

Original Article

Can Biochemical Markers Predict Bone Loss at the Hip and Spine?: A 4-Year Prospective Study of 141 Early Postmenopausal Women

R. W. Keen¹, T. Nguyen², R. Sobnack³, L. A. Perry³, P. W. Thompson⁴ and T. D. Spector¹

¹Rheumatology Department, St. Thomas' Hospital, London, UK; ²Garvan Institute of Medical Research, St. Vincent's Hospital, Sydney, Australia; ³St. Bartholomew's Hospital, London; ⁴The Royal London Hospital, London, UK

Abstract. A number of recent studies have suggested that non-invasive measures of bone turnover are associated with bone loss at the forearm in postmenopausal women. Whether bone turnover markers are predictive of bone loss from the clinically important sites of lumbar spine and femoral neck remain unclear, and was the aim of this 4-year prospective study. One hundred and forty-one normal, postmenopausal women (mean age 52.0 ± 3.3 years, mean menopause duration 20.4 ± 5.7 months) were recruited for the study in 1988. Fasting early morning samples of blood and urine were collected at the baseline visit and stored at -20°C prior to analysis. Serum was assayed for osteocalcin, oestradiol, oestrone, oestrone sulphate, testosterone, sex hormone binding globulin, dehydroepiandrosterone sulphate and total alkaline phosphatase. Urine was assayed for calcium, hydroxyproline, oestrone glucuronide and the collagen cross-links pyridinoline and deoxypyridinoline using high-performance liquid chromatography. Bone density was measured at the lumbar spine and femoral neck using dual photon absorptiometry at time 0, 12, 24 and 48 months. The mean annual percentage change in bone density (SE) was -1.41% (0.18) at the lumbar spine and -0.86% (0.22) at the femoral neck. There was no evidence of bimodality or a fast loser subgroup as the rates of change were normally distributed. Both simple and multiple stepwise regression analyses revealed no significant correlation between the rates of change in bone density with any biochemical marker, either individually or in combination, despite the study

having sufficient power (80%) to detect a correlation of 0.5 between any biochemical marker levels and bone loss. We conclude that single measurements of these markers of bone turnover and endogenous sex hormones appear unlikely to be clinically useful in predicting early postmenopausal bone loss from either the spine or the hip.

Keywords: Biochemical assay; Bone densitometry; Bone turnover; Menopause; Osteoporosis

Introduction

Bone mineral density (BMD) in later life is one of the major risk factors for osteoporotic fracture [1–3]. Bone mass is determined by both the peak value achieved during skeletal growth and subsequent loss, which is related both to age and to the menopause, as well as to other factors [4]. Postmenopausal bone loss is believed to be related to an overall increase in bone turnover that peaks between 1 and 3 years after cessation of ovarian function and slows down over the next 8–10 years – a pattern that is also illustrated by cross-section studies after oophorectomy [5]. Markers of bone formation such as serum osteocalcin, and those of resorption (i.e. urinary deoxypyridinoline), as well as other markers of bone turnover, are increased after the menopause and return to premenopausal levels after treatment with hormone replacement therapy (HRT) [6,7]. Non-invasive measurements of bone turnover at the time of menopause have been shown to be useful in identifying

women who subsequently experienced bone loss at a high rate at the forearm using single photon absorptiometry [8]. This finding has not, however, been confirmed at the spine or hip, sites which are of clinical importance in fracture prediction.

The aim of this study was to examine variations in bone loss in recently postmenopausal women over an average 4-year period and to assess whether baseline markers of bone turnover could be used to predict a high-risk group.

Materials and Methods

Subjects

One hundred and forty-one Caucasian women who were aged 45–62 years, within 5 years of the menopause and not on HRT, were examined prospectively for bone loss over a 4-year period. The women were normal volunteers who were participating in an ovarian cancer screening programme in 1988 and were selected on the basis of recent menopausal status, an intact uterus and non-HRT use. Menopausal status was confirmed by the absence of menstruation for more than 12 months. No women were taking HRT prior to the study onset but if hormonal treatment was initiated during the study period, results were included up to that time point. No women received concurrent treatment with medication known to affect bone metabolism such as steroids, calcium or vitamin D. All women gave informed consent to participate in the study and the protocol was approved by the local research ethics committee.

Bone Density Measurement

Consecutive BMD measurements were taken annually at the lumbar spine and left hip using a gadolinium-153 based Novo-BMC Lab 22a dual photon absorptiometer with peaks at 44 keV and 100 keV. Measurements were made at baseline, 12, 24 and 48 months. During the 4-year study period four radioactive sources were used and an aluminium spine phantom was used to monitor quality control over the duration of the study. An upward drift of +0.34% per annum was observed in the mean BMD of the phantom over the duration of the study, although this was probably artifactual and associated with the long-term precision error of the dual photon absorptiometry (DPA) scanning mode. Short-term reproducibility as assessed on 25 healthy, female volunteers who had repeat measures within 3 weeks was 0.8% at the spine and 1.6% at the hip [9]. Long-term precision of DPA over the 4-year study was calculated using the mean of the standard errors of the estimate for individual regression analyses of bone density against time [10]. These were found to be 3.92% at the spine and 4.69% at the hip.

Biochemical Markers

Serum and urine samples were collected between 0900 and 1600 hours after an overnight fast, spun immediately and stored at -20°C prior to analysis. Serum osteocalcin was measured using the Osteo-PR RIA kit (Cis UK); inter- and intra-assay variations were less than 10%. Serum total alkaline phosphatase was measured using routine methods with a SMAC III analyser; inter-assay variation was 0.9–1.8%. Serum oestradiol was measured by direct assay using a commercial kit (Diagnostic Products) with ^{125}I -oestradiol tracer and double antibody separation system with a lower detection limit of 20 pmol/l. Oestrone and oestrone sulphate were measured by radioimmunoassay following diethyl ether extraction and separation by column chromatography on Sephadex LH 20 as previously described [11]. Radioimmunoassay using standard techniques was also used to measure serum sex hormone binding globulin (SHBG), dehydroepiandrosterone sulphate (DHEAS) and testosterone [12]. The inter- and intra-assay variation for these assays varied between 8% and 12%. Urine samples were analysed for calcium on a RA 1000 discrete analyser (inter-assay variation 1.8–3.5%) and hydroxyproline with a multistep assay involving a standard chloramine T reaction and alkaline/organic extraction and subsequent spectrophotometric analysis at 550 nm [13]; the inter- and intra-assay variation were less than 15%. Urinary collagen cross-links were assayed using ion-pair reversed-phase high-performance liquid chromatography (HPLC) in the presence of 1-octanesulphonic acid (OSA) [14]. Inter- and intra-assay variations were less than 10% for both pyridinoline and deoxypyridinoline. This assay was validated against standard gradient systems [15], and correlations of 0.95 (pyridinoline) and 0.92 (deoxypyridinoline) were observed between the two techniques in 27 normal women. Urinary oestrone glucuronide was measured in diluted urine by radioimmunoassay and expressed relative to creatinine content [16]. Intra- and inter-assay coefficients of variation for this assay were 3.5% and 6.2% respectively.

Statistical Analysis

In calculating the rate of change in BMD it was assumed that the expected change in BMD is linear with time for each subject with variation in slopes and intercepts from subject to subject, and that the deviations of the measured BMD for a subject from the expected BMD have a zero mean and constant variance and are uncorrelated. Under these assumptions a linear regression equation, where BMD is a dependent variable expressed as a linear function with time (in years), was fitted for individual subjects. Under this model, the annual percentage change in BMD for each subject was then derived by dividing the regression slope by the intercept at time zero. Rates of change in BMD were calculated using separate models where the initial BMD

was either included or excluded from the slope calculation. Preliminary analysis suggested that biochemical marker values were not normally distributed. For subsequent analysis natural logarithmic transformation was required to normalize the data. We assessed the relationships between biochemical markers and rate of change in two ways. First, bivariate correlation analysis (Pearson's product moment correlation coefficient) was used to describe the association between the baseline value of each of the biochemical parameters and the annual percentage bone loss. Second, since these variables were likely to be correlated, and in order to search for a set of biochemical variables with maximum discriminatory power, we used the stepwise and backward algorithms to evaluate the significance of all the biochemical markers simultaneously. Multiple linear regression analysis was used to model the relationship between this set of variables and the rate of bone loss. Estimation and hypothesis testing based on this "final model" was done by the least squares method via the STATA statistical program.

Rates of change in BMD at the spine and hip, and individual biochemical levels, were subdivided into quartiles. The sensitivity and specificity of the markers for prediction of loss was assessed by the relationship between the lowest quartile of rate of BMD change (i.e. rapid loss) and highest quartile of marker level (i.e. high bone turnover).

Results

One hundred and forty-one women fulfilled the study entry criteria and participated in the study. A majority of these women (123, or 87%) did not start HRT during the 4-year period. The majority of women starting HRT did so within the first year of the study for menopausal symptom-relief, and bone density data were available only at baseline. Women who had two or more BMD

Table 1. Mean baseline characteristics of 123 women (SD)

Variable	Mean (SD)
Age (years)	52.3 (3.2)
Menopause age (years)	50.7 (3.2)
Menopause duration (months)	20.4 (5.7)
Height (m)	1.63 (0.06)
Weight (kg)	66.9 (11.3)
BMI (kg/m ²)	25.3 (4.2)
LS BMD (g/cm ²)	0.80 (0.11)
FN BMD (g/cm ²)	0.70 (0.10)
Annual change in LS BMD (%/year)	-1.41 (1.93)
Annual change in FN BMD (%/year)	-0.86 (2.24)

BMI, body mass index; FN, femoral neck; LS, lumbar spine.

measurements prior to the commencement of HRT were included in the analysis to reduce the potential bias that these women may have been subject to increased rates of bone loss. Data from four completed scans (0, 12, 24, 48 months) were available for 85 women at the lumbar spine and 75 at the femoral neck. At the lumbar spine an additional 22 women had only three scans and 7 only two scans, whilst at the femoral neck 21 women had three scans and 11 had two scans. Descriptive baseline details of the study population are shown in Tables 1–3.

At both skeletal sites no significant correlation was found between baseline BMD and demographic variables such as age, menopause duration, height or weight. A positive correlation was found, however, between the baseline BMD measurements at the spine and hip ($r = 0.41$, $p < 0.001$). There was no correlation between baseline BMD and individual biochemical markers, although spinal BMD at baseline correlated with DHEAS ($r = 0.27$, $p = 0.02$) when age, weight and menopause duration were fitted into a multiple regression model [9].

The mean annual percentage change in BMD (SE) for the total group when the initial BMD was included in the slope calculation was -1.41 (0.18)%/year for the

Table 2. Baseline biochemical markers in study group [mean (SD) unless otherwise stated]

Biochemical marker	Mean (SD)
Osteocalcin (ng/ml)	7.4 (2.4)
Alkaline phosphatase (IU)	78.9 (22.2)
Calcium/cr (mmol/mol)	450.3 (208.9)
Hydroxyproline/cr (mmol/mol)	10.4 (9.0)
Pyridinoline/cr (nmol/mmol)	40.9 (12.7)
Deoxypyridinoline (nmol/mmol)	12.8 (5.1)
Median oestradiol and interquartile range (pmol/l) ^a	36 (27, 76)
Testosterone (nmol/l)	1.8 (0.5)
DHEAS (μmol/l)	3.1 (2.3)
SHBG (nmol/l)	67.1 (26.0)
Median oestrone and interquartile range (pmol/l)	160 (117, 209)
Median oestrone sulphate and interquartile range (pmol/l)	601 (504, 802)
Median oestrone glucuronide and interquartile range (nmol/l)	8.7 (5.9, 12.7)

cr, creatinine; DHEAS, dehydroepiandrosterone; SHBG, sex hormone binding globulin.

^a $n=33$ (subjects with reading >20 pmol/l included).

Table 3. Intercorrelation among biochemical markers and sex hormone measurements

	1	2	3	4	5	6	7	8	9	10	11	12	13
1 Osteocalcin	1	0.24*	0.21*	0.23*	0.20*	0.17	-0.04	-0.15	-0.07	0.17	-0.17	-0.15	-0.02
2 Alkaline phosphatase		1	0.02	0.06	0.24*	0.22*	-0.16	0.06	-0.02	0.05	0.09	-0.00	-0.07
3 Urinary Calcium			1	0.17	0.10	0.21*	-0.24*	0.01	0.03	-0.12	-0.02	0.05	-0.12
4 Hydroxyproline				1	0.19*	0.12	0.09	0.11	0.01	0.05	0.05	0.04	-0.03
5 Pyridinoline					1	0.74**	0.13	-0.02	-0.05	-0.12	0.08	0.07	0.12
6 Deoxypyridinoline						1	-0.06	-0.02	-0.09	-0.09	-0.02	0.03	0.16
7 SHBG							1	0.10	-0.31**	0.15	0.22*	0.08	0.00
8 Testosterone								1	0.30**	0.15	-0.05	-0.07	-0.23
9 DHEAS									1	-0.13	0.03	-0.03	-0.08
10 Oestradiol										1	0.05	-0.05	-0.02
11 Oestrone											1	0.82**	0.23*
12 Oestrone sulphate												1	0.21*
13 Oestrone glucuronide													1

All parameters were logarithmic (natural) transformed. All values were calculated based on a minimum sample size of 97.

* $p < 0.05$; ** $p < 0.001$.

lumbar spine and -0.86 (0.22)/year for the femoral neck. Rates of change in bone density were normally distributed at both skeletal sites (Fig. 1), showed a linear pattern over the 4 years and no evidence of bimodality. There was no significant correlation between the loss rates at the two sites ($r = 0.13$). Similar results were obtained with the second model when the initial BMD were excluded from the slope calculation, although the number of subjects with full results was reduced ($n = 107$ at spine, $n = 95$ at hip) and the error associated with individual rates of change was increased.

Linear regression analysis revealed no significant correlation between annual percentage change in BMD at the spine or hip and any of the biochemical markers of bone turnover individually or in combination (Table 4; Figs 2, 3). Annual percentage change in BMD from the hip did correlate weakly with baseline BMD at that skeletal site ($r = -0.24$, $p = 0.05$), and a similar but non-significant trend was observed at the spine ($r = -0.20$, $p = 0.07$). The proportion of variance in rate of change accounted for by baseline BMD was 8.6% ($p = 0.0005$) at the lumbar spine and 18.3% ($p < 0.0001$) at the femoral neck. Addition of data from the 13 biochemical markers and sex hormones to the regression model increased this proportion of bone loss variance to 11% (spine) and 20% (hip) – a non-significant improvement. This relationship was seen, however, only in the model where the initial BMD measurements had been included in the slope calculation. Using BMD measures from year 2 to year 4 of the study showed no significant correlation between rate of change and BMD at the study onset at either the spine ($r = -0.13$, $p = 0.18$) or the femoral neck ($r = -0.13$, $p = 0.23$).

The lower quartile values (95% confidence intervals) for rates of change in BMD were -2.45 /year (-2.69 , -2.17) at the lumbar spine and -1.90 /year (-2.5 , 1.53) at the hip. The higher quartile values were -0.51 /year (-0.71 , -0.02) at the spine and 0.00 /year (-0.29 , $+0.37$). The sensitivity and specificity of

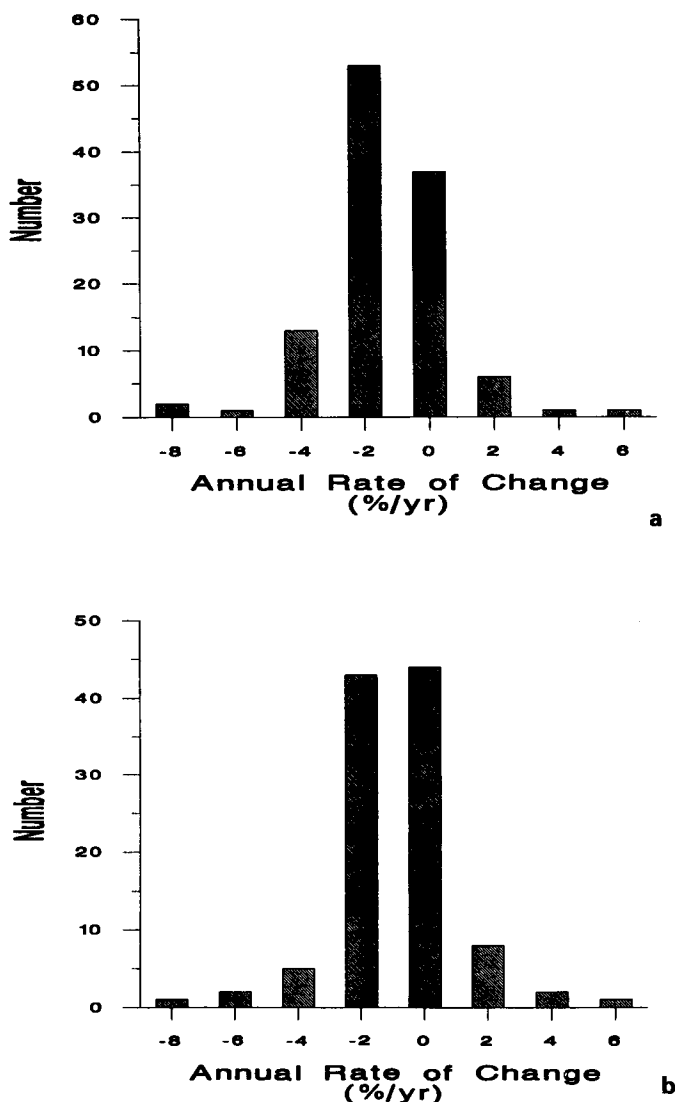


Fig. 1. Distribution of percentage bone loss over the 4-year study period at the lumbar spine (a) and femoral neck (b).

Table 4. Correlation between biochemical markers and annual percentage change in BMD (crude and baseline-adjusted) at the lumbar spine and femoral neck

	Correlation coefficient (<i>p</i> value) with bone loss at:			
	Lumbar spine		Femoral neck	
	Crude	Adjusted ^a	Crude	Adjusted ^a
Osteocalcin	0.02 (0.84)	-0.02 (0.82)	-0.03 (0.70)	-0.06 (0.51)
Alkaline phosphatase	-0.10 (0.24)	-0.12 (0.18)	-0.16 (0.06)	-0.13 (0.16)
Urinary calcium	-0.05 (0.53)	-0.04 (0.62)	-0.14 (0.12)	-0.11 (0.23)
Hydroxyproline	0.15 (0.10)	0.15 (0.10)	-0.08 (0.38)	-0.04 (0.64)
Pyridinoline	0.07 (0.45)	0.05 (0.53)	-0.00 (0.97)	-0.00 (0.95)
Deoxypyridinoline	0.03 (0.69)	0.02 (0.79)	-0.05 (0.59)	-0.07 (0.47)
Oestradiol	0.10 (0.27)	0.09 (0.34)	0.03 (0.73)	0.06 (0.57)
Oestrone	0.06 (0.47)	0.10 (0.25)	-0.12 (0.17)	-0.11 (0.27)
Oestrone sulphate	0.10 (0.28)	0.12 (0.16)	-0.11 (0.20)	-0.05 (0.58)
Oestrone glucuronide	0.18 (0.28)	0.17 (0.007)	-0.03 (0.72)	-0.10 (0.32)
Testosterone	0.02 (0.83)	-0.02 (0.80)	0.08 (0.40)	0.03 (0.73)
SHBG	-0.08 (0.36)	-0.07 (0.47)	0.02 (0.87)	0.04 (0.65)
DHEAS	0.15 (0.10)	0.14 (0.12)	0.11 (0.22)	0.06 (0.50)

^a Adjusted for baseline BMD.

the assays for predicting loss was poor at both the spine and the hip (sensitivity 33–58%, specificity 50–72%).

Discussion

Women who experience accelerated bone loss after the menopause may be at increased risk of subsequent osteoporotic fracture because of either lower bone mass or disorganization of skeletal microarchitecture [17]. Identification of these women at the time of menopause would allow targeting of preventive treatment to retard or reverse this loss, thereby reducing the risk of future fracture. To test whether biochemical markers are predictive of early postmenopausal bone loss we have studied longitudinally a group of 141 early postmenopausal women for an average of 4 years.

We found that the annual percentage rates of change in BMD from the femoral neck and lumbar spine appear to be normally distributed, and we were not able to demonstrate a subgroup of 'fast losers'. The mean annual rates of change at the spine were similar in magnitude to those reported in 38 women within 2 years of the menopause [18], but less than those of -2 to -3%/year previously reported from other studies [19]. Rates of change at the hip were also less than in previous reports [18,20]. There was no positive correlation between the rates of change at the hip and spine; this probably represents a statistical phenomenon. One possible explanation for the difference in rates of change in BMD between studies may be the dietary calcium intake, as high-calcium diets have been shown to retard the rate of both menopausal and age-related bone loss [21,22].

Our finding that baseline bone density at the spine or hip did not correlate with variables such as age, weight and years since menopause is of interest. This has

probably arisen as a result of the selection of women within a narrow age range, as many previous studies have included data from a wider range. Biochemical markers at baseline also did not correlate cross-sectionally with bone density at either site, although the weak relationship between spinal BMD and DHEAS suggests that adrenal sex hormone output may have a direct effect on bone. Similar previous studies have shown differing results depending on the markers tested and skeletal site involved [23,24]. Individual biochemical assays show a diurnal variation and exhibit significant fluctuation both within and between individuals [25]. This variation is most marked for the urinary markers of bone resorption, and a recent estimate suggests the long-term precision error is 12–17% for formation markers and 25–29% for urinary resorption markers [26]. Multiple measurements may reduce this error and more accurately reflect the true biochemical/hormonal status of a subject. Multiple measures of serum oestrone have been shown to correlate weakly with change in radial bone mass in 66 postmenopausal women over 3 years [27]. Urinary metabolites of sex hormones may also reduce the variability of a single measurement, although conflicting findings have been observed regarding their association with forearm BMD in postmenopausal women.

In our study we found no correlation between a range of biochemical markers of bone turnover, individually or in combination, and subsequent bone loss at hip and spine over a 4-year period. This contrasts with other studies which have shown positive correlations between baseline biochemical marker levels and subsequent bone loss at the forearm. We have estimated that this study had 80% power to detect a correlation of $r=0.5$ between bone loss and biochemical marker values, assuming a 50% error in the rate of loss and a 20% error in the marker values. Such high degrees of correlation

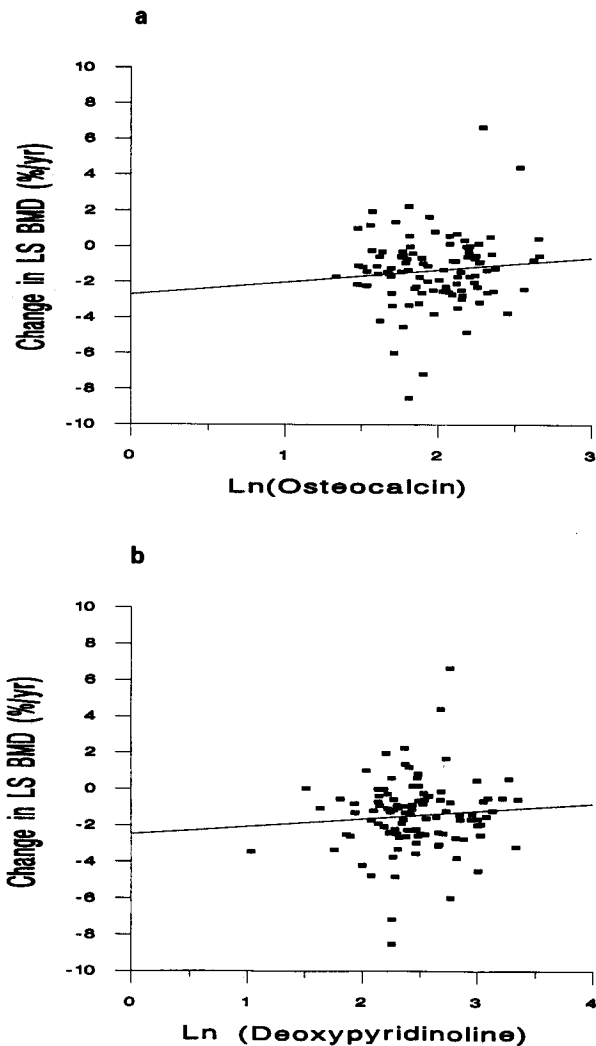


Fig. 2. Scatterplots of annual percentage change in BMD at the lumbar spine (LS) and serum osteocalcin (a) and urinary deoxyypyridinoline (b).

would be required for prediction of individual loss rates in the clinical setting and for decisions on therapeutic management. The sensitivity and specificity of these assays is also inadequate and would preclude the use of these markers in routine clinical use.

Christiansen et al. [8] documented forearm BMD loss over 2 years in 178 postmenopausal women. Loss rates appeared bimodal, with fast losers classified as losing $>3\%/year$. Measurement of body mass index, serum oestradiol, urinary hydroxyproline and calcium identified 79% of fast and slow losers. Further work from the same group showed that a single measurement of several markers (urinary calcium and hydroxyproline, serum alkaline phosphatase) and forearm BMD was predictive of women who had developed osteoporosis 12 years later, although it is probable that the initial bone mass accounted for this strong association [28]. Osteocalcin measured at baseline was found to correlate with bone loss at the forearm and lumbar spine in 83 women within 6–36 months of the menopause studied

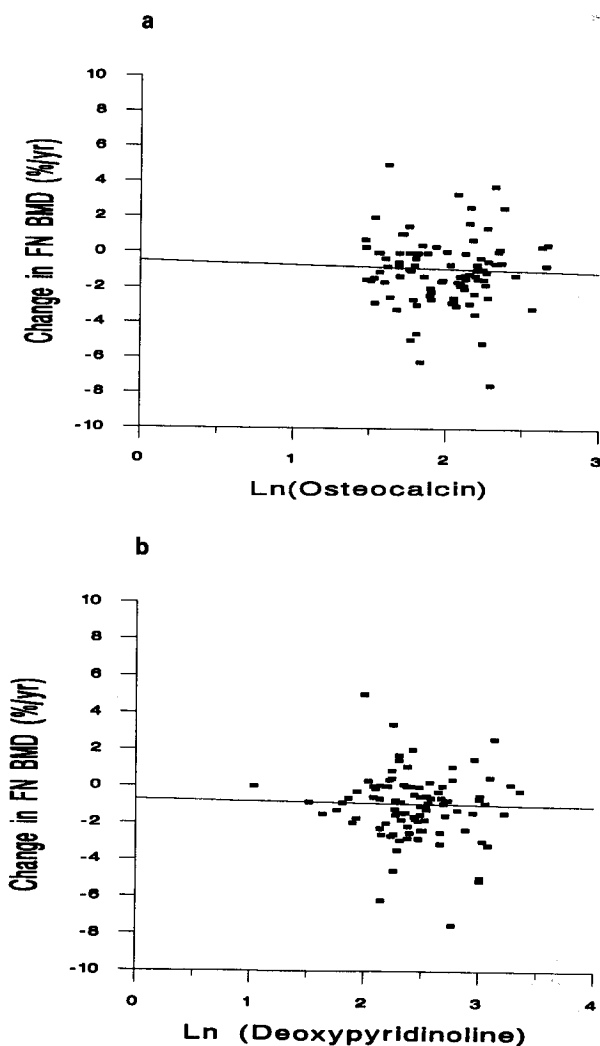


Fig. 3. Scatterplots of annual percentage change in BMD at the femoral neck (FN) and serum osteocalcin (a) and urinary deoxyypyridinoline (b).

for 2 years [7]. In a further small 2-year study of 37 postmenopausal women, rates of forearm bone loss correlated with baseline measurements of osteocalcin, urinary hydroxyproline, fasting deoxyypyridinoline and pyridinoline [6]. In this study the prediction of loss rate was increased either by taking the mean of three measurements of the urinary collagen cross-links obtained over a 9-month period or by combining osteocalcin, fasting pyridinoline and fasting urinary hydroxyproline levels in a multiple regression analysis. In efforts to reduce the error of single measurements, multiple samples over time of serum osteocalcin [27] and urinary excretion of oestradiol glucuronide [29] have been shown to correlate with change in radial shaft bone mineral content in early postmenopausal women.

Previous studies have described associations between initial bone mass and subsequent rate of change, yet studies regressing initial BMD value against rate of change are subject to statistical bias [30]. Several analytical approaches have been devised to try and overcome

this problem, and as our crude analysis had demonstrated a weak correlation between baseline BMD and subsequent loss we utilized the approach of Hui et al. [31]. Omitting the initial BMD measurement from the bone loss calculation removes the spurious negative correlations, but the estimate of the regression coefficient is attenuated as a result of the inherent measurement error of the baseline BMD. Our data suggest that the weak negative correlations observed in the crude results were statistically biased, and that if there is any true effect of initial BMD on the immediate postmenopausal rates of loss it is extremely small. It is also unlikely that patients with high BMD have persistently elevated rates of bone loss that persist into later life, as the short-term observation of the variance of age-specific rates of bone loss is much higher than that expected from the age-related increase in the population variance of BMD. This suggests a continual self-correction to rates of bone loss, and reduces the value of assessing loss rates over a short time period.

Our study has certain limitations and its results can be interpreted in a number of different ways. The biochemical markers utilized in this study are from the "first generation", and in the case of urinary hydroxyproline are not bone specific. These tests have now, however, been largely superseded by immuno-based assays which are more bone specific and further studies are required to assess whether these will have an improved predictive role. Power calculations for this study were based on a 20% error for the measurement of individual biochemical/hormonal assays, and although samples were centrifuged immediately after collection and stored at -20°C , it is probable that delay in performing the assays may have affected, in particular, the measurement of osteocalcin levels. This would have potentially biased our results towards the null hypothesis. Again, recent immunoassays have been developed for osteocalcin which recognize different molecular epitopes and are more suited to delayed storage and analysis – a setting more likely to occur in clinical practice. As stated previously the inherent variability of a single measurement at one time point may mean that such tests are insufficiently precise or sensitive to detect small differences in bone loss at the spine or hip, and although multiple measurements of markers may reduce the error estimates there are major implications for cost effectiveness and clinical utility. We also note that by obtaining samples between 0900 and 1600 hours the effect of diurnal variation may become apparent, and again introduce potential bias. We feel, however, that this would probably not mask a strong relationship such as would be required for clinical significance. The sampling procedure is also more similar to that encountered in daily clinical practice, where these markers would have to be utilized, than that applicable to a refined research setting.

Bone loss from the spine and hip appears less than previously reported, is normally distributed and no discrete "fast-loser" subgroup was identified. At

present our ability to detect these small changes in spine or hip BMD with DPA is limited due to the long-term precision of the scanning techniques (shown in this study to be 3–5%), which would again potentially bias our results towards the null hypothesis. We found, however, that in the small group of women with extreme spinal bone loss values (i.e. above the least significant change estimate) there was no significant correlation between marker values and the degree of bone loss. Long-term precision of forearm BMD is reported to be $<1\%$ and may explain the positive correlation that has been reported between markers and bone loss from this site. Longitudinal measures of forearm BMD are also less affected by the changes in position, medullary fat composition and osteophytes that can affect spinal and hip measurements. During the time period of this study DPA became outmoded and has been replaced by the more precise technique of dual-energy X-ray absorptiometry (DXA). Longitudinal measurement of BMD using DXA may more accurately detect small differences in rates of change, although if variation in loss rates is as small as this study suggests, identification of subgroups may not be cost effective. Alternatively studies may need to be of longer duration (i.e. 5–10 years) to overcome the long-term precision errors of DXA and DPA, and to fully test the sensitivity of markers, although this has financial implications for those supporting this research.

It is possible that biochemical markers may correlate with forearm (and therefore cortical) bone loss but not with bone loss from the spine or hip. It is likely that the better long-term precision of forearm BMD measurements accounts for the positive correlations observed, although it is possible that biochemical markers are reflecting changes in total body BMD and that cortical bone is a more representative component of this measure. It is important, however, that biochemical markers be shown to be predictive of bone loss at the clinically relevant sites such as hip and spine where the majority of osteoporotic fractures occur.

Our results overall suggest that single measurements of biochemical markers of bone turnover and endogenous sex steroids at the time of menopause are not predictive of subsequent bone loss measured by DPA over 4 years at the lumbar spine or hip, and appear unlikely to be clinically useful in this regard. Further studies are required, however, to identify whether the modern, bone-specific immuno-based assays may correlate better with bone loss measured with DXA, and to examine their role in predicting and monitoring response to therapy.

Acknowledgements. This study was funded by the Wellcome Trust. We are grateful to the following: Glen Blake, Keith Britton, Annie Edwards and Pat Harris for their help and assistance with the running of this study; Ian James, Hugh McGarrigle, Pat Mole, David Perrett and Chris Price for assistance with the biochemical marker assays. R.W.K. is an ARC Clinical Research Fellow.

References

- Hui SL, Slemenda CW, Johnston CC. Baseline measurement of bone mass predicts fracture in white women. *Ann Intern Med* 1989;111:355-61.
- Cummings SR, Black DM, Nevitt MC, Browner WS, Cauley JA, Genant HK. Appendicular bone density and age predict hip fracture in women. *JAMA* 1990;263:665-8.
- Cummings SR, Black DM, Nevitt MC, et al. Bone density at various sites for prediction of hip fractures. *Lancet* 1993;341:72-5.
- Johnston CC, Slemenda CW. Peak bone mass, bone loss and risk of fracture. *Osteoporosis Int* 1994;(Suppl 1):S43-4.
- Stepan JJ, Pospichal J, Presi J. Bone loss and biochemical indices of bone remodeling in surgically induced postmenopausal women. *Bone* 1987;8:279-84.
- Uebelhart D, Schlemmer A, Johansen JS, Gineyts E, Christiansen C, Delmas PD. Effect of menopause and hormone replacement therapy on the urinary excretion of pyridinium cross-links. *J Clin Endocrinol Metab* 1991;72:367-73.
- Johansen JS, Riis BJ, Delmas PD, Christiansen C. Plasma BGP: an indicator of spontaneous bone loss and of the effect of oestrogen treatment in postmenopausal women. *Eur J Clin Invest* 1988;18:191-5.
- Christiansen C, Riis BJ, Rodbro P. Prediction of rapid bone loss in postmenopausal women. *Lancet* 1987;1:1105-8.
- Spector TD, Thompson PW, Perry LA, McGarrigle HH, Edwards AC. The relationship between sex steroids and bone mineral content in women soon after the menopause. *Clin Endocrinol* 1991;34:37-41.
- Schlesselman JJ. Planning a longitudinal study. II. Frequency of measurement and study duration. *J Chron Dis* 1973;26:561-70.
- Selby PL, McGarrigle HHG, Peacock M. Comparison of the effects of oral and transdermal oestradiol administration on oestrogen metabolism, protein synthesis, gonadotrophin release, bone turnover and climacteric symptoms in postmenopausal women. *Clin Endocrinol* 1989;30:241-9.
- Wathen NC, Perry LA, Rubenstein E, Chard T. A relationship between sex hormone binding globulin and dehydroepiandrosterone sulphate in normally menstruating females. *J Gynaecol Endocrinol* 1987;1:47-55.
- Podenphant J, Larsen NL, Christiansen C. An easy and reliable method for the determination of urinary hydroxyproline. *Clin Chim Acta* 1984;142:145-8.
- James IT, Perrett D, Thompson PW. Rapid assay for hard tissue collagen cross-links using isocratic ion-pair reversed-phase liquid chromatography. *J Chromatol* 1990;525:43-57.
- Black D, Duncan A, Robins SP. Quantitative analysis of the pyridinium crosslinks of collagen in urine using ion-pair reversed-phase high-performance liquid chromatography. *Anal Biochem* 1988;169:197-203.
- Mole PA, Rae MH, Paterson CR. Urinary oestrogen excretion after the menopause in relation to age and body mass. *Ann Nutr Metab* 1989;33:246-51.
- Christiansen C, Hansen MA, Overgaard K, Riis BJ. Prediction of future fracture risk [abstract]. In: *Proceedings of the Fourth International Symposium on Osteoporosis*, 1993:52-4.
- Pouilles JM, Tremollieres F, Ribot C. Effect of menopause on femoral and vertebral bone loss. *J Bone Miner Res* 1995;10:1531-6.
- Nilas L, Christiansen C. Rates of bone loss in normal women: evidence of accelerated trabecular bone loss after the menopause. *Eur J Clin Invest* 1988;18:529-34.
- Stevenson JC, Cust MP, Gangar KF, Hillard TC, Lees B, Whitehead MI. Effects of transdermal versus oral hormone replacement therapy on bone density in spine and proximal femur in postmenopausal women. *Lancet* 1990;335:265-9.
- Dawson-Hughes B, Dallal GE, Krall EA, Sadowski L, Sayhoun N, Tannenbaum S. A controlled trial of the effect of calcium supplementation on bone density in postmenopausal women. *N Engl J Med* 1990;323:878-83.
- Reid IR, Ames RW, Evans MC, Gamble GD, Sharpe SJ. Effect of calcium supplementation on bone loss in postmenopausal women. *N Engl J Med* 1993;328:460-4.
- Siebel MJ, Cosman F, Shen V, et al. Urinary hydroxypyridinium crosslinks of collagen as markers of bone resorption and estrogen efficacy in postmenopausal osteoporosis. *J Bone Miner Res* 1993;8:881-9.
- Prince R, Dick I, Devine A, et al. Importance of bone resorption in the determination of bone density in women more than 10 years past the menopause. *J Bone Miner Res* 1993;8:1273-9.
- Eastell R, Simmons PS, Colwell A, et al. Nyctohemeral changes in bone turnover assessed by serum bone Gla-protein concentration and urinary deoxyypyridinoline excretion: effects of growth and ageing. *Cli Sci* 1992;83:375-82.
- Garnero P, Shih Wj, Gineyts E, Karpf DB, Delmas PD. Comparison of new biochemical markers of bone turnover in late postmenopausal osteoporotic women in response to alendronate treatment. *J Clin Endocrinol Metab* 1994;79:1693-700.
- Slemenda C, Hui SL, Longcope C, Johnston CC. Sex steroids and bone mass. *J Clin Invest* 1998;80:1261-9.
- Hansen MA, Overgaard K, Riis BJ, Christiansen C. Role of peak bone mass and bone loss in postmenopausal osteoporosis: a 12 year study. *BMJ* 1991;303:961-4.
- Walkinshaw MH, Mole PA, Paterson CR. Potential value of urinary oestrogen assays in the identification of fast bone losers after the menopause. *Osteoporosis Int* 1992;2:205-9.
- Davis JW, Grove JS, Ross PD, Vogel JM, Wasnich RD. Relationship between bone mass and rates of bone change at appendicular measurement sites. *J Bone Miner Res* 1992;7:719-25.
- Hui SL, Slemenda CW, Johnston CC. The contribution of bone loss to postmenopausal osteoporosis. *Osteoporosis Int* 1990;1:30-4.