Serial Assessment of Serum Bone Metabolism Markers Identifies Women with the Highest Rate of Bone Loss and Osteoporosis Risk

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Context: One of the important challenges in the management of osteoporosis is to identify women who are at high risk of developing osteoporosis and fragility fractures.

Objective: Our objective was to evaluate whether assessment of bone metabolism at multiple occasions can identify women with the highest risk for bone loss.

Design: The Malmö Osteoporosis Prospective Risk Assessment study is an ongoing longitudinal study. Participants have been evaluated at baseline and after 1, 3, and 5 yr.

Setting: We conducted a population-based study.

Participants: Participants included 1044 women, all 75 yr old at baseline.

Main Outcome Measures: Seven bone turnover markers were assessed at baseline and at 1, 3, and 5 yr (n = 573). The 5-yr change in areal bone mineral density (aBMD) was also determined.

Results: Baseline markers correlated weakly to change in total body aBMD. The associations were more pronounced when the average of the baseline and 1-yr measurements was used (standardized regression coefficients −0.12 to −0.23, $P < 0.01$). Adding the 3-yr and 5-yr measurement further strengthened the correlation (regression coefficients up to −0.30, $P < 0.001$). Women with constantly high turnover lost significantly more bone at total body assessment (−2.6%) than women with intermediate (−1.6%) or low turnover (−0.2%, P for trend < 0.001). They also had a greater decrease in hip BMD (−8.3, −6.0, and −5.1%, respectively, $P = 0.010$). Results were similar also in the subgroup of women with osteopenia.

Conclusions: Our results suggest that serial assessment of bone turnover improves the identification of women with the highest rate of bone loss and osteoporosis risk. **(*J Clin Endocrinol Metab* 93: 2622–2632, 2008)**

Osteoporosis is a major public health problem due to high fracture rates, decrease in the quality of life of affected individuals, and high healthcare costs (1). One of the important challenges in the management of osteoporosis is to identify individuals who are at high risk for future bone loss and fragility fractures, to better target pharmacological treatment to those who benefit most. Low bone mineral density (BMD) is a strong risk factor for fractures (2–5). However, only about half of the fractures occur in women who have BMD below the diagnostic threshold for osteoporosis defined by World Health Organization (T-score ≤−2.5 SD). Instead, a large number of fractures occur in women with moderately low BMD, *i.e.* osteopenia (T-

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Abbreviations: aBMD, Areal bone mineral density; BTM, bone turnover marker; CV, coefficient of variation; LSC, least significant change; OPRA, Osteoporosis Prospective Risk Assessment; S-boneALP, serum bone-specific alkaline phosphatase; S-cOC, serum γ-carboxylated osteocalcin; S-CTX-I, serum C-terminal cross-linked telopeptides of type I collagen; S-TotalOC, serum total osteocalcin; S-TRACP5b, serum tartrate-resistant acid phosphatase 5b; U-MidOC, urinary osteocalcin midfragment.

score between −2.5 and −1) (6–8). Although osteopenic women, as individuals, have a more moderate risk than women with osteoporosis, there are far more people in this diagnostic category (9). Treatment strategies are well defined for women with osteoporosis. For women with osteopenia, on the other hand, there is a need for other measures, in addition to BMD, to identify individuals who are at risk of developing osteoporosis and who might need treatment, despite not reaching the diagnostic threshold.

Combining BMD with other risk factors can potentially improve the identification of osteopenic women with high risk of developing osteoporosis. One risk factor is increased rate of bone remodeling, which has been shown to be associated with bone loss and fractures independently of BMD in several studies (10–12). Bone metabolism can be assessed by measuring bone turnover markers (BTMs) in serum or urine (13). Several studies have demonstrated moderate association between baseline levels of BTMs and subsequent change in BMD at various skeletal sites (10, 14–16). However, assessment of turnover over a longer period of time at multiple occasions should provide more precise measures of bone metabolism and take into account the high day-to-day variability in BTM measurements (17). The relationship between consecutive BTM measurements and bone loss has been evaluated in conjunction with pharmacological treatments (18–21). Information on untreated, elderly women is, however, limited to placebo groups in intervention trials and has not been evaluated in osteopenic women.

Our objective was to evaluate whether longitudinal measurements of BTMs at multiple occasions can improve the identification of elderly women with the greatest risk for bone loss. The association was particularly evaluated in the subgroup of osteopenic women in an attempt to identify individuals who are at highest risk of developing osteoporosis and who may need pharmacological intervention.

Subjects and Methods

Subjects

The Malmö Osteoporosis Prospective Risk Assessment (OPRA) study is a population-based cohort of elderly women, all 75 yr of age at inclusion. A total of 1604 women were randomly selected from the population files of the city of Malmö, Sweden, from November 1995 to May 1999, and 1044 women chose to attend (65%) (Fig. 1A). Informed consent was obtained from all participants, and the study was in all parts approved by the local ethics committee and in accordance with the Declaration of Helsinki. Full details of the OPRA population have previously been reported (22–25).

Women were invited for prospective follow-up visits after 1, 3, and 5 yr (Fig. 1B). Serum and urine samples were collected at baseline (n = 1040) and after 1 (n = 968), 3 (n = 849), and 5 yr (n = 711). Women who provided serum and/or urine sample at all four occasions were included (n = 654) (Fig. 1A). Information on smoking and the use of bone-active medication was collected by a questionnaire at all visits. Women taking bisphosphonates (n = 64) or potent estrogen (n = 13) or both (n = 4) during the prospective follow-up period or 2 yr before inclusion were excluded. After exclusions, there were 573 women eligible for the study (Fig. 1A). Sixty-five women (11%) were current smokers, and 72 (13%) had vitamin D supplementation.

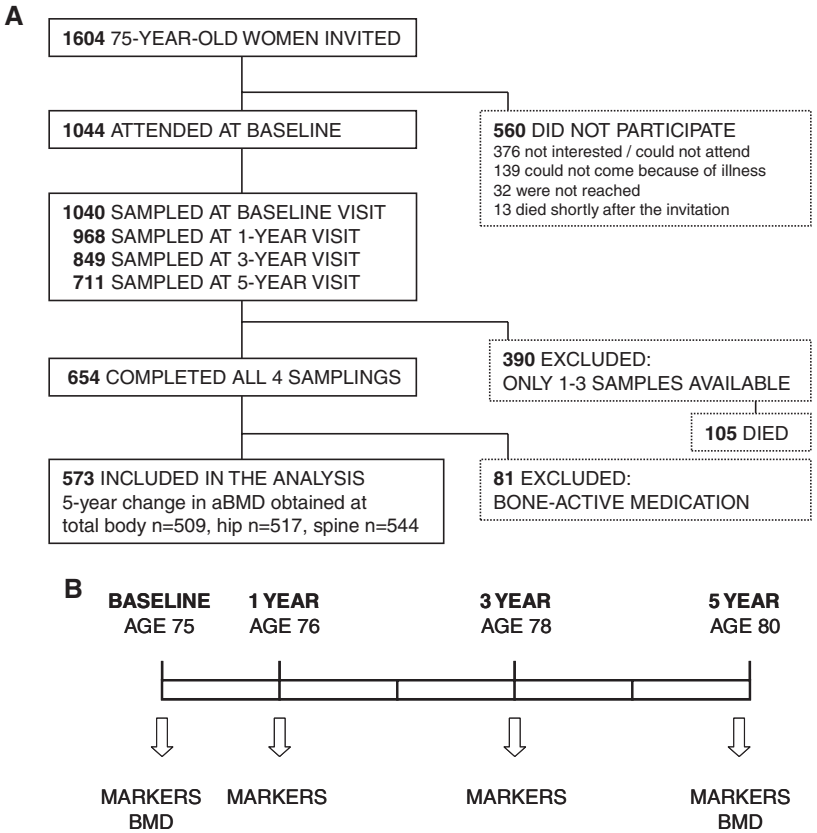

FIG. 1. A, Participating women. Participants of the Malmö OPRA study who attended the entire 5-yr follow-up and who did not take any bone-active medication were included in the analysis (n = 573). B, Overview of study design. Analyses at different time points are marked with *arrows*.

BMD

Areal BMD (aBMD) was measured at baseline and after 5 yr at the total body, total hip, femoral neck, and lumbar spine (L2–L4) by dual-energy x-ray absorptiometry (Lunar DPX-L). All women were measured using the same equipment. The stability of the equipment was checked every morning using a phantom provided by the manufacturer. Scan analysis at baseline was made with software versions 1.33 and 1.35 and at 5 yr with software version 4.7b, with the exception of hip scans, which were all analyzed with software version 4.7b. There was no drift in phantom measurement results during the study period. The precision as assessed by duplicate measurements after repositioning in 15 80-yr-old volunteers of the OPRA study was 0.5% for total body, 3.6% for total hip, 3.9% for femoral neck, and 1.2% for spine (26). The aBMD change over 5 yr was calculated as percent change from baseline aBMD value. We predominantly analyzed changes in total body aBMD because circulating BTMs should reflect total skeletal turnover. For comparison, change in hip aBMD was included in some analysis, because it is more widely used in clinical practice, particularly in elderly women. One-sided least significant change (LSCs) for BMD decrease at $P < 0.05$ (27) was calculated as 2.33 × coefficient of variation (CV) and was −1.2% for total body and −8.4% for total hip.

Both baseline and 5-yr results were available for 509 women at total body, for 517 at hip, and for 544 at lumbar spine. Baseline T-scores of femoral neck aBMD were used to define osteoporosis (T-score ≤ -2.5) and osteopenia ($-2.5 <$ T-score ≤ -1) according to World Health Organization guidelines (28). T-scores were obtained for 546 women at baseline and for 545 after 5 yr. At baseline, 23% had osteoporosis, 52% osteopenia, and 24% normal T-score (> -1). After 5 yr, 40% had osteoporosis, 47% osteopenia, and 13% normal T-score.

BTMs

Serum samples (nonfasting) were collected between 0800 and 1300 h. Urine samples were obtained as the first morning void, between 0230 and 1000 h. All samples were stored at −80 C. Serum tartrate-resistant acid phosphatase 5b (S-TRACP5b) was determined with BoneTRAP assay (SBA Sciences/Immunodiagnostic Systems IDS Inc., Bolton, UK). Serum C-terminal cross-linked telopeptides of type I collagen (S-CTX-I) were determined using Elecsys β-CrossLaps immunoassay (Roche Diagnostics, Indianapolis, IN) and serum bone-specific alkaline phosphatase (S-boneALP) with Metra BAP assay (Quidel Corp., San Diego, CA). Serum intact osteocalcin [S-OC(1–49)], total osteocalcin (S-TotalOC), and γ-carboxylated osteocalcin (S-cOC) were determined with previously described protocols (29). Urinary osteocalcin was determined with a two-site assay for osteocalcin midfragment (U-MidOC) (30). U-MidOC results were normalized for urinary creatinine determined with the alkaline picrate reaction and expressed as ratios. The within-assay (CVa) and between-assay (CVi) variations for the assays are 1.8 and 2.2% for S-TRACP5b; 5.9 and 5.8% for S-CTX-I; 3.6 and 4.4% for S-boneALP; <5 and <8% for S-OC(1–49), S-TotalOC, and S-cOC (29); and 1.7 and <12% for U-MidOC (30), respectively. One-sided LSC at $P < 0.05$ was calculated as $2.33\sqrt{CVa^2 + CVi^2}$ (31) and was 6.6% for S-TRACP5b, 19% for S-CTX-I, 13% for S-boneALP, 22% for S-OCs and 28% for U-MidOC. All analyses were performed blinded and in duplicates. The samples for each time point were analyzed simultaneously to minimize interassay variability.

Other measurements

Serum concentrations of PTH and 25-hydroxyvitamin D were measured at baseline with Elecsys PTH immunoassay (Roche Diagnostics) and Nichols Advantage assay (Nichols Institute Diagnostics, San Juan Capistrano, CA), respectively (25). Physical performance was assessed with gait speed and Romberg one-legged balance test as described (25). Briefly, walking speed was given in meters per second to walk 2 × 15 m, and the Romberg test consisted of four parts, standing on the left or the right leg with eyes open or closed.

Fractures

Fractures during the 5-yr follow-up were identified by hospital reports, as reported previously (24). Ninety-nine women sustained at least one fracture. Information on retrospectively sustained fractures was obtained from hospital records as described (32). Two hundred twenty-seven women (40%) had experienced a fracture before baseline evaluation.

Statistics

All markers were nonnormally distributed (Shapiro-Wilk test $<$ 0.95) and were used after logarithmic transformation. Time points were compared with *t* test for dependent samples (aBMD) or repeated-measures ANOVA (BTMs). Standardized linear regression coefficients (β_{std}) were determined between BTMs and 5-yr change in total body aBMD (percentage of baseline aBMD). BTMs were used as single measurements (baseline) or as average of two (baseline and 1-yr), three (baseline, 1-yr, and 3-yr), or four (baseline, 1-yr, 3-yr, and 5-yr) measurements of the same BTM. Longitudinal changes were assessed using slopes for BTMs over 1 yr (baseline and 1-yr sample), 3 yr (baseline, 1-yr, and 3-yr) or 5 yr (baseline, 1-yr, 3-yr, and 5-yr). Because the time gap between the first and last women enrolled in the study was 3.6 yr, the effect of storage time was evaluated by linear regression between baseline BTMs and days in freezer before the assay.

Longitudinal BTM results were used to identify women with constantly high, intermediate, or low bone turnover. The level of turnover was defined in comparison with other women of the study, *i.e.* a population-based sample of elderly women of the same age. Classification was done separately for each BTM. Briefly, women were classified into high, middle, or low BTM tertile at each time point. Those in the lowest (highest) tertile at three or four samplings were considered to have constantly low (high) turnover over the 5-yr period (see Fig. 3A). All other women were classified into the intermediate group. BTM measurement at all four time points was available in 511–540 women, depending on BTM. A *t* test for dependent samples was used to evaluate total body and total hip aBMD change in each group. Differences between groups were assessed with a *P* value for trend. Comparisons between groups concerning progression to osteoporosis or to LSC of BMD change were performed with χ^2 test. We also assessed turnover only at baseline (tertiles) and performed a classification over the first 1-yr period only, *i.e.* women at the highest (lowest) tertile at both baseline and 1 yr were classified into the high (low) turnover group and all others into the intermediate group.

Statistica for Windows 7.1 (StatSoft Inc., Tulsa, OK) was used for statistical analysis, except for slopes, which were calculated using SPSS for Windows 14.0 (SPSS Inc., Chicago, IL). *P* values < 0.05 were considered statistically significant.

Results

Changes in aBMD and bone turnover

The difference between baseline and 5-yr aBMD was statistically significant at all skeletal sites evaluated ($P < 0.001$, Table 1). There was a decrease in total body (−1.5%, n = 509), total hip (−6.3%, n = 517), and femoral neck (−7.4%, n = 531) and an increase in lumbar spine (+2.0%, n = 544).

There were significant differences between BTMs measured at different time points ($P < 0.001$, Table 1). S-CTX-I and S-boneALP were increasing during follow-up, whereas both increases and decreases were observed for other BTMs. Approximately half of the women (43–57%, depending on BTM) remained in the same BTM tertile at baseline and after 5 yr. Of those at the highest tertile at baseline, 50–64% remained at the highest tertile also 5 yr later.

BTMs assessed at multiple occasions and change in aBMD

Baseline levels of some BTMs were weakly correlated to 5-yr change in total body aBMD. The association was more pronounced and statistically significant for all BTMs (except S-boneALP) when we used the average of two measurements (Fig. 2, A–C and E–G). Adding a third and a fourth BTM measurement further strengthened the correlation. With four measurements, β_{std} ranged from −0.16 (U-MidOC) to −0.29 (S-TRACP5b) and were all significant at $P < 0.001$. S-boneALP was not associated with aBMD change either as a single measurement or an average of multiple measurements (Fig. 2D). The longitudinal changes in BTMs (slopes) did not correlate to aBMD change as strongly as did the average values of multiple measurements of each marker (Fig. 2, A–G). Associations between 5-yr slopes and aBMD change were, however, significant for all BTMs (except S-boneALP) and ranged from −0.11 (S-TRACP5b) to −0.21 (S-cOC).

TABLE 1. Descriptive data for aBMD and bone turnover markers at baseline and 1, 3, and 5 yr in 573 75-yr-old women in the OPRA study

	Baseline	1 yr	3 yr	5 yr	5-yr change
Age (yr)	75.2 (0.1)	76.2 (0.1)	78.2 (0.1)	80.2 (0.1)	
Body mass index (kg/m^2)	26.2 (23.6–28.9)	NA	NA	26.4 (4.1)	
Total body aBMD (g/cm^2)	1.02 (0.09)	NA	NA	1.00 (0.10)	−1.5% (3.2)
Total hip aBMD (g/cm^2)	0.87 (0.14)	NA	NA	0.81 (0.14)	−6.3% (9.0)
Femoral neck aBMD (g/cm^2)	0.78 (0.13)	NA	NA	0.72 (0.12)	−7.4% (10.8)
Lumbar spine aBMD (g/cm^2)	1.00 (0.19)	NA	NA	1.02 (0.20)	+2.0% (7.2)
S-OC(1–49) (μg/liter)	5.1 (3.7–6.3)	5.7 (4.5–7.5)	3.4 (2.0–4.6)	4.0 (2.7–5.9)	
S-TotalOC (μg/liter)	8.3 (6.4–10.4)	7.7 (5.9–10.0)	8.0 (6.2–10.6)	6.4 (4.9–8.9)	
S-cOC (μg/liter)	7.2 (5.6–9.1)	7.5 (5.8–9.7)	7.7 (5.7–10.2)	7.3 (5.6–9.8)	
S-boneALP (U/liter)	22 (18–26)	26 (22–33)	28 (22–36)	28 (23–35)	
S-CTX-I (ng/liter)	268 (190–398)	282 (211–396)	301 (220–410)	323 (229–437)	
S-TRACP5b (U/liter)	3.3 (2.6–4.0)	2.5 (1.8–3.6)	3.5 (2.8–4.5)	4.7 (3.6–6.2)	
U-MidOC/creat (μg/mmol)	1.05 (0.72–1.55)	0.90 (0.56–1.31)	1.58 (1.05–2.28)	1.31 (0.81–1.99	
S-PTH (pM)	4.2 (3.3–5.5)	NA	NA	NA	
S-25(OH)D (ng/ml)	37 (30–45)	NA	NA	NA	

OPRA participants who completed all four serum/urine samplings and did not take potent estrogen and/or bisphosphonates were included in the analysis (Fig. 1A). Values are means (SD) or medians (interquartile range), and the change in aBMD is shown as percent change of baseline value. All changes in aBMD (*t* test for dependent samples) and BTMs (repeated-measures ANOVA) are significant at $P < 0.001$. NA, Data not available.

When the regression coefficients were adjusted for baseline aBMD (total body), β_{std} values were only marginally improved (Fig. 2H). Adjustment to baseline body weight, which could be an independent predictor of bone loss, resulted in associations that were only slightly weaker than the unadjusted associations (Fig. 2H). We also adjusted results for sample storage time. S-CTX-I or S-boneALP levels were not correlated to storage time ($P > 0.05$), and their β_{std} values were not substantially changed after adjustment (Fig. 2H). Levels of S-OCs, U-MidOC, and S-TRACP5b correlated to time in freezer, and a few of their β_{std} values became less significant after adjustment for storage time. Because a recent fracture may increase BTM levels (32, 33), we also evaluated β_{std} after excluding women who sustained a fracture during the follow-up period (n = 99). The association with bone loss was, however, of similar magnitude also after excluding women with incident fractures (Fig. 2H).

Bone loss in women having constantly high, intermediate, or low turnover

The change in aBMD was evaluated in subgroups of women with constantly low, intermediate, or high turnover over 5 yr (Fig. 3A). Decrease in total body aBMD was significantly greater in women who had constantly high turnover when compared with women who had constantly low or intermediate turnover (P for trend < 0.01, except for S-boneALP, Table 2). Five-year bone loss in the high turnover group was −2.3 to 2.7%, whereas in the low turnover group it varied from −0.2 to −1.0%, depending on BTM. The differences were most pronounced when classification was based on S-CTX-I or S-TRACP5b. (Table 2 and Fig. 3B). Results were similar also if high turnover was defined as being in the highest tertile at all four measurements (data not shown). Women with constantly high S-CTX-I had also greater decrease in hip aBMD (−8.3%) when compared with women with intermediate (−6.0%) or low levels (−5.1%) (P for trend = 0.01, Fig. 3D). There was a similar tendency also for other BTMs, particularly U-MidOC, but without reaching statistical significance (Table 2). The number of women who had a significant decrease in aBMD, considering LSC of bone loss at total body, was 66% in the high S-CTX-I group and 25% in the low S-CTX-I group ($P < 0.001$). For bone loss at the hip, the percentages of women were 42 and 30%, respectively ($P = 0.074$).

Baseline levels of S-PTH were significantly higher (5.3 pM) in women who had constantly high turnover (S-CTX-I) when compared with women with intermediate (4.5 pM) or low turnover (4.2 pM, P for trend < 0.001). Results were similar if turnover was measured with other BTMs, but the difference in S-PTH did not reach the level of statistical significance when turnover was defined with S-TRACP5b and U-MidOC. There was no difference between the groups of high, intermediate, or low turnover with regard to vitamin D levels ($P = 0.39$), walking speed ($P = 0.72$), or balance ($P = 0.67$).

Bone loss in osteopenic women with constantly high, intermediate, or low turnover

Change in BMD in women with constantly low, intermediate, or high turnover was also evaluated within the subgroup of osteopenic women (n = 285). In osteopenic women, the P values for trend between low, intermediate, and high groups were significant for all BTMs (except S-boneALP) and less than 0.001 for S-CTX-I and S-TRACP5b (Table 2). The difference was most pronounced for S-CTX-I. The aBMD decreased in the high S-CTX-I group by −3.0% ($P < 0.001$) and in the intermediate group by −1.9% ($P < 0.001$), and there was no decrease in the low S-CTX-I group (−0.2%, $P = 0.65$) (Fig. 3C). Osteopenic women with high S-CTX-I also had greater decrease in hip aBMD (−9.6%) when compared with those with intermediate (−6.0%) or low levels (−6.0%) (P for trend = 0.027, Fig. 3E). The result was similar also for S-TotalOC, S-boneALP, and U-MidOC (Table 2).

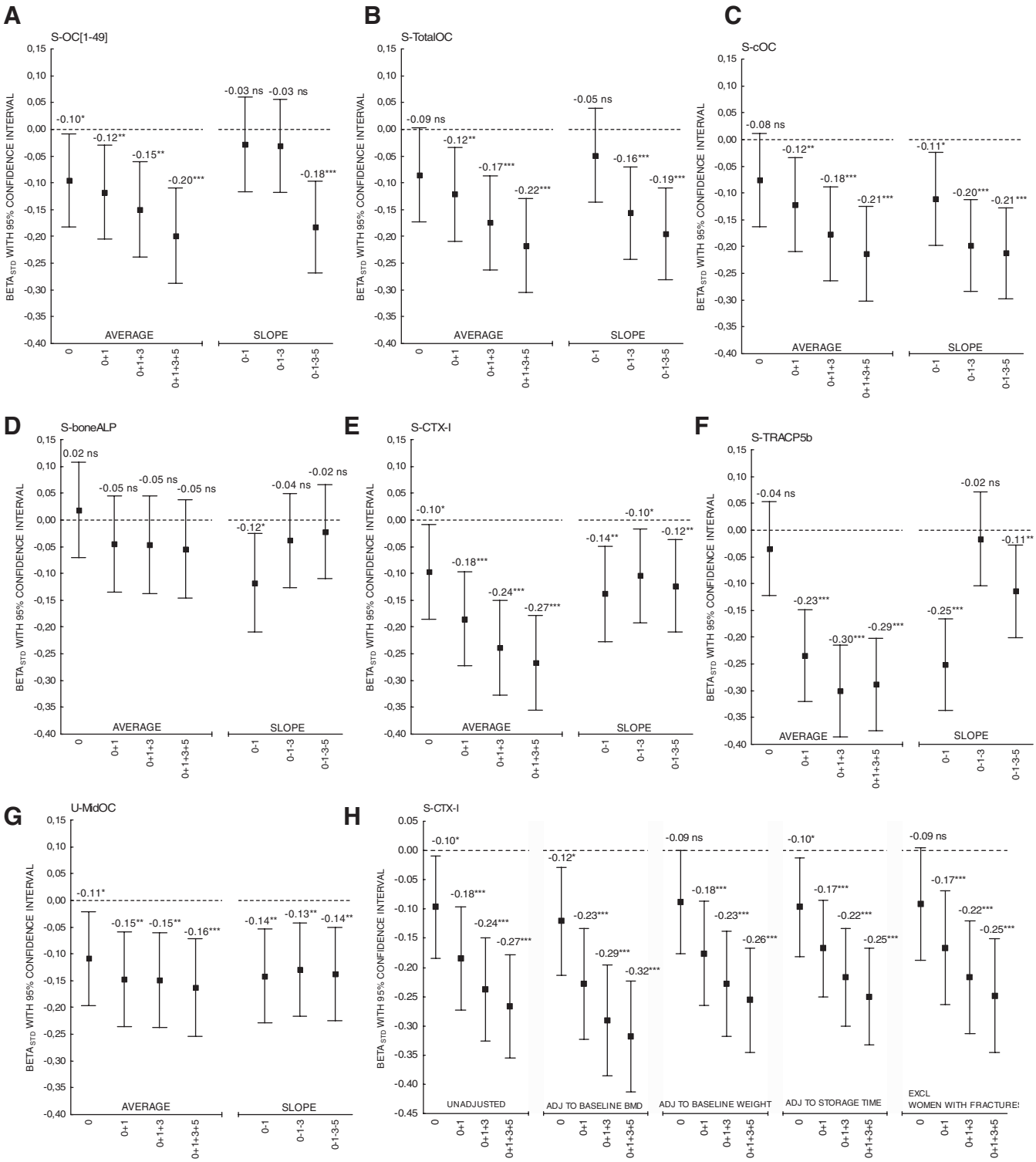

FIG. 2. Standardized linear regression coefficients (β_{std}) between BTMs and 5-yr change in total body aBMD. Results are shown for S-OC(1–49) (A), S-TotalOC (B), S-cOC (C), S-boneALP (D), S-CTX-I (E), S-TRACP5b (F), U-MidOC/creat (G), and for S-CTX-I (H) also after adjustment to baseline total body aBMD, baseline body weight, and storage time and after excluding women with incident fractures. BTMs were used as single measurements (baseline = 0) or average of two (0 + 1), three (0 + 1+3), or four measurements (0 + 1+3 + 5). In addition, slopes over 1 yr (0–1), 3 yr (0–1-3), or 5 yr (0–1-3–5) were analyzed. *Squares* indicate the value for β_{std}, and the *whiskers* represent 95% confidence interval. The *P* values are indicated with *asterisks*: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant.

The average baseline femoral neck T-score for osteopenic women was −1.8. T-score was similar for osteopenic women with constantly high (−1.7), intermediate (−1.8), or low S-CTX-I (−1.6, $P > 0.05$). After 5 yr, the average T-score was decreased to −2.3. Almost half (49.1%) of osteopenic women who had constantly high S-CTX-I had progressed to an osteoporotic level of femoral neck BMD. In intermediate and low S-CTX-I groups, the incident rates of progression were 38.3 and 27.8%, respectively.

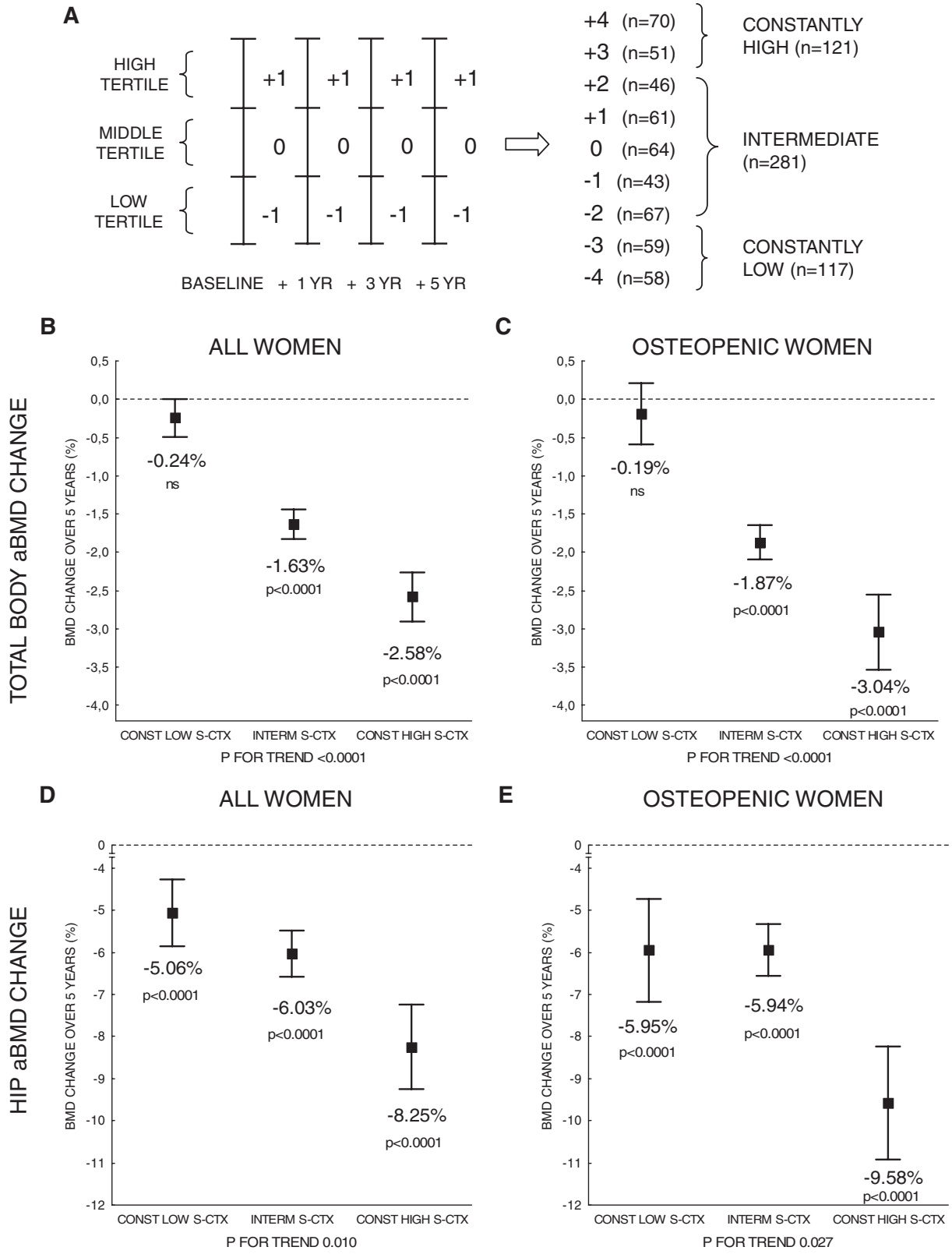

FIG. 3. A, Definition of women with constantly high, intermediate, or constantly low bone turnover. The classification was done separately for each BTM, but the number of women in each category shown is based on S-CTX-I. Measurements at all four time points were available for classification in 511–540 women (of 573), depending on BTM. B and C, Total body aBMD change (percentage of baseline) in all women and osteopenic women with constantly high, intermediate, or low S-CTX-I. D and E, Total hip aBMD change (percentage of baseline) in all women and osteopenic women with constantly high, intermediate, or low S-CTX-I. Results are shown as means ± SE, and the *P* values refer to BMD change from baseline to 5 yr (*t* test for dependent samples). *P* value for trend is given on x-axis.

TABLE 2. The 5-yr aBMD change (total body and total hip) in women with constantly low, intermediate, or high bone turnover over 5 yr (Fig. 3A)

	All women (n = 573)[1]				Osteopenic women (n = 285)[2]			
	Bone turnover over 5 yr (baseline+1 yr+3 yr+5 yr)				Bone turnover over 5 yr (baseline+1 yr+3 yr+5 yr)			
	Constantly low	Intermediate	Constantly high	*P* trend	Constantly low	Intermediate	Constantly high	*P* trend
Total body aBMD change (%)								
S-OC[1–49]	−0.66(0.30)[a]	−1.50(0.19)[c]	−2.29(0.31)[c]	<0.001	−0.96(0.45)[a]	−1.83(0.25)[c]	−2.39(0.42)[c]	0.019
S-TotalOC	−0.67(0.27)[a]	−1.60(0.19)[c]	−2.30(0.30)[c]	<0.001	−0.69(0.41)	−2.02(0.25)[c]	−2.29(0.41)[c]	0.006
S-cOC	−0.61(0.28)[a]	−1.51(0.19)[c]	−2.48(0.30)[c]	<0.001	−0.75(0.43)	−1.87(0.23)[c]	−2.49(0.43)[c]	0.002
S-boneALP	−1.25(0.31)[c]	−1.54(0.19)[c]	−1.94(0.32)[c]	0.115	−1.22(0.39)[b]	−1.84(0.25)[c]	−2.17(0.50)[c]	0.113
S-CTX-I	−0.24(0.25)	−1.63(0.19)[c]	−2.58(0.33)[c]	<0.001	−0.19(0.40)	−1.87(0.23)[c]	−3.04(0.49)[c]	<0.001
S-TRACP5b	−0.21(0.28)	−1.63(0.18)[c]	−2.71(0.36)[c]	<0.001	−0.52(0.35)	−1.96(0.24)[c]	−2.70(0.46)[c]	<0.001
U-MidOC	−0.98(0.30)[b]	−1.51(0.20)[c]	−2.25(0.33)[c]	0.004	−0.41(0.39)	−2.38(0.27)[c]	−2.10(0.39)[c]	0.013
Hip aBMD change (%)								
S-OC[1–49]	−6.61(0.82)[c]	−6.06(0.56)[c]	−7.04(0.94)[c]	0.700	−6.34(1.12)[c]	−6.14(0.73)[c]	−8.44(1.08)[c]	0.163
S-TotalOC	−6.37(0.77)[c]	−5.89(0.55)[c]	−7.37(0.93)[c]	0.402	−5.79(1.03)[c]	−6.15(0.68)[c]	−8.72(1.17)[c]	0.049
S-cOC	−6.26(0.74)[c]	−5.82(0.56)[c]	−7.57(0.93)[c]	0.270	−6.25(1.00)[c]	−5.96(0.69)[c]	−8.73(1.20)[c]	0.092
S-boneALP	−5.17(0.87)[c]	−6.43(0.57)[c]	−6.81(0.85)[c]	0.199	−4.10(1.13)[c]	−7.06(0.69)[c]	−7.89(1.16)[c]	0.022
S-CTX-I	−5.06(0.79)[c]	−6.03(0.55)[c]	−8.25(1.00)[c]	0.010	−5.95(1.22)[c]	−5.94(0.62)[c]	−9.58(1.34)[c]	0.027
S-TRACP5b	−5.69(0.78)[c]	−6.58(0.53)[c]	−6.28(1.05)[c]	0.628	−4.98(0.88)[c]	−7.27(0.72)[c]	−7.06(1.21)[c]	0.189
U-MidOC	−5.18(0.72)[c]	−6.18(0.60)[c]	−7.26(0.90)[c]	0.093	−4.49(0.94)[c]	−7.12(0.74)[c]	−7.79(1.08)[c]	0.046

The number of women in each category (constantly low/intermediate/constantly high) for all women was 109/291/122 [S-OC(1–49)], 131/282/127 (S-TotalOC), 116/245/117 (S-cOC), 112/294/115 (S-boneALP), 117/281/121 (S-CTX-I), 108/335/94 (S-TRACP5b), and 112/279/120 (U-MidOC), depending on the marker used for classification. The number of osteopenic women in each category was 48/147/64, 57/146/67, 58/144/66, 54/155/54, 55/151/56, 58/151/59, and 51/145/66, respectively. Values are means (SE). aBMD change from baseline to 5-yr visit is shown as percentage of baseline aBMD value.

[a] $P < 0.05$.

[b] $P < 0.01$.

[c] $P < 0.001$.

Bone loss in women with high, intermediate, or low turnover for time period less than 5 yr

When baseline BTM tertiles alone were used for classification, only one *P* value < 0.05 was observed (Table 3). The use of both baseline and 1-yr results clearly improved the association between turnover and total body bone loss, also in osteopenic women, suggesting an advantage for assessing BTMs at multiple time points (Table 3). The association was, however, most consistent when results from all four visits were used (Table 2).

Discussion

In this population-based study of elderly women, we found that women with constantly high bone turnover lost significantly more bone than women with constantly low turnover. This was observed also in the subgroup of women with osteopenia.

Women with constantly high bone metabolism have greater bone loss

Among randomly selected elderly women without bone-active medication, a subgroup of women who had constantly high levels of bone turnover was identified. These women lost significantly more bone than women with intermediate or low turnover, also in the hip, which should be of particular interest from a clinical perspective. These results suggest that high turnover at consecutive measurements should be considered a risk factor for bone loss. Importantly, high turnover over the first 1-yr period alone was associated with decrease in BMD (although not as strongly as turnover over 5 yr), suggesting that an even shorter time period may be used for identification of bone loss. This finding is of importance from a clinical point of view, because only BTM results obtained at the beginning of follow-up period should be used if the goal is to predict 5-yr bone loss. This particularly applies to bone loss at total body because high turnover over the 1-yr period was significantly associated with total body BMD change. The association with hip BMD change was not significant unless turnover was assessed at all four time points, limiting the utility of BTMs to predict hip bone loss. Continuously high levels of bone turnover and greater bone loss in such high-turnover women may be, at least in part, due to elevated levels of S-PTH.

BTMs improve the identification of osteopenic women with the highest rate of bone loss

Osteopenia is a broad category in terms of BMD and fracture risk. It is not obvious whether intervention is indicated for this heterogeneous group of women, and there is a need for more sensitive tools to know which osteopenic women are at highest risk for bone loss and who might benefit most from therapy (9). We found that among women with osteopenia, individuals with constantly elevated BTMs had significantly greater bone loss in total body and hip than women with intermediate or low turnover. Elevated turnover at consecutive assessments could thus provide an additional tool assisting the decision making for osteopenic women. The utility of BTMs in clinical decision-making

TABLE 3. The 5-yr aBMD change (total body) in women with low, intermediate, or high bone turnover in baseline sampling or over the first 1-yr period

	Bone turnover at baseline				Bone turnover over 1 yr (baseline + 1 yr)			
	Low	Middle	High	*P* trend	Constantly low	Intermediate	Constantly high	*P* trend
All women (n = 573)								
Total body aBMD change (%)								
S-OC[1–49]	−1.08(0.24)[c]	−1.62(0.23)[c]	−1.81(0.25)[c]	0.038	−1.03(0.30)[c]	−1.44(0.18)[c]	−2.03(0.31)[c]	0.019
S-TotalOC	−1.29(0.26)[c]	−1.44(0.23)[c]	−1.79(0.24)[c]	0.150	−1.05(0.29)[c]	−1.47(0.19)[c]	−2.00(0.29)[c]	0.020
S-cOC	−1.23(0.25)[c]	−1.58(0.23)[c]	−1.71(0.25)[c]	0.168	−0.88(0.28)[b]	−1.60(0.19)[c]	−1.87(0.29)[c]	0.014
S-boneALP	−1.52(0.25)[c]	−1.43(0.22)[c]	−1.64(0.27)[c]	0.728	−0.99(0.29)[b]	−1.69(0.19)[c]	−1.69(0.30)[c]	0.096
S-CTX-I	−1.22(0.24)[c]	−1.47(0.24)[c]	−1.89(0.25)[c]	0.054	−0.55(0.28)	−1.64(0.19)[c]	−2.20(0.31)[c]	<0.001
S-TRACP5b	−1.34(0.25)[c]	−1.77(0.22)[c]	−1.40(0.26)[c]	0.886	−0.29(0.33)	−1.54(0.16)[c]	−2.54(0.41)[c]	<0.001
U-MidOC	−1.26(0.25)[c]	−1.42(0.23)[c]	−1.92(0.26)[c]	0.063	−0.94(0.31)[b]	−1.55(0.18)[c]	−2.12(0.34)[c]	0.008
Osteopenic women (n = 285)								
Total body aBMD change (%)								
S-OC[1–49]	−1.31(0.36)[c]	−2.21(0.33)[c]	−1.78(0.31)[c]	0.365	−1.36(0.47)[b]	−1.83(0.24)[c]	−2.11(0.40)[c]	0.208
S-TotalOC	−1.74(0.39)[c]	−1.81(0.31)[c]	−1.81(0.30)[c]	0.891	−1.39(0.45)[b]	−1.85(0.25)[c]	−2.02(0.38)[c]	0.278
S-cOC	−1.71(0.37)[c]	−1.94(0.30)[c]	−1.70(0.32)[c]	0.977	−1.11(0.40)[b]	−2.04(0.26)[c]	−1.92(0.39)[c]	0.154
S-boneALP	−1.49(0.32)[c]	−1.97(0.28)[c]	−1.76(0.41)[c]	0.586	−0.98(0.34)[b]	−2.11(0.27)[c]	−1.78(0.42)[c]	0.165
S-CTX-I	−1.44(0.34)[c]	−1.74(0.32)[c]	−2.09(0.34)[c]	0.167	−0.68(0.45)	−1.84(0.24)[c]	−2.61(0.43)[c]	0.001
S-TRACP5b	−1.81(0.33)[c]	−2.06(0.32)[c]	−1.46(0.34)[c]	0.443	−0.99(0.41)[a]	−1.79(0.23)[c]	−2.62(0.54)[c]	0.020
U-MidOC	−1.35(0.37)[c]	−2.32(0.32)[c]	−1.74(0.31)[c]	0.452	−0.76(0.44)	−2.09(0.25)[c]	−2.06(0.41)[c]	0.053
Hip aBMD change (%)								
S-OC[1–49]	−6.62(0.90)[c]	−6.40(0.86)[c]	−7.03(0.86)[c]	0.725	−6.62(1.16)[c]	−6.12(0.66)[c]	−8.22(1.10)[c]	0.276
S-TotalOC	−7.15(0.89)[c]	−5.80(0.87)[c]	−7.11(0.87)[c]	0.980	−6.32(1.02)[c]	−6.14(0.68)[c]	−8.21(1.11)[c]	0.184
S-cOC	−6.56(0.87)[c]	−6.53(0.89)[c]	−6.97(0.87)[c]	0.736	−5.92(0.97)[c]	−6.54(0.71)[c]	−7.65(1.07)[c]	0.233
S-boneALP	−5.68(0.94)[c]	−7.10(0.84)[c]	−7.08(0.88)[c]	0.284	−5.13(1.10)[c]	−6.82(0.69)[c]	−7.80(1.08)[c]	0.086
S-CTX-I	−6.90(0.89)[c]	−5.81(0.88)[c]	−7.29(0.88)[c]	0.749	−7.47(1.27)[c]	−6.12(0.64)[c]	−7.34(1.16))[c]	0.992
S-TRACP5b	−6.02(0.86)[c]	−7.49(0.91)[c]	−6.48(0.85)[c]	0.732	−5.35(1.24)[c]	−6.95(0.62)[c]	−6.64(1.19)[c]	0.521
U-MidOC	−6.44(0.82)[c]	−6.56(0.92)[c]	−7.29(0.89)[c]	0.497	−5.36(1.06)[c]	−6.68(0.67)[c]	−8.13(1.14)[c]	0.091

The level of turnover was defined in comparison with all women of the study, *i.e.* the random population-based sample of elderly women of the same age. The number of women (for all women) in each category (tertiles) at baseline (low/middle/high) was 189/191/189 [S-OC(1–49)], 189/191/189 (S-TotalOC), 189/191/189 (S-cOC), 178/194/184 (S-boneALP), 185/186/185 (S-CTX-I), 189/189/190 (S-TRACP5b), and 188/188/188 (U-MidOC). The number of women (for all women) in each category at baseline plus 1 yr (constantly low/intermediate/constantly high) was 122/316/123, 132/298/133, 136/282/145, 121/310/114, 121/301/123, 79/409/74, and 121/310/121, respectively. The number of osteopenic women in each category at baseline was 85/98/101, 86/96/102, 87/97/98, 85/106/88, 92/92/95, 89/101/94 and 87/96/100, respectively. The number of osteopenic women in each category at baseline plus 1 yr was 56/164/63, 58/156/69, 63/147/73, 60/158/58, 55/156/65, 39/202/42, and 48/170/62, respectively. Values are means (SE). aBMD change from baseline to 5-yr visit is shown as percentage of baseline aBMD value.

[a] $P < 0.05$.

[b] $P < 0.01$.

[c] $P < 0.001$.

needs, however, to be verified in further studies. Previously, in the JPOS study, osteopenic women in the highest tertile for baseline S-OC had a greater incidence of rate of progression below threshold for osteoporosis at lumbar spine and distal radius over 3 yr when compared with women in the lowest tertile (34). In the osteopenic women of the OFELY study, increased baseline S-boneALP levels were also associated with increased fracture risk (35). For comparison, we evaluated total body BMD change using baseline BTM tertiles alone. Only a few associations were close to the level of significance, and in the osteopenic subgroup and for hip BMD, associations were all nonsignificant (Table 3). This suggests a clear advantage from assigning BTMs over at least two (Table 3) or preferably multiple time points (Table 2).

Multiple serial measurements of BTMs enhance the correlation between turnover and bone loss

Although the baseline levels of BTMs were weakly correlated with 5-yr aBMD change at total body, the correlation was clearly enhanced when we used the average of two, three, or four measurements of each marker. The advantage of multiple measurements may be due to increased precision by averaging duplicate or multiple samples. The reproducibility of BTMs depends on both the analytical features of assays and biological fluctuations, and averaging the results from multiple samples will diminish the variation in sampling, storage, or assay procedure (17, 36). Long-term bone turnover should also provide a more reliable index of bone metabolism compared with turnover assessed at single occasion. We found that the changes in BTMs did not correlate with bone loss as strongly as did the average value of multiple measurements. Weaker association may be, at least in part, due to the relative stability of bone metabolism in elderly women. Our data suggest that constantly high turnover at repeated samplings is a more important risk factor for bone loss than increases in turnover, at least in elderly women randomly selected from the population. It would have been of interest to identify which BTM is most consistently associated with bone loss. Multiple regression analysis was, however, not possible due to the strong correlation between BTMs. The association with aBMD change was, however, fairly similar for all BTMs, except for S-boneALP, but tended to be most consistent for resorption markers, particularly S-CTX-I.

In contrast to a decrease in total body and hip aBMD, we observed an increase in spine aBMD. There were no significant associations between BTMs and changes in spine aBMD (data not shown) (26). At higher ages, spinal vertebral compression fractures, osteophytes, and aortic calcification may mask aBMD loss in lumbar spine. Therefore, it is understandable that spine aBMD increased during the follow-up, from the age of 75 yr to the age of 80 yr, and that the lumbar spine may not be an optimal site for bone loss assessment in the elderly.

A weak association between baseline levels of BTMs and change in BMD has been demonstrated in several studies (14–16), including OPRA (26). In general, markers have been moderately associated with change in BMD, particularly at the forearm, but the predictive value at other skeletal sites, such as the hip and lumbar spine, has been limited (10). The relationship between consecutive BTM measurements and bone loss has been evaluated in conjunction with antiresorptive (18, 21) and anabolic treatments (19, 20), in which short-term changes (months) in BTMs were correlated with long-term changes (years) in BMD in response to therapy. In alendronate and/or hormone replacement therapy trials, the 6-month changes in BTMs correlated with 3-yr changes in hip or spine BMD, with Spearman correlations up to −0.46 (18, 21). In a teriparatide intervention trial, improvement in hip or spine BMD was more correlated with short-term changes in markers than with baseline levels, with r values up to 0.65 (19). The changes in BTMs and aBMD will, however, be of considerably greater magnitude in preselected subjects after pharmacological intervention than in untreated elderly women from an average population. In line with this, the correlations in the placebo groups of intervention studies were markedly lower, with r values reaching −0.29 and −0.32 at best, and not consistently significant (19, 21).

Stability of bone metabolism in elderly women

Approximately half of the women belonged in the same BTM tertile at age 75 and 80, suggesting that bone metabolism is rather stable in elderly women who are well beyond the menopausal ages and do not take any bone-active medication. In the OFELY study (women, 50–81 yr), the agreement between being in the highest tertile at baseline and after 4 yr was 71% for S-OC and 76% for S-CTX-I (37). In our study, the agreement being in the highest tertile at baseline and after 5 yr was 50–64%, depending on BTM. We were unable to observe consistent longitudinal patterns applicable to all BTMs. The minor fluctuations in BTMs may reflect small changes during aging, but assay variability may influence as well. To reduce the assay variability, we standardized BTMs at each time point. Using standardized BTMs did not, however, markedly change β_{std} values and significances (data not shown), suggesting that changes in BTMs are not only explained by assay variability but also influenced by biological variability, such as diet, physical activity, or immobility (17).

Strengths and limitations

The strengths of this study include the prospective design, the size of cohort, and the random selection as well as that all women were the same age and that none was using bone-active medication. By using several BTMs reflecting different aspects of bone metabolism and by measuring them at four time points, we can base our evaluation on a comprehensive view of bone metabolism. Furthermore, we evaluated bone loss over a long time period, 5 yr. There are also some limitations. When the study was initiated, information on the effect of feeding on BTMs was not available. Baseline samples were collected without fasting, and we chose not to change the protocol during the study. Nonfasting status may have affected particularly results on S-CTX-I (38). However, S-CTX-I was one of the best predictors of bone loss, and sampling after fasting could even further improve its association with bone loss. Because all women were of the same age and ethnic background, caution must be exercised when the results are transferred to other than 75-yr-old Caucasian women. In addition, the associations of BTMs were greatest with change in total body BMD, not hip BMD. Hip BMD loss is the current standard for clinical decision making, and the information on the

clinical utility of serial measurements of total body BMD for fracture prediction is limited. Finally, we did not study fractures, which are the clinically most relevant outcome in osteoporosis, and it will be of interest to evaluate the value of long-term bone turnover for fracture prediction when long-term fracture data for the OPRA study are available. Short-term BTM changes have already been shown to predict reduction in vertebral fracture risk with antiresorptive therapy (39, 40).

Conclusions

We conclude that BTMs are correlated with aBMD change in postmenopausal women who do not take bone-active medication. Averaging multiple consecutive measurements improves the precision and strengthens the correlation. Our results suggest that women, also osteopenic women, who have constantly high bone turnover lose significantly more bone than women with constantly low turnover. High bone metabolism at serial assessments could improve the identification of women with the highest rate of bone loss and osteoporosis risk and assist in targeting preventive measures, which is of special clinical interest in women with osteopenia.

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