Determinants of the first decade of bone loss after menopause at spine, hip and radius


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Summary

This study documented bone loss at three different sites in the early postmenopausal period, and examined potential predictors. Forty-three women underwent repeated measurements of bone density at the lumbar spine, proximal femur and distal radius for up to 14 years. Individual rates of bone loss were calculated for the spine and hip; for radial trabecular bone, rates were calculated separately for two time periods, earlier and later after menopause. In the spine and radius, initially high rates of loss diminished with time after menopause. No positive correlations for bone loss were found between the three sites, suggesting that faster than average bone loss was specific to individual bones. High body mass index (BMI) was significantly protective against fast bone loss at the spine and radius; in the spine, each unit increase in BMI was associated with a ~5% reduction in the rate of bone loss. Of the other variables measured (maximum oxygen consumption, lean body mass, fat mass, mean psoas muscle area at the L3 level, hand grip strength as well as anthropometry) only bone densitometry was sufficiently predictive to help guidance on hormone replacement or other prophylactic therapy. The data suggest that the known relationship between excessive lean-ness and risk of osteoporosis and vertebral fractures after menopause might in part be due to fast postmenopausal bone loss. Because bulk of psoas muscle was associated with low spine loss rates, the data also support a role for applied muscular loading in local maintenance of bone density.

Introduction

Women lose bone from the spine after the menopause at a rate several-fold faster than that in men of the same age or premenopausal women. Furthermore, after age 50, rates of vertebral deformity increase faster in women than in men. This difference can be largely attributed to the faster rate of decline in spinal bone mineral density in women.

There has been much interest in the possibility of preventing osteoporotic fractures by reducting postmenopausal bone loss. Hormone replacement therapy (HRT) prevents both bone loss and fractures, but not all women wish to take HRT for prolonged periods after the menopause, and after 5 years of HRT the perceived risk of breast cancer increases appreciably. Many women are interested in taking HRT for prevention of osteoporosis only if they are at higher than average risk of an osteoporotic fracture. Certain pharmaceutical approaches have been shown to prevent postmenopausal bone loss, such as bisphosphonates and selective oestrogen receptor modulators (SERMs) such as tibolone and raloxifene. However there remain concerns about the long-term use of these agents in prophylaxis as distinct from treatment of disease.

Future fracture risk is related to current bone density, which in turn is a function of premenopausal bone density and its subsequent rate of loss. Black calculated that by age 70, premenopausal bone density
density and cumulative postmenopausal bone loss contribute about equally to the between-individual variation in fracture risk. Hence there has been considerable interest in developing methods for predicting rates of bone loss in the two decades after menopause.

There have been a number of long-term prospective studies of forearm bone density in perimenopausal and postmenopausal women, but the technology of dual-photon absorptiometry, which made precise spinal measurements possible, became available more than a decade after single-photon absorptiometry, delaying the generation of results from long-term cohort studies on the spine and hip. The Harrow postmenopausal bone loss study was begun in 1984 with the aim of documenting rates of spinal bone loss in normal women recruited within three years of the menopause, in a community setting. Because rates of bone loss after the menopause are attenuated quite rapidly, so that women >5 years after the menopause are losing bone less quickly than women within 2 years of the menopause, we previously related bone loss to years since menopause rather than chronological age. In our first report, after 5 years of study, which included seven dual-photon absorptiometry (DPA) measurements per subject, differences between women in their individual rates of spinal bone loss were on the borderline of statistical significance. The purpose of the present paper is to provide a description of spinal, femoral and radial trabecular bone loss over the first 11–14 postmenopausal years in the same population sample, to demonstrate the extent to which individual women differed in their rates of bone loss after menopause, and if possible, to identify predictors of bone loss from among variables measured at baseline which related to lifestyle, muscle mass, muscle strength and anthropometry.

Methods

Patients

Women between 42 and 52 years of age from four primary-care practices who had not had a hysterectomy were invited to participate in a study of how women lose spinal bone at the menopause. Women who had a history of malignancy were not interviewed, and the remainder were asked about the date of their last menstrual period. Those consenting who were between 9 and 36 months of their last menstrual period and in good general health were assessed by additional cytology of their vaginal cells at the same time as a routine cervical smear. All those showing parabasal cells, which are a marker of diminished ovarian hormone stimulation, and a random 50% of those not showing parabasal cells were invited to join the study. Sixty-four accepted, giving an 80% response rate. Over the ensuing years until their final bone density measurement, 17 received hormone replacement therapy for 3 months or more at some stage. None had cervical cancer. The daily calcium intakes of these women were comparatively stable and ranged from 466 to 1882 mg/day (median 916) when assessed by weighed intake on two occasions approximately 5 years apart. At the conclusion of these measurements, the subjects were a median age of 64 (mean 63.5, SD 2.7) years of age. The study was approved by the Harrow District Ethical Committee.

Lifestyle, anthropometric and muscle-related variables

At recruitment, each woman answered a simple questionnaire which included questions on self-assessed smoking (yes/no; how many cigarettes per day), alcohol consumption (none/moderate/more than moderate) and exercise (recreational exercise yes/no). Height was measured on the same stadiometer and weight on the same balance. Weight, and on some occasions height, were remeasured at each densitometry visit.

Total body potassium was measured by whole body scintillation counting (40K) on three occasions over the first 5 years of the study. On up to four occasions at yearly intervals, each woman had a CT scan of the trunk at L3 level for measurement of BMD by QCT. These studies were begun before it was realized that the GE 9000 series machine used was sensitive for the measurement of BMD to table height, so the BMD trend data were not considered reliable. Because of the reported association of psoas muscle weight with bone density, each scan was also analysed for the sum of the cross-sectional areas of the left and right belly of the psoas muscle by manually tracing round the images of the two muscles to generate mean psoas area. Because maximum oxygen consumption, measured as VO2max, has been reported to be protective of bone density after menopause, on one occasion at approximately 3.5 years post-recruitment, each compliant woman who was passed medically fit undertook a treadmill test under medical supervision. After an habituation session followed by at least 5 min rest, subjects began walking at their pre-selected speed on a Powerjog treadmill with zero gradient. After 3 min, gradient was increased in increments of 1% every 2 min until the target heart rate was achieved (85% of maximum, which was calculated as 210−0.65*age in years). The test was discontinued prior to this if the test had reached 13 min in duration, if the subject wished to terminate it, or if she exhibited any of the standard symptomatic medical indications for discontinuation of an exercise...
ECG as required by the Ethical Committee. While the test was performed, heart rate was monitored through the three standard chest leads of an exercise ECG, and the subject breathed through a mouthpiece connected to a Mijnhardt Oxycon 4 on-line gas analysis system for measurement of oxygen uptake. Grip strength was measured using a RKK Grip Dynamometer at about the same stage in the study as the best of three attempts, on the non-dominant hand because of its reported association with bone density.\(^{17}\)

**Bone densitometry**

Six-monthly measurements of the lumbar spine over the first 2 years with further measurements at 3.5 years and in all but 15 women at 5 years were made using the Novo BMC Lab 22a dual photon absorptiometer (DPA).\(^8\) Three-and-a-half-year measurements were also made on most subjects, and 5-year measurements were made on all subjects using a Hologic QDR-1000 which eventually replaced the Novo BMC Lab 22a; all duplicate measurements on the Hologic and on the Novo at the 3.5 and 5 year time-points were made on the same day. Spine density data from the Novo and Hologic densitometers were expressed in g/cm\(^2\). At the time of transfer from the Novo to the Hologic, individual conversion factors were calculated from the ratios of the paired measurements to allow an individual’s Hologic data as well as her Novo data to be expressed in g/cm\(^2\) (Hologic units). Appendix 1 contains details of the methods used to confirm that the Novo and Hologic data could be validly combined for the purpose of individual longitudinal analyses of the bone density data, after application of these conversion factors.

The images from the bone density scans (Hologic) were reviewed as individual series. In some cases there was unequivocal evidence of degenerative spinal osteoarthritis, with patchy increases in bone density, irregularity of vertebral body outline and loss of intervertebral disc spaces. These subjects were classified as having osteoarthritis for the purposes of this paper before any analysis was begun.

As described previously, we made 6-monthly measurements of trabecular bone density in the distal radius\(^9,18\) using the ‘Isotom’ peripheral quantitative computed tomography (pQCT) system. After all subjects had had their fifth measurement, this system became defunct due to irremediable hardware problems. The ‘Isotom’ system was eventually replaced by the considerably more precise X-ray-based ‘Densiscan’ (Scanco) pQCT system, which was used to make measurements on the same radius twice, at approximately 5 years and 7 years post recruitment, before it was relocated to support a different project.

## Analysis of densitometry time-trend data

### General procedures for evaluating DPA/DXA data

The following procedure was followed in each series of Hologic spine scans to test for the homogeneity of response of the individual lumbar vertebrae L2, L3 and L4. For each vertebra in each woman, the BMD was plotted against time to give three individual vertebral time trends. Co-variance analysis was then used to test for differences in time trend between vertebrae. Subjects in whom a significant difference in time trend between vertebrae was identified were excluded from the analysis of the combined L2–L4 data, on the grounds that for some reason (e.g. local degenerative disease) one or more vertebrae may be behaving atypically, and that our intention was to track spinal bone loss in three vertebrae that behaved similarly.

For the remaining women, the analysis centred on the 35 who did not take HRT for at least 3 months. The bone density data for vertebrae L2–L4 combined were plotted against years since menopause, and fitted by means of regression analysis.

Because in some previous studies rates of bone loss were related to initial bone mass, statistical models incorporating bone density values log-transformed before analysis were compared with similar models in which the bone density data were treated untransformed. The guiding principles in the choice of statistical model were that it would be: biologically plausible; mathematically simple, only incorporating additional terms to describe differences between individuals on good biological grounds or on grounds of goodness of fit; and amenable to computation. For the spine, we chose a mathematically linear model. The simplest such model which adequately fitted the data used individual starting values for the logarithm of BMD at menopause, individual rates of loss of log(BMD) for each woman and a final term representing an initially slow rate of attenuation in the rate of loss of BMD which was the same for each woman and proportional to (time from menopause).\(^2\) If BMD loss was modelled without being first log-transformed, the model needed to be considerably more complicated to fit the data adequately (Appendix I).

### Procedures for fitting pQCT radius data

The radius data obtained from the ‘Isotom’ pQCT machine were presented previously, and the results of the previous analysis used, in which ‘trabecular bone plus soft tissue density’ data were first converted to ‘trabecular bone only’ data, log-transformed and analysed as described above to calculate relative rates of decline per year. Because the ‘Densiscan’
does not measure precisely the same volume of trabecular bone as the 'Isotom', the subsequent radius data obtained with the new pQCT machine had to be analysed separately. The data were first converted into 'trabecular bone only' data using conversion equations derived from multiple cross calibrations with the European Forearm Phantom (EFP)\cite{19,20} and relative rates of decline in bone density per year were then calculated from the ratio of each measurement pair after these data were first log-transformed.

The precision of the estimates of the rate of bone loss was obtained by calculating the 95% CI for the rate of loss. For models with individual rates of loss, the 95% CIs were based on the average of the standard errors for the individual rates of loss.

**Plasma and urine biochemistry**

Serum osteocalcin (Incstar UK) was measured at yearly intervals for 2 years, then at each densitometry visit until 7 years post-recruitment. Urine was collected at 6-month intervals after a 24 h gelatine-free diet for the first 2 years and then again at 3.5, 5 and 9 years post-recruitment. Urinary hydroxyproline\cite{21} was measured in a fasting early-morning urine specimen after discarding the first voiding of the morning. The ratio of hydroxyproline to creatinine was calculated. Changes in the biochemical data were examined by the same statistical regression techniques as those employed with the spinal bone densitometry data. The osteocalcin\cite{22} and hydroxyproline\cite{21} assays, as performed in our laboratory, were previously compared with reference methods for measuring whole-body bone formation and bone resorption using $^{85}$Sr as a tracer for calcium and making corrections for long-term exchange of radiotracer. A single measurement of osteocalcin predicted bone formation with a coefficient of variation of $\pm 30\%$ (equivalent to a bone formation rate of 1 mmol/day, $n=58,22$) and hydroxyproline predicted bone resorption with a similar coefficient of variation when measured repeatedly and averaged.\cite{21,22} However, the coefficient of variation of the mean of 18 successive hydroxyproline estimations was 5% compared to an estimated 21% for a single estimation at an excretion rate typical for a normal postmenopausal woman,\cite{21} so the predicted coefficient of variation in estimating bone resorption from a single hydroxyproline estimation in the present study was 36% of a typical subject’s average value in the population studied, equivalent to 1.2 mmol/day.

**Statistical analysis of the data determinants of bone loss**

It was possible to calculate two coefficients per woman representing, respectively, each individual’s rates of spinal bone loss and total hip bone loss in the early post-menopause. These coefficients were treated as dependent variables, and simple regression analysis was used to determine the statistical significance of relationships between these bone loss coefficients and continuous variables representing lifestyle, anthropometric and muscle-related characteristics. The significance of dichotomous variables as determinants of loss coefficients were assessed by unpaired Student's t tests. There is extensive documentation in the literature relating many of these variables to bone density or rates of bone loss. Therefore, in developing multiple regression models, a step-backwards approach was used, in which all variables significant at $p<0.05$ were entered together with those reported significant in the literature. The least significant was removed in sequence until all remaining variables were individually significant at $p<0.10$. Because the analyses for the total hip and the spine gave different results, MANOVA was used to calculate the significance of the contrasts between the two coefficients for each independent variable significantly related to either hip or spine bone loss rates. All statistical calculations were implemented on JMP v3.1 (SAS Institute).

**Results**

Three women withdrew before the second bone density measurement and are not reported on. Seventeen at some stage took HRT for 3 or more months, of whom one was judged to have OA of the spine, and one had non-parallel trends in the three measured lumbar vertebrae, leaving 15 sets of spine data which could be analysed. There were sixteen sets of hip data with three or more valid measurements in the HRT group. Of the 44 who did not take HRT, four were judged to have spine OA. A further four had non-parallel trends in their three measured lumbar vertebrae. One provided insufficient data for analysis, leaving 35 sets of spine data which could be analysed. An example is shown in Figure 1. Four of the 44 were excluded from the hip analysis because of insufficient data. Loss of one or more bone density results was due to death in one case; emigration to other countries in three cases; and removal to other parts of the country in four cases, making them only occasionally available for measurement.

**Women who did not take HRT**

Table 1 shows the characteristics at recruitment of the subjects studied. Using data from the European Quantitative assessment of Osteoporosis (QAO) study as our referent,\cite{19,23,24} Table 2 shows at two time-
Bone loss after menopause

**Figure 1.** Spine BMD data from one subject fitted by an exponential plus a constant (a class 2 model as defined in the appendix).

**Table 1a** Lifestyle and medical history responses

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyroid disease</td>
<td>1</td>
<td>42</td>
<td>43</td>
</tr>
<tr>
<td>Smoker</td>
<td>8</td>
<td>35</td>
<td>43</td>
</tr>
<tr>
<td>Cigarettes/day</td>
<td>–</td>
<td>–</td>
<td>ranged from 3–30</td>
</tr>
<tr>
<td>Any alcohol</td>
<td>34</td>
<td>9</td>
<td>43</td>
</tr>
<tr>
<td>Regular exercise</td>
<td>23</td>
<td>20</td>
<td>43</td>
</tr>
</tbody>
</table>

Table 1a points the proportions among women who did not take HRT, who were classified as having osteopenia (BMD at the measurement site less than the $-1SD$ value for young normal subjects) or osteoporosis (BMD at the measurement site less than the $-2.5SD$ value for young normal subjects). In the case of the spine data, the results were analysed for women who did not appear to have OA or discordant rates of individual bone loss in their three measured vertebrae.

Figure 2 shows the distribution of bone loss rate coefficients calculated from the spine data and Table 3 shows data on cumulative losses. The model predicts that these initial annual loss rates are attenuated slowly in each woman as time passes after menopause; but that later they become attenuated increasingly rapidly. Since some of our women had reached 14 years after menopause, their loss rates were calculated to have reduced by 36% at the end of the study compared to the beginning. The calculated imprecision (as a 95% CI) associated with any individual’s spine loss rate coefficient, was just under

**Table 1b** Descriptive statistics of muscle-related and anthropometric variables

<table>
<thead>
<tr>
<th></th>
<th>Min.</th>
<th>Max.</th>
<th>Median</th>
<th>Mean</th>
<th>SD</th>
<th>Normal distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean psoas area</td>
<td>330.2</td>
<td>919.0</td>
<td>588.4</td>
<td>601.9</td>
<td>129.4</td>
<td>yes</td>
</tr>
<tr>
<td>Grip strength</td>
<td>18.75</td>
<td>33.00</td>
<td>25.13</td>
<td>24.99</td>
<td>3.32</td>
<td>yes</td>
</tr>
<tr>
<td>VO$_2$ max</td>
<td>20.8</td>
<td>37.2</td>
<td>26.5</td>
<td>27.5</td>
<td>4.0</td>
<td>yes</td>
</tr>
<tr>
<td>Mean height (cm)</td>
<td>151.2</td>
<td>173.7</td>
<td>161.5</td>
<td>161.3</td>
<td>6.1</td>
<td>yes</td>
</tr>
<tr>
<td>Predicted BMI at 4 ysm</td>
<td>15.7</td>
<td>30.9</td>
<td>23.8</td>
<td>23.7</td>
<td>3.4</td>
<td>yes</td>
</tr>
<tr>
<td>Predicted weight at 4 ysm (kg)</td>
<td>50.16</td>
<td>83.58</td>
<td>63.50</td>
<td>64.44</td>
<td>8.23</td>
<td>yes</td>
</tr>
<tr>
<td>Rate of weight change (kg/year)</td>
<td>$-0.57$</td>
<td>$1.69$</td>
<td>$0.34$</td>
<td>$0.43$</td>
<td>0.47</td>
<td>yes</td>
</tr>
<tr>
<td>% of fat in body mass at 4 ysm</td>
<td>26.0</td>
<td>48.8</td>
<td>37.4</td>
<td>37.1</td>
<td>5.5</td>
<td>yes</td>
</tr>
<tr>
<td>Total body K+ at 4 ysm</td>
<td>1958</td>
<td>2831</td>
<td>2411</td>
<td>2407</td>
<td>224.5</td>
<td>yes</td>
</tr>
</tbody>
</table>

ysm, years since menopause.
Table 2  Numbers (percentages) of women with osteopenia and osteoporosis

<table>
<thead>
<tr>
<th></th>
<th>L2–4 at 4 ysm</th>
<th>L2–4 at 12 ysm</th>
<th>Neck at 4 ysm</th>
<th>Neck at 12 ysm</th>
<th>Troch at 4 ysm</th>
<th>Troch at 12 ysm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>17</td>
<td>6</td>
<td>19</td>
<td>13</td>
<td>27</td>
<td>24</td>
</tr>
<tr>
<td>(48.6%)</td>
<td>(17.1%)</td>
<td>(47.5%)</td>
<td>(32.5%)</td>
<td>(67.5%)</td>
<td>(60%)</td>
<td></td>
</tr>
<tr>
<td>Osteopenia (expected 16%)</td>
<td>17</td>
<td>25</td>
<td>19</td>
<td>26</td>
<td>13</td>
<td>16</td>
</tr>
<tr>
<td>(48.6%)</td>
<td>(71.4%)</td>
<td>(47.5%)</td>
<td>(65%)</td>
<td>(32.5%)</td>
<td>(40%)</td>
<td></td>
</tr>
<tr>
<td>Osteoporosis (expected 1%)</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>(2.9%)</td>
<td>(11.4%)</td>
<td>(5%)</td>
<td>(2.5%)</td>
<td>(0%)</td>
<td>(0%)</td>
<td></td>
</tr>
</tbody>
</table>

expected: in women aged 20–30 years.

half (0.48) of the 95% CI for the population distribution, suggesting that 23% of the apparent variance in the population distribution may be attributable to measurement imprecision.

Femur and radius BMD loss rates

We could not validly measure bone loss rates in the femur until the Hologic densitometer became available. In the case of most women, this was 3.5 years after recruitment; so we had less hip than spine data. The model which gave the best fit to the change in total hip BMD over time was a linear model of a similar form to that used in the spine, which had individual starting values and individual rates of loss of log (BMD), but no (time since menopause)$^2$ term. Twenty-eight per cent of the cent of the between-individual variance in rates of hip bone loss was attributable to measurement imprecision. Since the coefficient of the (time since menopause)$^2$ term in the spine model was the same for all women, the two models were comparable. The distribution of the hip coefficients is also shown in Figure 2.

For the two sets of radius trabecular bone measurements (made on non-identical sites), we used models of bone loss in which loss was calculated as a proportion of the initial value. The first set of measurements gave results suggesting that 40% of the inter-individual variance in loss rates was attributable to measurement imprecision. It was not possible directly to estimate the contribution of measurement imprecision to the second set of radius results, but the single-measurement in vivo precision of the ‘Densiscan’ was improved in relation to the earlier machine by an order of magnitude. For these exclusively trabecular bone sites in the radius, it is clear that rates of bone loss after menopause are proportionately much higher than in the other two sites in the spine and femur which are composed of both cortical and trabecular bone (Figure 2).

We then compared calculated loss rate coefficients between measurement sites. For the two adjacent, but not identical, radius sites studied at different times, there was a weak positive correlation ($R^2$ adjusted 0.14; $p=0.024$). When spine and hip loss rate coefficients were compared there was, unexpectedly, an inverse correlation ($R^2$ adjusted $-0.14$; $p=0.019$) (Figure 3). The radius coefficients were unrelated to those for the other two sites ($0.6 < p < 0.9$).

Statistical determinants of bone loss

Several factors related to anthropometric and muscle measurements, although not VO$_2$max (our measure of cardiovascular fitness), correlated significantly with bone loss (Table 4). Unexpectedly, however, rate of hip bone loss correlated inversely and significantly with rate of spine bone loss (Figure 3) and its determinants were different (Table 5).

The group contained only five smokers, yet a possible effect of smoking on spinal bone loss was detected. Since the small number of smokers made any further analysis of their data unreliable, it was decided to present data from the stepwise regression analyses on the non-smokers only. The analysis of this subset showed height ($p=0.0038$, negative estimate) and psoas area ($p=0.0031$, positive estimate), which were not themselves significantly related ($p=0.15$) as independent, significant determinants of spine loss rate ($R^2$ adjusted $=0.279$). Psoas area could be substituted by lean body mass ($p<0.05$).

Analysis of the hip loss rate coefficients showed that indices related to body mass index ($R^2$ adjusted $=0.193$, $p=0.0061$) were the only significant determinants when stepwise regression was applied. MANOVA, simplified to include just body mass index (weight/height$^2$), and applied to the hip, spine and the two sets of consecutive radius data showed a significant contrast between the hip and the other two sites ($p=0.019$). The estimated positive effect of BMI in reducing bone loss was, in relative terms, respectively 11 times and six times larger over the two successive measurement periods at the radius than at the spine.

As described previously,$^9$ we modelled the evolution with time of plasma osteocalcin (a biochemical marker of bone formation) and found it to rise
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improved when each women had her own individual intercept, although no further improvement occurred when women were allowed their own individual rates of decline. Therefore, rates of bone loss from the spine, hip and radius were modelled with the calculated hydroxyproline:creatinine intercept at menopause as the independent variate. However, none of the four regressions was statistically significant ($0.053 < p < 0.3$).

Baseline cervical cytology was not predictive of bone loss rates ($0.15 < p < 0.90$ according to measurement site) and all women not on HRT showed increasing proportions of basal cells on subsequent samples taken biannually on two further occasions.

**Women who took HRT**

This was a group who started taking HRT at various times after entry into the study. Some have continued to the present time and others have already stopped taking HRT. Overall in this sub-group, there was no trend in spine bone density or in hip bone density in either direction.

**Discussion**

The precise measurement of rates of postmenopausal bone loss rates in the spine and hip is not straightforward. It seems likely that with the advent of DXA, future improvements in precision will be modest unless ways can be found to improve the reproducibility of positioning, since photon fluxes generated by current generation densitometers allow very precise measurements in vitro over short time periods. Of the equipment used in the present study, the Novo DPA densitometer is now obsolescent and gives relatively poor precision, which may help to explain the modest separation of subjects with respect to postmenopausal bone loss rates reported after 5 years of the study.

We have extended the study, and with over a decade’s worth of data in most participants, have measured individual bone loss rates in the three key fracture sites of spine radius and hip with much better precision than was previously possible. Nevertheless, according to measurement site, from 23–40% of the estimated between-subject variance in the calculated individual loss rate coefficients was attributable to measurement imprecision, reducing somewhat the potential of our explanatory variables to predict rates of bone loss precisely.

The data for the spine and radius, but not that for the hip, suggest an attenuation in the rate of bone loss with time after menopause. For the radius, this confirms earlier work of Johnston and his colleagues but we were not able to confirm the observations of Harris and Dawson.

Figure 2. Distributions of loss rate coefficients from: a spine; b hip; c radial trabecular bone (earlier measurements); and d radial trabecular bone (later measurements). Note different scales. Negative coefficients indicate bone loss as fractions of bone per year. The two radius distributions were significantly non-normal (Shapiro-Wilk’s test) and negatively skewed.
Table 3 Descriptive statistics of bone loss after menopause

<table>
<thead>
<tr>
<th>Site</th>
<th>Years post menopause (span)</th>
<th>Min. % change</th>
<th>Max. % change</th>
<th>Median % change</th>
<th>Mean % change</th>
<th>SD % change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spine</td>
<td>3–13 yrs</td>
<td>−19.0</td>
<td>4.0</td>
<td>−8.6</td>
<td>−8.3</td>
<td>4.87</td>
</tr>
<tr>
<td>Hip</td>
<td>6–13 yrs</td>
<td>−13.4</td>
<td>4.3</td>
<td>−2.9</td>
<td>−2.8</td>
<td>3.96</td>
</tr>
</tbody>
</table>

Both distributions are normal ($p > 0.5$).

Table 4 Correlations between muscle-related measures and the coefficient of lumbar spine bone loss

<table>
<thead>
<tr>
<th></th>
<th>$p$</th>
<th>Estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean psoas area</td>
<td>0.0467</td>
<td>0.0000136</td>
</tr>
<tr>
<td>Grip strength</td>
<td>0.3104</td>
<td></td>
</tr>
<tr>
<td>VO$_2$ max</td>
<td>0.9792</td>
<td></td>
</tr>
<tr>
<td>Mean height (cm)</td>
<td>0.0584</td>
<td>−0.000301</td>
</tr>
<tr>
<td>BMI (wt/ht$^2$)</td>
<td>0.0113</td>
<td>0.000698</td>
</tr>
<tr>
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<tr>
<td>change (kg/year)</td>
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<tr>
<td>% of body mass</td>
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<td>0.000440</td>
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<tr>
<td>fat</td>
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<tr>
<td>Hydroxyproline/creatinine</td>
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</table>

Estimate, coefficient relating the determinant of interest to log(BMD) change per year, expressed in units of measurement.

Hughes$^{26}$ and Pouilles$^{27}$ that bone loss rate declines significantly with time since menopause in the proximal femur. In Pouilles’s study, women were recruited at different times after menopause and studied for an average interval of 42 months; examination of his data suggests that much of the attenuating effect on femoral bone loss of time since menopause occurred in the first 5 postmenopausal years, which in our study had passed for all our subjects by the time we were equipped to measure the proximal femur. Harris$^{26}$ used a similar design, but included additionally much older women. From the data in Figure 1, it may be calculated that relative to the mean value for the total hip, the initial rate of spinal bone loss after menopause appeared to be three times larger, while the rate of bone loss from the distal trabecular bone of the radius an average of 4 years after menopause was 10-fold larger, declining to 6-fold larger some 4 years after that. This was unsurprising in view of the fact that the radius measurement site was exclusively trabecular (cancellous) bone, and bone turnover is much less rapid in cortical bone. The data for hydroxyproline:creatinine was consistent with the concept that, in at least parts of the skeleton, bone resorption slows gradually as time passes after menopause.

In other respects, the biochemical data using markers developed at least a decade and a half ago offered no prospect that such simple tests could offer a useful guide to the identity of women with fast bone loss. However, our data provide some insights. Because we have shown that rates of bone loss are so variable in normal women after the menopause when contrasted between measurement sites, it is
unrealistic to expect biochemical markers, however closely they reflect bone formation or resorption in the skeleton as a whole, to reflect bone loss in specific skeletal sites, unless these sites represent a high proportion of total bone turnover. In particular, the radius, as a bone of comparatively modest size which is largely composed of cortical, rather than trabecular bone, is unlikely to contribute much to the circulating pools of biomarkers that are sampled in pursuit of the biochemical prediction of fast bone loss. This probably explains the mixed results obtained by different workers who have performed prospective marker studies using this bone as a referent.\(^{28}\)

Although we found no close predictors of fast bone loss, we were able to demonstrate that spinal bone loss is related to several indices, which might help to explain part of the epidemiology of vertebral fracture, and contribute to the development of population-based as distinct from patient-based prevention strategies. Body mass index was associated positively with reduced bone loss in the spine and radius. In recent epidemiological work we have shown that body mass index is quite strongly protective against vertebral deformity in European women and men,\(^{29}\) and that this probably has its effect through increasing bone density.\(^{2}\) Our data suggest that for the spine and radius (but not the hip), body mass index, probably as a measure of relative obesity, is associated with a skeleton that is relatively protected against postmenopausal bone loss in its first decade and a half. This is consistent with the concept that after menopause, adipose tissue as the main source of oestriol may contribute usefully to slowing bone loss, although another explanation is that heavier women subject their skeletons to increased mechanical loading.

In support of this, psoas area (after adjusting for body height) was a second protective determinant for the spine. The psoas is one of the main flexors of the lumbar spine as well as contributing to hip flexion. The biological significance of this finding is that it might indicate a continued role for mechanical loading of the spine in its protection against bone loss after menopause, even though there is increasing evidence that experimentally oestrogens are important in modulating the effects of loading on bone.\(^{30}\) There is evidence in young athletes who develop athletic amenorrhoea that increased mechanical loading can provide some site-specific, if incomplete, protection against bone loss.\(^{31}\)

A negative finding of our study was the absence of a protective effect of high VO\(_2\)max. We only measured this once in the cohort, at an average of 5–6 years post-menopause. This index of aerobic fitness is reported after adjustment for body size and is affected by the relative proportions of body fat (having a low aerobic metabolism) which contributes to a substantial dispersion within the normal population. Perhaps if it had been possible to measure VO\(_2\)max a second time and estimate its rate of decline, a relationship with bone loss would have been revealed. However, the suggestion of Pocock et al.\(^{15,16}\) that VO\(_2\)max was protective, was based on indirect methods of estimating fitness rather than direct measurements such as we performed.

We did not expect the bone loss rates for spine and hip to show a negative correlation. Pouilles,\(^{27}\) with his different study design, found positive correlations between femoral and spinal loss rates, but if it is accepted that bone loss declines with time since menopause, this was an inevitable consequence of his study design, which included fast-losing women studied in the first three post-menopausal years and slow-losing women studied a decade later in the evolution of their menopause. Hansen et al.\(^{7}\) used a similar recruitment design to ours, but did not report on correlations between individual rates of bone loss at different sites. Nor, like us, were they able to begin DXA hip measurements until relatively late after menopause. We have no explanation for our finding that when adjusted for time since menopause, hip and spine loss rates tend to correlate inversely. The only other circumstance under which we have encountered a similar phenomenon is during treatment of women with postmenopausal osteoporosis with parathyroid peptide hPTH 1-34 when no oestrogen replacement is given concurrently.\(^{32}\)

This study had a number of limitations. Its size was comparatively small, being governed initially by resource constraints associated with the expense of the slow procedure of DPA with its expensive isotope sources. It was not designed to measure precisely attributable risks in populations, but rather to identify any common determinants of fast bone loss which might prove useful in managing individual patients. Therefore, our observations on the protective effects of BMI for the spine need confirmation in a larger population study. There were a number of changes of equipment, which were inevitable, in light of the limited lifespan of such equipment in relation to the study’s intended duration. This led to an interruption in the radius measurements and the need for cross-calibration procedures between equipment at the other sites. The success of the cross-calibration between the Hologic and Novo equipment in retrospect was fortuitous, in the light of the problems experienced in a similar comparison by Peel and Eastell\(^{33}\) between Hologic and Lunar DXA equipment. In Peel and Eastell’s study, both sets of equipment were capable of considerably better measurement precision than our Novo machine, making
their Bland and Altman analysis more critical than ours. Inevitably in a long duration cohort study, many other investigations performed at baseline used techniques that are no longer state of the art 12–15 years later. However, retention of our cohort has been high, and initial compliance was also good. This is the first longitudinal study which has followed spinal bone loss after menopause for as long as 13 years in some individuals, and the first to compare in individuals its long term evolution with that of bone loss at other key sites.

In conclusion, bone loss can be considerable in normal women after menopause, the fastest-losing 20% of normal women showing more than a 13% decline in their spinal bone density in the decade from the third to the thirteenth postmenopausal year. The equivalent figure for the hip was over 6.5%. In the radius, our data are consistent with those of both Hui and He in suggesting variability over time in its rate of bone loss. We were not able to show similar unpredictability of loss rates with time in the spine and hip, perhaps because of their slower rates, and the limitations as to measurement precision of DXA applied to the axial skeleton. Our study has explained in part the intrinsic difficulties of predicting fast bone loss from biochemical measurements in normal individuals, who clearly lose bone at different relative rates in different parts of the skeleton. Finally, our results provide a possible explanation for the role of body leanness, in predisposing to vertebral deformity, and suggest that mechanical loading modulated by muscle contraction could be important in protecting the postmenopausal skeleton. A previous study has found a protective effect of obesity against spinal bone loss, but in our study more than 75% of subjects had a body mass index under 26, indicating that this was not an obese population. From a public health perspective, there might be advantages after menopause for the skeleton, particularly the spine, in avoiding excessive leanness. Although cardiovascular fitness was unrelated to rates of bone loss, our subjects showed a rather narrow range of VO2 max values, with not one subject having a value above the bottom quartile of the range seen in a group of postmenopausal veteran endurance athletes studied contemporaneously (R. Wolman, unpublished observations). Maintenance of muscle power may be more relevant to bone health than cardiovascular fitness.

Acknowledgements

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References

Bone loss after menopause


Appendix I: procedures for validating and combining the longitudinal analysis of bone-density time-trend data obtained on the Novo and Hologic machines

Effects of measurement imprecision

In our hands, there was an approximately three-fold difference in short-term precision (coefficient of variation) between measurements of lumbar spine bone density obtained with the Hologic and Novo equipment. To avoid biasing our estimates of rates of change in bone density, it was therefore necessary to adjust for this difference in precision by weighting the data statistically to allow for the lesser precision of each DXA datum. The desired weighting factor was the ratio of the long-term variances of the two techniques adjusted to the same scale, so this was calculated as the square of the ratio of the two coefficients of variation. The long-term imprecision of L2-4 DXA densitometry determined in post-menopausal women was taken as the referent and each DXA datum was assigned a weight of 1. It was
assumed that DXA and DPA imprecision had two components, a short term and a long term, which were independent, so that their variances were additive. For DPA the short-term imprecision component (c.v.%) used in our calculations was the figure of 2.6% obtained by the technique’s originators Kröllner and Pors Nielsen, although considerably higher than for DXA. We assumed the long-term component was the same for both DXA and DPA, because it was thought to be principally dependent on biological factors and positioning of the subject. For DXA, we used our own short-term precision data, and the long-term variance component was calculated by difference between long- and short-term overall variances in the L2–4 data of Fuleihan for post-menopausal women. The weighting factor for the DPA data relative to the DXA data calculated in this way was 0.3054, being inversely proportional to the ratio of the calculated long-term scaled variances for the two techniques.

Validity of combining Hologic and Novo spine data

Next, we considered whether it was valid to combine Novo and Hologic data in the same time trend analysis, since there is some evidence that this may not be valid with other combinations of instruments. A regression analysis was performed on the short-term (1–2.4-year) rates of change in bone density based on data taken at just the two time points from women who had both DXA and DPA measurements at 3.5 and 5 years. This showed no significant difference from the line of identity in the regression equation relating DPA-derived rates to DXA-derived rates. We therefore performed a Bland and Altman analysis of the relationship between the individual differences in slope for the paired Novo and Hologic data. We used the best estimate of the true slope by weighting these calculated means to allow for their different measurement precisions, as recommended by Altman. We first excluded three women who were judged to have developed probable osteoarthritis on the basis of inspecting the Hologic images. This was because the procedures for editing scan images were different on the two systems, and this diagnosis was the main trigger to image editing in our study prior to analysis.

Our Bland and Altman analysis, which was based on 25 subjects who never took HRT and 8 who did (so included both subjects whose bone density was increasing and subjects in whom it was decreasing), showed no significant trend ($p = 0.68$) and no significant difference in trend between HRT and non-HRT groups ($p = 0.06$). The median difference between the DPA and the DXA trend data was 0.000 g.cm$^{-2}$y$^{-1}$ (non-HRT group).

Statistical time-trend models for calculating rates of bone loss in the spine and hip

Two classes of model were compared which described the decline in bone density with time after menopause. Both classes were previously compared by Johnston and his colleagues in cross-sectional and longitudinal studies of radial bone loss in post-menopausal women. The first class of model (class 1) fitted a polynomial function of time since menopause (or chronological age) to the BMD data, before or after log-transformation. In these class 1 models, each woman’s data were fitted with an individual starting value to allow for the individual differences in BMD between women. To allow for the differences in initial bone density values between individuals, co-variance analysis was applied. The simplest model was investigated first, in which bone loss, which was the same for each woman, was assumed to be a simple function of time since menopause. Tests were then applied using ANOVA to see if the data were better fitted by allowing individual rates of bone loss to differ. A further analysis was performed in which bone loss was allowed to be curvilinear. This was achieved by fitting the data to the two independent variables ‘time’ and ‘time squared’. Again, tests were performed to see if the data were better fitted by allowing the individual patients to have different rates of bone loss as functions of the square of time since menopause. In comparing models in which BMD and the logarithm of BMD were alternative dependent variables, preference was given to models that could be fitted with fewer terms (and thus used fewer statistical degrees of freedom) unless a more complex model significantly improved the fit to the data.

In the other class of model (class 2), data from each individual woman was fitted to a non-linear function comprising an exponential decline in BMD after menopause becoming asymptotic to a post-menopausal constant value. To allow a comparison of the two classes of model, for the class 2 model the residual sums of squares were calculated by cumulating the residuals derived from the individual fits to the data of each woman.

Choice of model to fit lumbar BMD loss rates

When the various models of BMD time trend were compared in women classified as not having taken HRT, it was found among the models of L2–4 spinal bone loss that a considerably simpler model could be fitted when the logarithm of BMD was fitted in place of BMD untransformed, because the latter required individual terms for each woman in (time since menopause)$^2$ whereas for the model fitting the
logarithm of BMD, including such individual terms did not improve the fit, and the adjusted $R^2$ value was improved compared to the model fitting the untransformed BMD data. The simplest class 1 model which adequately fitted the data incorporated individual starting values for $\log(BMD)$ at menopause, individual rates of loss of $\log(BMD)$ for each woman and a final term representing an initially slow rate of attenuation in the rate of loss of BMD which was the same for each woman and proportional to (time since menopause)$^2$. This model gave a near-identical goodness of fit to the class 2 (non-linear) model (Figure 1), so it was not possible to establish a preference on those grounds, but the class 1 model was much more tractable computationally, since it did not require iterative fitting.