Bone Mineral Density and Biochemical Markers of Bone Turnover in Healthy Elderly Men and Women

Elizabeth A. Krall, Bess Dawson-Hughes, Kathryn Hirst, J. C. Gallagher, Sheryl S. Sherman, and Gail Dalsky

Background. Osteoporosis risk in middle-aged women is twofold greater than that in men, and the difference increases with age. Gender differences in bone mineral density, estimated rates of bone loss, and usefulness of markers of bone metabolism for predicting bone density have not been well described in healthy elders aged 65 and above. The purpose of this cross-sectional analysis was to describe associations of bone mineral density at the hip, spine, and whole body with age, serum osteocalcin, and urinary N-telopeptide crosslinks of Type I collagen in healthy elderly men and women.

Methods. A total of 1,087 healthy adults (273 men and 814 women) aged 65 to 87 years were enrolled in a collaborative study at 3 sites: Tufts University (Boston, MA), University of Connecticut Health Center (Farmington, CT), and Creighton University (Omaha, NE). Bone mineral density (BMD) at three regions of the hip, the lumbar spine, and whole body was determined by dual-energy x-ray absorptiometry. Serum osteocalcin was measured by immunoassay, and measurement of N-telopeptide crosslinks (Ntx) in urine was made using an enzyme-linked radioimmunoassay (ELISA).

Results. Among women, the age-related decline in BMD at all non-spine skeletal sites was significantly different from zero, with the largest decline seen at the femoral neck (−.0038 g/cm²/y, p < .001) and the smallest at the trochanter of the hip (−.0023 g/cm²/y, p = .03). Among men, the changes at all non-spine sites were not significant. In both sexes, spine BMD tended to increase with age (men, +.0045 g/cm²/y, women, +.0003 g/cm²/y). Serum osteocalcin and urinary Ntx were inversely related to BMD at all skeletal sites, but the weakest associations were observed at the spine. Individuals whose values of both osteocalcin and Ntx were in the lowest quartiles of the respective sex-specific distributions had mean femoral neck BMD that were 11% higher than individuals with marker values in the highest quartiles.

Conclusions. These findings suggest that age-related decreases in BMD may vary by gender and skeletal site. Determinations of osteocalcin and N-telopeptide crosslinks at a single point in time may potentially be used as indicators of current bone status, particularly at non-spine skeletal sites.

The risk of osteoporotic fracture rises dramatically after age 65. Bone mineral density (BMD), an important predictor of fracture risk, decreases with age in both men and women, but it is not clear if the loss of bone density among the elderly occurs intrinsically or if it is related to increased prevalence of other risk factors. Previous studies consistently suggest that hip density continues to decrease throughout life (1-8). However, it is not certain if rates of decline are equivalent in men and women. No gender differences were observed in two cohorts of elderly subjects (3,4), but other data indicate that bone loss from the femoral neck and trochanter may occur in women at approximately twice the rate in men (6,7).

Age-related patterns in BMD of the spine are more difficult to define. In the anterior-posterior projection of the spine, soft tissue calcifications in the scan region erroneously elevate the apparent bone mineral density. Significant inverse relationships were observed between spine BMD and age among women in many (6-9), but not all cross-sectional analyses (5). No decrease in spine BMD with age has been observed in men (6,7). Longitudinal studies indicate there may be no significant loss in BMD at the spine in persons over age 65 of either gender (3,5). Few data are available on whole body BMD in this age group.

Several biochemical markers of bone turnover have been identified. Osteocalcin, a vitamin K-dependent protein synthesized by the osteoblast, is the most abundant noncollagenous protein in the bone matrix. A portion of newly made osteocalcin is released into the circulation and serves as a valid marker of bone formation. Serum osteocalcin level is correlated with histologic measures of bone formation (10) and is increased in diseases with accelerated bone turnover rate (11,12). Markers of bone resorption include cross-linked amino-terminal peptides (N-telopeptide crosslinks) of Type I collagen, byproducts of the degradation of mature bone collagen that are excreted in the urine. Urinary excretion of this resorption marker increases at menopause (13) and shows marked decreases in response to treatment with bone resorption inhibitors (14-17). The usefulness of osteocalcin and N-telopeptide crosslinks in predicting bone density in a healthy population is less well established.

We present here a description of the associations of bone mineral density at the hip, spine, and whole body with age.
and with levels of serum osteocalcin and N-telopeptide crosslinks in healthy men and women over age 65 who participated in the National Institute on Aging’s STOP/IT study (Sites Testing Osteoporosis Prevention/Intervention Treatment).

METHODS

Study design and subjects. — The purpose of the STOP/IT study was to determine the effectiveness of three different interventions to reduce bone loss from the femoral neck among healthy men and women aged 65 and older. The interventions and study sites were calcium and vitamin D supplementation (Tufts University), weight-bearing aerobic exercise (University of Connecticut), and hormone replacement therapy with estrogen and progestin with or without 1,25-dihydroxyvitamin D (Creighton University, Omaha, NE). Prior to enrollment, each subject underwent laboratory tests, medical history, and a physical examination. Exclusion criteria common to all sites included current or terminal illnesses such as cancer or renal disease; nonambulation; abnormal urine calcium and creatinine clearance; and conditions or medications that affect calcium metabolism or bone mineral density, including estrogen, progestin, androgen, systemic fluoride, or bisphosphonate. Each study site had additional exclusion criteria related to the specific intervention. For inclusion at Tufts, femoral neck BMD at screening was not more than 2 standard deviations below an age-matched reference mean (i.e., BMD not lower than .53 g/cm² in women and .63 g/cm² in men). The acceptable range of femoral neck BMD at Connecticut was an average of the right and left hip BMD between 0.6 and 0.9 g/cm² and the range at Creighton was .55 to .885 g/cm². The study subjects who met all eligibility criteria were comparable among the three study sites with respect to the prevalence of selected chronic conditions. Diabetes was reported by 3–7% of men and 3–4% of women, hyper- or hypothyroidism by 1–3% of men and 17–20% of women, and a history of any cancer by 16–22% of men and 10–15% of women.

Measurements. — Bone mineral density of the femur, whole body, and lumbar spine was measured by dual-energy x-ray absorptiometry (Lunar Model DPX-L) using standardized protocols for uniform subject positioning, scan mode, and scan analysis. Femur scans were performed in duplicate and the mean value was used in all analyses. In vitro quality control procedures were performed to ensure comparability of BMD measurements across study sites. A single hip phantom consisting of bone ash embedded within a 12 cm-thick block of epoxy material in a hip-shaped configuration was scanned on each densitometer at multiple time points in the trials. The results of five repetitions of the femoral neck region of the hip phantom were 1.039 ± .009 g/cm² (Tufts), 1.031 ± .006 (Connecticut), and 1.038 ± .006 (Creighton). Trochanter and Ward’s triangle BMD varied by no more than 1.1% and 1.9%, respectively, among study sites. In vivo BMD variability was estimated quarterly from paired hip scans of 10 subjects. Within-subject coefficients of variation of femoral neck BMD for these paired scans were similar across sites and never exceeded 1.53%.

Blood was drawn by venipuncture after a minimum 8-hour fast. To reduce diurnal variation, all blood samples were collected between 7:00 and 9:30 a.m. Urine was collected over a 24-hour period. Aliquots of serum and urine were frozen at −70°C for up to 12 months and subsequently shipped without thawing to a central laboratory for analysis. Serum osteocalcin was measured in triplicate samples by immunoassay (Incstar Corp., Stillwater, MN). The intra- and intra-assay coefficients of variation (CV) are each 4%, and the lower limit of detectability is .72 ng/ml. Evaluation of six serum samples stored at −70°C for up to 120 weeks showed no significant deterioration of osteocalcin activity over time. Because the enrollment periods at each study site extended over different periods of time, the osteocalcin measurements were made in 16 batches, each of which contained control samples to monitor reliability. Urinary creatinine concentration was measured with standard laboratory procedures. Measurement of N-telopeptide crosslinks in urine was made using ELISA-based (enzyme-linked radioimmunoassay) Osteomark kits (Ostex International, Seattle, WA). The inter- and intra-assay CVs were each less than 10%. The assay is calibrated with standard amounts of human bone collagen and the results are expressed as nanomoles of bone collagen equivalent corrected for creatinine concentration (nmol bce/mmol CR).

Statistical methods. — Relationships of BMD values with age, serum osteocalcin, and N-telopeptide crosslinks were evaluated with Pearson correlation coefficients and linear regression models. Differences in means among the study sites were evaluated with t-tests and analysis of variance. P-values less than .05 were considered statistically significant.

Significant differences in baseline BMD, observed among the three study sites, likely arose from different eligibility criteria (Table 1). To evaluate these differences and justify pooling the data, femoral neck BMD was regressed on age, study site, and the interaction of age and site under three case scenarios. The first scenario included all subjects from all sites with valid femoral neck BMD data (273 men, 814 women). The second analysis was restricted to 224 men and 751 women whose femoral neck BMD fell between the minimum and maximum values that were common to all three sites (.655 to 1.0485 mg/cm² in men, and .575 to .8985 mg/cm² in women). The third analysis included individuals with comparable femoral neck BMD as stated above, as well as a common range of age (78 y or less), body mass index (less than 33 kg/m²), and smoking status (all nonsmokers) and was performed on 199 men and 604 women. Study site was a significant predictor of BMD among males and females in the case with no restrictions, and the interaction between site and age was significant in females. By restricting femoral neck BMD, all site and age-site interaction terms became nonsignificant and remained so in the third case, when similar eligibility criteria were applied. We concluded that the differences in femoral neck BMD among study sites were due to the differences in eligibility and that pooled analyses unadjusted for study site were appropriate.
Table 1. Mean Baseline Bone Mineral Density of Men and Women by Study Site (Mean ± SD)

<table>
<thead>
<tr>
<th></th>
<th>Women</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>Men</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tufts</td>
<td>Connecticut</td>
<td>Creighton</td>
<td></td>
<td></td>
<td></td>
<td>Tufts</td>
<td>Connecticut</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>246</td>
<td>79</td>
<td>489</td>
<td></td>
<td></td>
<td></td>
<td>199</td>
<td>74</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>71 ± 5</td>
<td>70 ± 4</td>
<td>71 ± 4</td>
<td>n.s.</td>
<td></td>
<td></td>
<td>70 ± 5</td>
<td>70 ± 4</td>
<td>n.s.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Femur BMD (g/cm²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neck</td>
<td>0.81 ± 0.11†</td>
<td>0.76 ± 0.08</td>
<td>0.76 ± 0.09</td>
<td>&lt;.001</td>
<td></td>
<td></td>
<td>0.96 ± 0.13</td>
<td>0.91 ± 0.09</td>
<td>0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ward’s triangle</td>
<td>0.65 ± 0.13†</td>
<td>0.62 ± 0.10</td>
<td>0.62 ± 0.11</td>
<td>.003</td>
<td></td>
<td></td>
<td>0.77 ± 0.14</td>
<td>0.72 ± 0.10</td>
<td>.002</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trochanter</td>
<td>0.72 ± 0.13†</td>
<td>0.69 ± 0.10</td>
<td>0.69 ± 0.12</td>
<td>.027</td>
<td></td>
<td></td>
<td>0.95 ± 0.15</td>
<td>0.91 ± 0.12</td>
<td>.068</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole body BMD (g/cm²)</td>
<td>1.02 ± 0.09</td>
<td>1.01 ± 0.09</td>
<td>1.01 ± 0.09</td>
<td>n.s.</td>
<td></td>
<td></td>
<td>1.20 ± 0.09 II</td>
<td>1.18 ± 0.08</td>
<td>.030</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lumbar spine BMD (g/cm²)</td>
<td>1.05 ± 0.20§</td>
<td>0.99 ± 0.17</td>
<td>1.01 ± 0.18</td>
<td>.009</td>
<td></td>
<td></td>
<td>1.29 ± 0.21</td>
<td>1.18 ± 0.19</td>
<td>&lt;.001</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*p-value* for difference among means.
†BMD at Tufts is significantly different from both other locations.
§BMD at Tufts is significantly different from Creighton.

RESULTS

Men in the STOP/IT population had higher mean bone mineral density than women at all skeletal sites (Table 2). In contrast, the average levels of osteocalcin and N-telopeptide crosslinks were lower in men by 17% and 38%, respectively. All of the differences in BMD and biochemical markers between the sexes were significant at p < .001.

Bone mineral density declined with age but the estimated rates of change varied by gender and skeletal site. The magnitude of decline in BMD at the femoral neck and Ward’s triangle was similar in men and women. Among women, femoral neck BMD decreased by 0.0038 g/cm² (p < .001) for each year of age and among men, by 0.0028 g/cm²/y (p = .11), as shown in Figure 1. The estimates of the rate of decline in BMD at Ward’s triangle were identical in men and women (slope = −.0034 g/cm²/y), but the decline was statistically significant only among the women (r = −.12, p < .01 in women; r = −.11, p = .11 in men). These slopes represent annual declines of approximately 0.3% to 0.5% of the average BMD at these skeletal sites.

At the trochanter and whole body sites, opposite patterns were observed between the sexes. BMD at the trochanter declined by 0.4% per year of age in women (slope = −.0034 g/cm²/y), but minimal nonsignificant changes were observed in men (slope = .0002 g/cm²/y, r = .01, p > .50). Whole body BMD data of individuals are shown in Figure 2, and averages by age group in Table 3. Whole body BMD decreased with age in women (slope = −.0038 g/cm²/y, p < .001) but not in men (slope = −.0010 g/cm²/y, p > .50). Spine BMD tended to increase as one got older (Figure 3), but the slopes were not significantly different from zero in either gender (men, slope = −.0045 g/cm²/y and women, .0003 g/cm²/y).

Body mass index (BMI) accounted for a small portion of the variability in the relationship between BMD and age, but the results were not changed substantially by including BMI in the regression.
Serum osteocalcin and urinary Ntx levels were each inversely related to BMD in men and women; however, the associations with spine BMD tended to be consistently weaker than with other skeletal sites (Table 4). The difference in mean femoral neck BMD between individuals with osteocalcin (OC) values in the lowest quartile and those with values in the highest quartile was 8% in each sex. The corresponding differences between quartiles of the Ntx distribution were 7% in men and 8% in women. When examined by joint distributions of OC and Ntx, the difference in femoral neck BMD between low OC-low Ntx and high OC-high Ntx groups increased to 11% in both women (Figure 4) and men (Figure 5). Differences in BMD at the other regions of the hip, the whole body, and spine between extreme categories of OC and Ntx were also within the range of 8% to 16%.

DISCUSSION

The results of this study suggest that loss of bone mineral density (BMD) from the femoral neck and Ward’s triangle regions of the hip occurs at similar rates in older men and women. The decrements in BMD at the femoral neck, Ward’s triangle, and trochanter represent estimated annual losses of 0.3 to 0.5% of the average bone mass that remained at age 65. There appeared to be no gender differences in the
magnitude of the age-related decrease in femoral neck and Ward’s BMD. However, lack of statistical significance in the men’s values may be due to the smaller sample size and reduced statistical power. These findings are in general agreement with previous cross-sectional studies of elderly men and women, although the estimates of decline in BMD are somewhat lower in the STOP/IT population. In the Framingham, MA community over age 65, estimated decreases in femur BMD ranged from .45% to .88%/year in men and from .53% to .94%/year in women (4). Greenspan et al. (5) reported decrements in BMD of .76% per year at the femoral neck and .71% per year at Ward’s triangle in cross-sectional analyses of healthy ambulatory women aged 66 to 93 years. In other populations, estimates of annual decline in BMD from the hip among older men and women have approximated or exceeded 1% per year (2,8). Longitudinal studies have confirmed that bone loss from the hip continues into advanced ages (1,3,5), and a recent study reported accelerated rates of femoral BMD loss among women over 75 compared to those aged 65 to 69 years (1).

Cross-sectional studies of loss of BMD, such as the present one, may underestimate the rate of BMD loss. This inaccuracy may be partly due to biases in subject selection. All STOP/IT subjects were volunteers and are not necessarily representative of elderly individuals in the population. These volunteers were medically healthy and were willing to commit to 2 or 3 years of participation. Thus, they likely represent the most healthy segment of the elderly population, especially at the upper end of the age range. However, volunteer and healthy subject biases do not entirely explain why estimated rates of decline in the STOP/IT men and women were lower than in other studies that also comprised healthy elderly volunteers (5). A cohort effect might also have produced the results observed in the present study. For example, secular trends in height and body habitus, two strong determinants of bone density, would predict that individuals born in successive years will be taller, heavier, and have denser bones throughout their lifetime compared to those born earlier. Adjustment for body mass index had no substantial effect on the patterns of BMD change at the hip or any other skeletal site.

Age-related decreases in spine BMD in this age group have not been consistently observed (5-9). Data from longitudinal studies suggest that lumbar spine BMD values fail to decrease, probably because of artifacts and calcifications in the spine scan field (3,5). The results of the present study support these prospective findings.

The decline in whole body BMD varied by gender in this study. Among women, whole body BMD decreased by 0.4% per year. In contrast, the change in whole body BMD among men was negligible. The disparity in patterns of whole body BMD loss by gender may be a true observation, or it is possible that men and women self-selected differently for this study. Older women may be more conscious than men of their own risk of osteoporosis and more willing to seek information about their bone density, with the result that female volunteers could be overrepresented by those who have one or more risk factors for low bone density. If this were a substantial bias, however, we should have also seen a sex difference in the decrease of BMD at all regions of the hip. Prospective studies will provide more informative data on rates of whole body bone loss in the elderly and determine
if women do lose bone mineral more rapidly than men. To our knowledge, these are the first whole body BMD data presented for a large group of men and women over age 65.

The strength of the inverse relationship between initial BMD and serum osteocalcin in the STOP/IT population is similar to findings reported by others (7,18). These consistent findings in various studies suggest that serum osteocalcin is an indicator of bone status at most skeletal sites. Correlation coefficients of variance in BMD and osteocalcin appeared strongest at the whole body and hip, and the proportion of variance in BMD explained by a single determination of osteocalcin ranged from 11.7% at the whole body to 2.7% at the spine. The tendency for correlations between biochemical markers and BMD to be weaker at the spine than other skeletal sites also supports the assumption that non-bone, calcified soft tissue was detected in the spine scans. Garnero et al. (13) reported strong significant correlations between BMD at several sites and Ntx among women more than 20 years postmenopause. Although correlations of BMD with the bone resorption marker, N-telopeptide crosslinks, were weaker in the STOP/IT population and generally less strong than with osteocalcin, the combined marker measurements detected an 8 to 16% difference in mean BMD between individuals with low values of both markers compared to those with high levels of both markers. Even in these cross-sectional data, a difference of about 1 to 1.5 standard deviations in both biochemical markers was paralleled by a 1–1.5 standard deviation difference in BMD. The ability of these markers to discriminate larger differences in BMD was attenuated by the BMD criteria for this study. All subjects were free of metabolic bone diseases and had femoral neck bone density that was not less than 2 standard deviations below the average for their age. As a result, the range of BMD did not include the extremely low values that would be found in an unselected population, and this limited our ability to evaluate the diagnostic value of osteocalcin and N-telopeptide crosslink measurements. The value of an osteocalcin measurement may be greater when the rate of bone loss, as opposed to cross-sectional bone density, is the outcome. For example, Sowers et al. (19) observed that BMD loss from the radius among postmenopausal women was greatest in those whose initial osteocalcin value was in the highest 25% of the distribution.

By age 65, bone mineral density has been influenced by the peak bone mass attained in early adulthood and by years of age-related bone loss. An individual who starts out with a low peak bone mass will tend to have a lower than average BMD when he or she reaches old age. The individual with greater than average bone loss from middle age onward will also have a low BMD. One could speculate that older individuals with the lowest BMD values at a given point in time may not only have experienced low peak mass but also have current high rates of bone loss. This hypothesis would be consistent with the high levels of markers of bone formation and resorption, which reflect recent bone turnover, observed in this study.

In summary, the results of this study suggest that BMD at the femoral neck and Ward’s triangle decline by about 0.3% to 0.5% per year in both men and women aged 65 and older. Among women, bone density at the trochanter and whole body also declined by approximately 0.3% per year of age, but among men, BMD at these skeletal sites showed no age-related decrease. Spine BMD tended to remain constant with advancing age in both men and women. Biochemical markers of bone turnover, serum osteocalcin, and urinary N-telopeptide crosslinks were moderately correlated with
BMD of the femur and whole body, but less strongly at the spine.

ACKNOWLEDGMENTS

This study was supported by Grant AG-10353 from the National Institutes of Health. The authors acknowledge the contributions of G. Falconer, S. Harris, C. Bovest, and the staff of the Metabolic Research Unit at the Human Nutrition Research Center on Aging (Tufts University); K. Shoukri, K. Prestwood, B. G. Biskup, M. J. Davies, and S. Warner (University of Connecticut Health Sciences Center). The contents of this publication do not necessarily reflect the views or policies of the U.S. Department of Agriculture, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

Address correspondence to Dr. Elizabeth A. Krall, Human Nutrition Research Center on Aging, Tufts University Medical School, 711 Washington Street, Boston, MA 02111.

REFERENCES


Received February 5, 1996
Accepted August 1, 1996