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Bone turnover markers after the menopause: T-score approach

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ABSTRACT

Bone turnover increases at the menopause and is associated with accelerated bone loss. However, it is not known to what extent there is an imbalance between the processes of bone resorption and bone formation, nor whether it is the rate of bone turnover or the bone balance that is most closely associated with the rate of bone loss.
We studied 657 healthy women ages 20 to 79 from five European cities (the OPUS Study) and divided them into two premenopausal age groups, 20 to 29 ($n = 129$), 30 to 39 years ($n = 183$), and three postmenopausal groups 1 to 10 years ($n = 91$), 11 to 20 years ($n = 131$) and 21+ years since menopause ($n = 123$). We measured collagen type I C-telopeptide (CTX, a marker of bone resorption) and procollagen I N-propeptide (PINP, a marker of bone formation). We used these two markers to calculate the overall bone turnover and the difference between bone formation and resorption (bone balance) using the results from the women ages 30 to 39 years to calculate a standardised score (T-score). We found that the CTX and PINP levels were higher in the women ages 20 to 29 and in the women in the three menopausal groups as compared to women ages 30 to 39 years ($p < 0.001$). For example, the CTX and PINP levels were 80 and 33% higher in women 1 to 10 years since menopause as compared to women ages 30 to 39 years. In this group of postmenopausal women, the bone turnover expressed as a T-score was 0.72 (0.57 to 0.88, 95%CI) and the bone balance was −0.37 (−0.59 to −0.16). There was greater rate of bone loss from the total hip in all the groups of women after the menopause compared to women before the menopause. We conclude that the bone loss after the menopause is associated with both an increase in bone turnover and a negative bone balance and that bone loss was most clearly associated with overall bone turnover.

The menopausal period is associated with an increase in bone turnover and accelerated bone loss, with an average of 11% spine bone loss over the 10 years after menopause (Greendale 2012 [1]).This bone loss is associated with an increase in bone turnover and most biochemical markers of bone formation and bone resorption increase, with the exception of pro-collagen I C-propeptide [2,3].

The IOF/IFCC have recommended CTX and PINP as reference bone turnover markers [4]. It is important to better understand how these markers change across life.

Postmenopausal bone loss is believed to occur as a result of an increase in the rate of bone remodelling and a negative imbalance between bone formation and resorption. Thus, it may be more informative to describe bone turnover markers in terms of whole body bone turnover and bone balance. We describe a new approach for estimating overall bone turnover and balance by comparing bone turnover in older women to that in younger women after log transforming the data, a T-score approach. We use this term as the scores are based on the number of standard deviations from the expected level in healthy young women and so the terminology is borrowed from descriptions of bone mineral density results. The purpose of estimating bone turnover and balance was to better understand the mechanisms underlying bone loss, but not for application to clinical practice as the calculations are too complex.

In postmenopausal women, there is evidence that bone turnover marker levels are positively related to rates of bone loss [5], but we don't know whether this is true for bone balance.

The aims of our study were to: 1) describe the changes in PINP after the menopause; 2) describe the changes in bone turnover and bone balance after the menopause; and 3) to relate bone turnover and bone balance to the rate of bone loss in postmenopausal women.

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1. Methods

For this analysis, we used the results of the Osteoporosis and Ultrasound (OPUS) study, which is a multi-centre population-based study coordinated Medizinische Physik in Kiel [6]. The study was carried out in Sheffield, Aberdeen, Berlin, Kiel and Paris and comprised Caucasian women ages 20–79 years who were recruited from random population samples. Each subject in the OPUS study population was given the European Vertebral Osteoporosis Study (EVOS) risk factor questionnaire to collect their medical history and lifestyle habits. Non-fasting blood samples were taken between 1200 and 1500 h and stored at −80 C [7].

1.1. Measurements

Several analytes were measured from these samples using automated systems in the Bone Biochemistry Laboratory (Sheffield). These included serum CTX, intact PINP and 25-hydroxy-vitamin D which were measured using the IDS-iSYS automated immunoassays (Immunodiagnostic Systems, Boldon, UK). The inter-assay CVs for CTX and intact PINP were 6.5 and 7.2%, respectively.

Additionally, bone mineral density at the lumbar spine (LS) and total hip (TH) was measured using dual-energy X-ray absorptiometry (DXA) at baseline and at 6 years [8]. We used both Hologic and Lunar devices and so used standardised BMD, expressed as mg/cm^2, are recommended for the spine [9] and for the total hip [10]. We calculated the percentage difference per year for each group.

1.2. Study subjects

We studied 657 women ages 20–79 excluding those with diseases known to affect bone metabolism or taking any drugs that have substantial effects on bone turnover markers. The inclusion criteria were the same as we have used before [7] of absence of vitamin D deficiency (25D > 30 nmol/L), absence of renal impairment (eGFR > 30 mL/min/1.73m^2), BMD T-score at both the lumbar spine and total hip > −2.5, premenopausal women were non-pregnant and postmenopausal women were not taking anti-resorptive therapy, including menopausal hormone therapy.

1.3. Statistical analysis

The women were divided into two premenopausal age groups, 20–29 (n = 129), 30–39 years (n = 183), and three postmenopausal groups 1–10 (n = 91), 11–20 (n = 131), 21+ years post-menopause (n = 123). The age band 30–39 years was used as the reference group. We used the Shapiro-Wilk normality test to check if the data for CTX and PINP was normally distributed. The test proved the data to be skewed and so the data were log transformed to base 10. In most cases, the CTX and PINP levels were normally distributed as a result of this transformation. The Wilcoxon signed-rank test was used to compare the relative increases in intact PINP and CTX.

The T-scores for each of the bone turnover markers were used to calculate bone turnover and bone balance. Data for CTX and PINP were skewed so these were $\log_{10}$ transformed. The mean and SD for the reference group were calculated. The T-scores for all groups were then calculated from the mean and standard deviation for the reference group:

- T-score = ((BTM − mean BTM)/standard deviation).

Bone turnover and bone balance were calculated from the T-scores for bone resorption and bone formation:

- Bone turnover = (T-score bone formation + T-score bone resorption)/2
- Bone balance = (T-score bone formation − T-score bone resorption)

Using ANOVA with Tukey as the post hoc test we compared the mean percentage differences in change in BMD between groups. All statistical analyses were performed using Prism 7 for MacOS X (version 7c, GraphPad Software Inc., CA, USA).

We examined the relationship between change in BMD at the LS and TH and the bone turnover and bone balance T-scores in all postmenopausal women and then in just the women up to 10 years postmenopausal. In the latter group, we dichotomised change in BMD as rapid loss (4% or more loss, [11]) or not, and as high bone turnover (T > 1) or not, and negative or positive bone balance.

2. Results

2.1. Bone turnover markers by group

There were significant differences in both BTMs between the groups ($p < 0.0001$) (Fig. 1). The reference group had the lowest levels of CTX and PINP (Table 1). Compared to them, the women who were 1–10 postmenopausal years had higher PINP and CTX levels (33% and 80%, respectively, $p < 0.0001$). The Wilcoxon test showed a significant difference between these relative increases of CTX and PINP ($p < 0.0001$). There were no significant differences in CTX or PINP levels among the three groups of postmenopausal women.

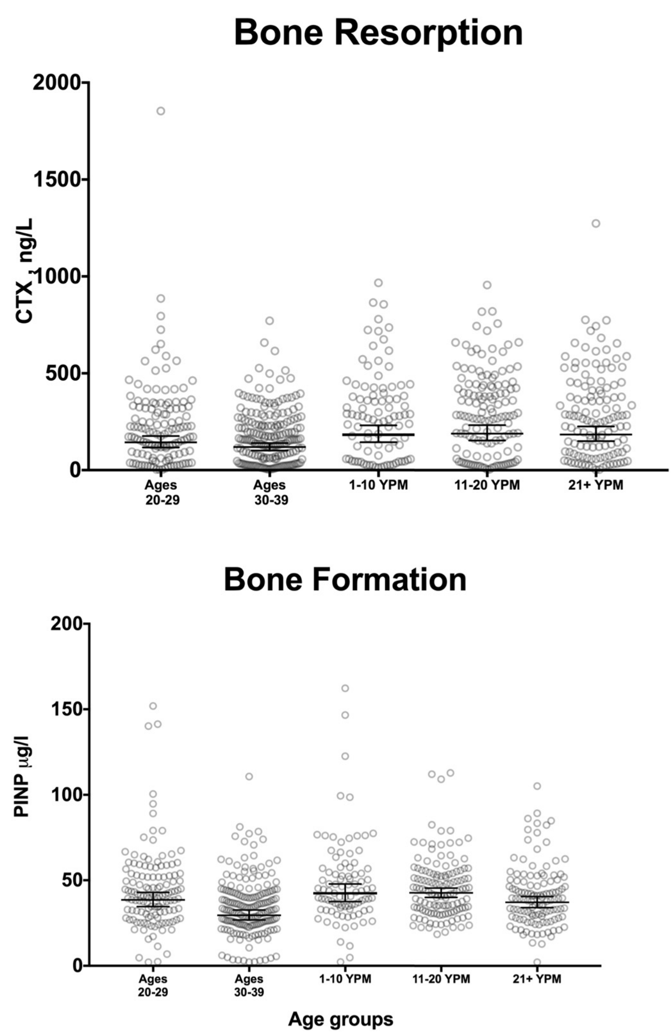

Fig. 1. Serum CTX and PINP levels in healthy women from the different age groups of the OPUS study. The horizontal lines represent the geometric means and their 95% confidence intervals. All groups differed from the reference group, the group ages 30 to 39 years ($p < 0.0001$, analysis of variance; $p < 0.05$ for all pairwise comparisons with the reference group using Dunnett's Multiple Comparisons Test).

Table 1
Baseline characteristics (mean, SD except for bone turnover markers for which geometric mean and 95% CI are given).

Group	Ages 20 to 29 years	Ages 30 to 39 years	1 to 10 YPM	11 to 20 YPM	21+ YPM
n	129	183	91	131	123
Age, years	26.3 (2.6)	36.1 (2.7)	59.8 (3.0)	65.4 (4.4)	72.6 (4.9)
YPM	–	–	7.5 (2.7)	15.9 (2.7)	27.4 (4.9)
BMD, total hip, mg/cm^2	971 (129)	1004 (135)	949 (126)	896 (116)	881 (119)
BMD, lumbar spine, mg/cm^2	1159 (125)	1184 (145)	1087 (156)	1055 (141)	1063 (142)
CTX, ng/L	245 (220 to 272)	185 (169 to 202)	325 (287 to 367)	318 (289 to 350)	300 (268 to 336)
PINP, μg/L	38.6 (34.6 to 42.9)	29.6 (26.8 to 32.6)	42.4 (37.5 to 47.9)	42.7 (40.1 to 45.5)	37.2 (34.1 to 40.6)

2.2. Bone turnover and bone balance by group

In the postmenopausal women who were 1–10, 11 to 20 and 21+ years since menopause, the bone turnover estimates expressed as a T-score were 0.72 (5% CI, 0.57 to 0.88), 0.71 (0.60 to 0.82) and 0.57 (0.44 to 0.71); and the bone balance estimates were − 0.37 (−0.59 to −0.16), −0.32 (−0.45 to −0.19), and − 0.47 (−0.64 to −0.31), respectively (Fig. 2a). The higher level of bone turnover was a consequence of increases in both CTX and PINP, and the more negative bone balance was a consequence of a greater relative increase in CTX than PINP (Fig. 2b).

In the younger women ages 20 to 29, the bone turnover expressed as a T-score was 0.44 (0.30 to 0.58) and the bone balance was −0.06 (−0.25 to 0.12) (Fig. 2a). The higher level of bone turnover was a consequence of higher levels of both CTX and PINP, and the neutral bone balance was a consequence of similarly high levels of CTX and PINP (Fig. 2b).

2.3. Rate of bone loss

At the total hip, there was a small but significant bone loss in the younger women, and this rate more than doubled after the menopause and remained increased long after the menopause (Fig. 3). At the lumbar spine, there was no significant bone loss in the younger women and this rate increased 1–10 years since menopause, to levels similar to the total hip, but at later times after the menopause there was no significant bone loss from the spine.

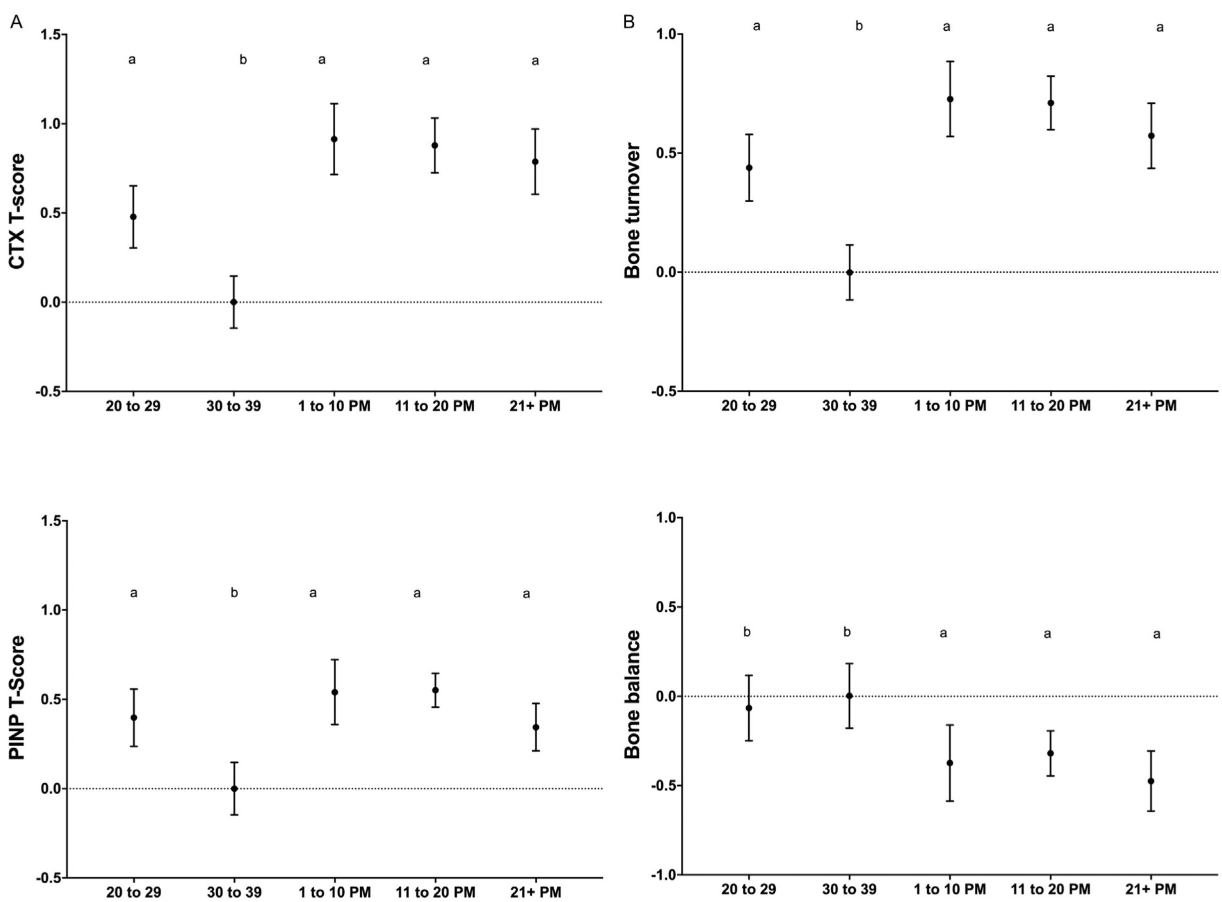

Fig. 2. a. Serum CTX and PINP and b. bone turnover and bone balance all expressed in SD units as T-scores. The point estimate is the geometric mean and the range the 95% confidence interval. For the CTX and PINP T-scores, the effect of group was significant (p < 0.0001, analysis of variance) and the groups all different from the reference group (p < 0.01, Dunnett's Multiple Comparisons Test). For the turnover, the effect of group was significant (p < 0.0001, analysis of variance) and the groups all different from the reference group (p = 0.0001, Dunnett's Multiple Comparisons Test). For the balance, the effect of group was significant (p = 0.0002, analysis of variance) and the postmenopausal (but not the young group) groups all different from the reference group (p < 0.05, Dunnett's Multiple Comparisons Test). Letters indicate significant differences p < 0.05) those groups with the different letter are significantly different.

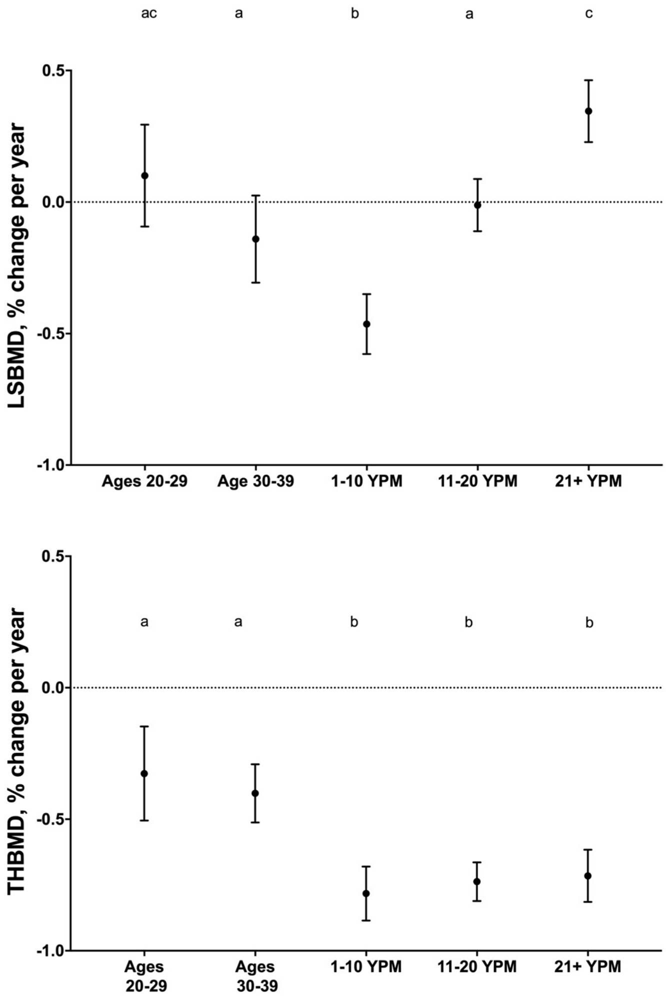

Fig. 3. Annual rate of change in BMD at the lumbar spine and total hip, mean and 95% confidence intervals. For lumbar spine and total hip, the effect of group was significant ($p < 0.0001$, ANOVA) and the multiple comparison test (Tukey) is shown by letters, those groups with the different letter are significantly different ($p < 0.01$).

In the entire group of postmenopausal women, the only significant relationship between bone turnover or bone balance and bone loss was between bone turnover and TH rate of change and this was weak ($r = -0.13$, $p = 0.04$). In women 1–10 years postmenopausal, there was a significant association between bone turnover and change at the LS ($r = -0.43$, $p < 0.0001$) but not with bone balance (there were no associations with bone loss from the TH). Rapid bone loss from the LS was associated with a higher bone turnover (mean T-score 1.08 as compared to 0.57 with slow loss) and the mean rate of change at the spine if bone turnover was high was −5.3% over 6 years as compared to gain of 0.6% if the bone turnover was not high, but no such relationships were found with TH. Negative balance was not associated with change in BMD at the LS or TH.

3. Discussion

We found higher mean levels of CTX and PINP after the menopause by about 80 and 33% (respectively) as compared to women ages 30 to 39 years. An increase of between 32 and 86% was reported by others for CTX [12–15] and between 17 and 53% for PINP [13–15].

Most bone turnover markers are found to be higher after the menopause as compared to before. In prospective studies, the increase in bone resorption begins almost 2 years before and is maximal one year after the final menstrual period [16]. Bone resorption remains elevated for several decades after the final menstrual period [17]. PICP does not increase at the menopause and so is the exception [2,3]. PINP is secreted in equimolar amounts with PICP, so it is surprising that it increases as PICP does not. However, the clearance mechanisms for PINP and PICP differ and PICP is cleared by the mannose receptor in the liver and this is affected by hormone status (PINP is cleared by the scavenger receptor in the liver and this is not affected by hormone status) [18].

It is well recognised that there is bone loss from the proximal femur with ageing into old age. However, it has recently been appreciated from a longitudinal study of 614 young women that hip bone loss begins as early as age 19 year [19]. This would explain why we found some bone loss from the total hip (but not the lumbar spine) in premenopausal women in our study. The rate of this bone loss doubled after the menopause in the present study and this was associated with higher bone turnover and negative bone balance. It is surprising that there was only a weak correlation between rate of bone loss and TH rate of bone loss, but studies that have shown a clear association have been based on women recently menopausal [20]. Another explanation for the weak association is the long follow-up period; the BTM may be associated with bone loss in the short term (2 years) but not the long-term (6 years). The absence of an association of spine BMD rate of bone loss and bone turnover in all postmenopausal women might be due to the degenerative changes that obscure the bone loss with ageing as has long been recognised [21]. This is supported by the strong relationship between bone turnover and bone loss from the LS in women within 10 years of the menopause.

Bone Marker Balance has been estimated previously using several approaches by: i) calculating the *Z*-score for the postmenopausal women relative to an age-matched control group [22,23]), ii) using the multiple of the medians of the BTM to calculate bone turnover and bone balance [24], and iii) calculating the bone balance index of postmenopausal women based on the relationship between urinary NTX and serum osteocalcin in women more then 5 years before final menstrual period [25]. The advantages of our method over these other ones are that we relate to healthy young women (T-score), we allow for the skewed distribution of CTX and PINP (by log transformation), we standardise the results (by using standard deviation units) and we calculate separately bone turnover and bone balance.

These other approaches to bone balance have been studied in relation to menopausal bone loss. Rogers [23] found no greater prediction from the estimate of balance than from bone turnover markers alone. Shieh [25] used osteocalcin and N-telopeptide of type I collagen to develop a bone balance index and found a relationship to menopausal bone loss at the spine, but not the femoral neck.

High bone turnover is not always associated with bone loss. In the present study, we found that women ages 20 to 29 had higher bone turnover than women ages 30 to 39 years. Yet, the younger women did not have bone loss unlike the postmenopausal women. This observation of higher bone turnover markers in young women (and men) has been reported before [26] [13–15,27,28]). It is likely the higher levels relate to the consolidation of cortical bone [29] and to the contributions from the growth plates, for example the medial epiphysis of the clavicle closes in men and women mean age 25 years [30].

There are limitations to this study. All the subjects in the study were Caucasian which means the results may not apply to other ethnic groups. The blood samples were obtained from women in a non-fasting state during the afternoon. Studies have shown that bone resorption markers are lowest during the afternoon and there is a decrease in bone resorption markers after food intake, especially with the marker CTX [31,32].

The study supports the use of exploring a T-score approach to studying osteoporosis. The information about bone turnover across life has been well described but the approach to calculating bone balance proved helpful in this study to adjust for the increase in bone turnover markers in the third decade when there is no bone loss from the spine, and to identify the changes at the menopause that are associated with accelerated bone loss. In the future, the T-score approach might prove useful in predicting fractures and in monitoring the effects of anabolic and anti-resorptive therapy for osteoporosis.

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Disclosure statement

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