	41.71±17.91	42.77±15.53	0.84
Urine Calcium/Creatinine mol/mol (0.10-0.58)	0.31±0.22	0.45±0.36	0.1
Urine Calcium Excretion umol/L GF	21.15±15.05	30.35±21.20	0.08
Renal Phosphate (P04) Threshold mmol/L (0.75-1.35)	1.17±0.22	1.06±0.26	0.1

* p<0.05, ** Mann-whitney U-test, the rest were independent t-test Normal ranges presented in brackets

The univariate regression model adjusting the mean bone turnover marker units for weight (Kg) and serum creatinine determined that P1NP was significantly higher in the postmenopausal group compared to the old- old group. The postmenopausal group had a higher mean P1NP marker by 22.79ug/L (95%CI 7.72, 37.86), than the old-old group. There was no statistically significant difference in either the ALP marker or the NTx/Cr markers with the adjustment for weight alone and serum creatinine, respectively.

Table 5 demonstrates the association between bone turnover markers (dependent variables) and selected anthropometric and biochemical data (independent variables). We conducted a multivariate regression analysis. In the first iteration of the model the variables utilised were age, BMI, albumin, serum creatinine, ALP, P1NP, and NTx/Cr. This model demonstrated that ALP (dependent variable), with an adjusted r2 of 0.80, was influenced positively by P1NP (β=0.86), albumin (β=8.98) and the NTx/Cr ratio (β=2.88). P1NP (dependent variable) was not statistically significantly influenced by the adjustment with the anthropometric and biochemical data. The NTx/Cr ratio, with an adjusted r2 of 0.94, was found to be influenced negatively by albumin (β=-3.00), and positively with ALP (β=0.29) levels.

In the second iteration of the model the variables utilised were age, weight, albumin, serum creatinine, ALP, P1NP, and NTx/Cr. This

model demonstrated that ALP (dependent variable), with an adjusted r2 of 0.84, was influenced positively by weight (β=2.41), NTx/Cr (β=3.28), and the P1NP level (β=0.74). P1NP (dependent variable), with an adjusted r2 of 0.79, was not statistically significantly influenced by the independent variables. The NTx/Cr ratio, with an adjusted r2 of 0.96, was found to be influenced negatively by weight (β=- 0.71), and positively with associated with ALP level (β=0.26).

Table 4 Univariate Comparison of Means (adjusted for BMI and Serum Creatinine, and Weight & Urinary Creatinine)

All BTMs in the model were adjusted for BMI. NTx/Cr was also adjusted for serum creatinine			**95% CI**		**Outcome**
	Difference in means (PM vs OO)	**p-value**	**Lower**	**Upper**	
P1NP	20.54	0.016*	4.88	36.21	PM > OO
ALP	14.05	0.078	-1.69	29.79	PM = OO
NTx/Cr	-1.87	0.705	-11.95	8.21	PM = OO
All BTMs in the model were adjusted for weight. NTx/Cr was also adjusted for serum creatinine			**95% CI**		**Outcome**
Model	**Difference in means (PM vs OO)**	**p-value**	**Lower**	**Upper**	
P1NP	22.79	0.008*	7.72	37.86	PM > OO
ALP	13.16	0.131	-4.19	30.5	PM = OO
NTx/Cr	-0.08	0.988	-10.878	11.037	PM = OO

Table 5 Multiple linear regression analysis with ALP, P1NP, and NTx/Cr as dependent variables and select anthropometric and biochemical data entered as predictors

Model	**Adjusted r2**	**F**	**p-value**	**Significant predictors**
ALP	0.8	7.6	0.04	P1NP (β=0.86) Albumin (β=8.98) NTx/Cr (β=2.88)
P1NP	0.68	4.53	0.08	-
NTX/Cr	0.94	25	<0.01	Albumin (β=-3.00) ALP (β=0.29)

Predictors: age, BMI, Albumin, NTx/Cr, serum creatinine, P1NP, & ALP

Model	**Adjusted r2**	**F**	**p-value**	**Significant predictors**
ALP	0.84	9.72	0.02	Weight (β=2.41) NTx/Cr (β=3.28) P1NP (β=0.74)
P1NP	0.67	4.35	0.09	-
NTX/Cr	0.96	39.08	<0.01	Weight (β=-0.71) ALP (β=0.26)

Predictors: age, weight, Albumin, NTx/Cr, serum creatinine, P1NP, & ALP.

Discussion

This study is based on the postulate by Riggs et al in 1982 that postmenopausal osteoporosis (younger) is characterized by excessive and disproportionate trabecular bone loss and is associated mainly with vertebral fractures compared with "senile osteoporosis" (older) which is characterized by proportionate loss of both cortical and trabecular bone. This study compares a younger postmenopausal group with an older group referred to a tertiary clinic for osteoporosis review. It looked at differences in bone turnover markers as a possible window to the difference in the pathogenesis of younger predominantly trabecular bone loss and vertebral fractures to older people with a higher cortical bone loss and predominant hip fracture risk.

To increase the reliability of the findings, this cohort was calcium and vitamin D replete and excluded patients with other disease that may have affected the findings. However, the main factor that may have influenced the findings was that the older individuals were significantly shorter with a trend to lower weight. However BMI was similar to the younger group suggesting no disproportionate loss of height or weight through disease or degeneration. This might represent a cohort effect, and introduce a bias to the study's findings and generalisability.

Currently, P1NP is one of the best available markers of bone formation. It was significantly lower in the older cohort, consistent with the hypothesis of reduced bone formation in older cohorts. ALP, the alternative but less reliable formation marker was similar in the 2 groups. This study measured total rather than bone ALP, which may be a short coming. The former is significantly produced in the liver and is elevated in diseased live states. The latter is more specific for bone and warrants separate study. Neither formation markers were changed after adjusting for other parameters including height which was significantly different, weight which showed a trend or BMI which was similar.

We found no significant difference in bone resorption markers between the two groups. This was unexpected, given our hypothesis that the predominant pathophysiology in PM osteoporosis is one of predominant resorption mediated via osteoclastic activity at these sites when compared to senile osteoporosis, where both formation and resorption were expected to be reduced resulting in stasis of born turn over. The similar and relatively high normal resorption marker (NTX/Cr) in both groups was unexpected especially with the difference between PINP in the 2 groups and based on the expectation of coupling between osteoblastic and osteoclastic activity. This finding was not affected by adjustment for BMI and weight (muscle bulk) or

creatinine (muscle bulk and renal function) which may help explain this difference. It may suggest either loss of coupling in older cohorts or that although bone activity at each site is reduced for formation and resorption (stasis), the increase number and size of remodeling sites and resorption pits in older cohorts may have increased this marker of resorption. It may also imply that trabecular bone loss, which is the main source of resorption, may continue at similar rates in older cohorts.

This net imbalance between formation and resorption has significant implications for the ageing skeleton. The balance between bone resorption and bone deposition is determined by the activities of two principle cell types, osteoclasts and osteoblasts, which are from two different origins. Osteoblasts produce a matrix of osteoid, which is composed mainly of Type I collagen. Osteoblasts are also responsible for mineralization of this matrix. The coupling and uncoupling between the osteoclasts and osteoblasts is central to the concept of bone turnover. Bone turnover is the process of resorption followed by replacement of bone with little change in shape. Osteoblasts and osteoclasts, coupled together via paracrine cell signaling are referred to as bone remodeling unit. Osteoclasts have highly active ion channels in the cell membrane that pump protons into the extracellular space, thus lowering the pH in their own microenvironment. This drop in pH dissolves the bone mineral. Osteoblasts, through an as yet poorly characterized mechanism, lay down new bone mineral. The balance between the activities of these two cell types governs whether bone is accrued, maintained, or lost. The activities of these cells are also intimately intertwined. In a typical bone remodeling cycle, osteoclasts are activated first, leading to bone resorption. Then, after a brief reversal phase, during which the resorption pit is occupied by osteoblast precursors, bone formation begins as progressive waves of osteoblasts form and lay down fresh bone matrix.

Furthermore, the bone formation phase typically takes much longer than the resorption phase. Hence, any increase in remodeling activity, especially with suppressed formation, tends to result in a greater net loss of bone in older compare to younger people. Ageing appears to be a significant factor as postulated by Riggs et al and supported by these findings.

The main strengths of this study are that it specifically looks at a cohort referred for assessment of their bone health. Secondly it excluded patients with other factors that may have influenced or biased the findings. There was consistency in terms of the clinical assessment (single clinician practice and setting) and all tests were done at a single laboratory which reduces the variability and improves the reliability and comparability of the results in these cohorts.

The main limitation of this study is the relatively small sample size. However what was lost in quantity was improved by the quality of the data.

Conclusion

The findings in this study of lower bone formations markers, but equivalent resorption markers support the hypothesis that in older cohorts the dominant problem is one of reduced bone formation, senile osteoporosis hypothesis. There is uncoupling between formation and resorption. This suggests that the former is more

important in cortical bone and may explain the higher risk of hip and non-vertebral fracture risk in older cohorts. Measuring P1NP may be more important and useful than NTX/Cr in risk assessment in older cohorts. The utility of bone ALP as opposed to total ALP as an alternative to P1NP needs to be explored further. This finding has obvious implications for treatment in older cohorts and the need for anabolic rather than predominant antiresorptive agents to treat this cohort.

Acknowledgments

Wee Yang Pang Anupaum Chauhan

Conflicts of interests

The authors of this manuscript have no competing interests.

References

1. Wheater G1, Elshahaly M, Tuck SP, et al. The clinical utility of bone marker measurements in osteoporosis. *Journal of Translational Medicine*. 2013;11(1):201.
2. Riggs BL, LJ Melton. Evidence for two distinct syndromes of involutional osteoporosis. *Am J Med*.1983;75(6): 899–901.
3. Carey JJ, AA Licata, MF Delaney. Biochemical markers of bone turnover. *Clinical Reviews in Bone and Mineral Metabolism*. 2006;4(3):197–211.
4. Vega D, NM Maalouf, K Sakhaee. The Role of receptor activator of nuclear factor-κB (RANK)/RANK Ligand/Osteoprotegerin: clinical implications. *The Journal of Clinical Endocrinology & Metabolism*. 2007;92(12):4514–4521.
5. Meier,C, MJ Seibel, ME Kraenzlin. Use of bone turnover markers in the real world: are we there yet?. *Journal of Bone and Mineral Research*. 2009;24(3):386–388.
6. Stokes FJ, Petko Ivanov, Lisa M Bailey, et al. The effects of sampling procedures and storage conditions on short-term stability of blood-based biochemical markers of bone metabolism. *Clinical Chemistry*. 2011;57(1):138–140.
7. Blumsohn A, RA Hannon, R Eastell. Apparent instability of osteocalcin in serum as measured with different commercially available immunoassays. *Clin Chem*. 1995;41(2):318–319.
8. Woitge HW, MJ Seibel, R Ziegler. Comparison of total and bone-specific alkaline phosphatase in patients with nonskeletal disorder or metabolic bone diseases. *Clin Chem*. 1996;42(11):1796–804.
9. Vasikaran S, Eastell R, Bruyère O, et al. Markers of bone turnover for the prediction of fracture risk and monitoring of osteoporosis treatment: a need for international reference standards. *Osteoporos Int*, 2011;22(2):391–420.
10. Bjarnason NH, Henriksen EE, Alexandersen P, et al. Mechanism of circadian variation in bone resorption. *Bone*. 2002;30(1):307–313.
11. Baxter I, Rogers A, Eastell R, et al. Evaluation of urinary N-telopeptide of type I collagen measurements in the management of osteoporosis in clinical practice. *Osteoporos Int*, 2013;24(3):941–947.
12. Glover SJ, Gall M, Schoenborn KO, et al. Establishing a reference interval for bone turnover markers in 637 healthy, young, premenopausal women from the united kingdom, france, belgium, and the united states. *Journal of Bone and Mineral Research*. 2009;24(3):389–397.
13. Biver E, Chopin F, Coiffier G, et al. Bone turnover markers for osteoporotic status assessment? A systematic review of their diagnosis value at baseline in osteoporosis. *Joint Bone Spine*. 2012;79(1):20–25.