0013-7227/02/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 87(4):1539–1543

Bone Loss at the Femoral Neck in Premenopausal White Women: Effects of Weight Change and Sex-Hormone Levels

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To investigate whether bone loss occurs in the premenopause, we measured the bone mineral content (BMC), bone mineral density (BMD), and bone area in the spine (L2–L4), femoral neck, and total hip, as well as the sex hormone levels of 130 healthy premenopausal white women (age, 31–50 yr) at least three times over 1–9 yr. We found an increase in all three bone measurements at the spine but no change in volumetric density. Neither could we detect any age-related changes in any of the three measurements in the total hip. In contrast, we detected a significant decrease in femoral neck BMD over time, due to a decrease in BMC and increase in bone area. Greater loss in femoral neck BMD was associated independently with weight loss and lower levels of estrone sulfate or E2. Separating the women into those with FSH spikes (>20 IU/liter) and women with consistently low FSH, we found the latter group had smaller decrease in BMD and that the decrease was due less to a decline in BMC and more to an increase in bone area. In summary, femoral neck BMD decreases in premenopausal women, particularly those with lower levels of estrogens resulting from slowing ovarian function despite regular menses. This decrease can be offset by more rapid weight gain. (*J Clin Endocrinol Metab* 87: 1539–1543, 2002)

THERE ARE CONFLICTING findings in the literature on bone loss before the onset of menopause. Some studies (1, 2) find no detectable bone loss, whereas others show significant bone loss, both cross-sectionally (3–8) and longitudinally (9–11) in different populations of premenopausal women. Determining whether premenopausal bone loss occurs is important because even if the annual loss is only 0.2%, the cumulative bone loss from age 30 to 50 would contribute a decrease of about 0.5 SD from peak bone mass in the population before onset of menopause.

Several factors may contribute to the discrepant findings from different studies. These include: 1) choice of the study population and design, 2) definition of menopause, and 3) choice of the skeletal site and measurement of bone mass. Compared with longitudinal studies, cross-sectional studies do not directly measure changes with age, but the simpler logistics of this design allow larger and more representative samples to be studied. Menopausal status has been defined variably by age, menopausal symptoms, and sex hormone levels, or combinations of these criteria. Another source of discrepant findings is the choice of different bone mass measurements in different studies. Bone loss at different skeletal sites starts at different ages (11). Even for the same skeletal site, the unit of measurement may differ across studies. The most fundamental unit is bone mineral content (BMC), measured in grams of calcium, but more commonly used is areal bone mineral density (BMD), obtained by dividing BMC by the projected area of the scan. BMD partially corrects for bone size and reduces the noise in longitudinal measurements (11). Although volumetric bone density (BMC divided by bone volume) more directly reflects the mass within a defined volume, biomechanical calculations show that BMD is a better indicator of bone strength because the size of a bone also contributes to strength (12). Seeman (13) emphasizes the importance of appropriate interpretation of findings on the basis of different measurements. This is especially important in studying premenopausal bone loss because some authors have argued that the apparent bone loss in premenopausal women is due not to a decline in BMC but to an increase in bone size (8), making the decrease in BMD less of a problem.

If BMC in fact decreases with age in a substantial segment of premenopausal women, then it is important to identify risk factors for premenopausal bone loss. Although the search for risk factors for bone loss in postmenopausal women has been extensive (14, 15), much less is known in premenopausal women. Two biological factors, sex hormones (16–18) and body weight (19, 20), have been consistently linked to postmenopausal bone loss, but it is not clear whether they have the same effects in premenopausal women.

In this study, we examine the longitudinal changes of BMC, the bone area, and BMD at both the spine and the hip in a group of premenopausal white women. Where we find age-related decline in BMD, we find out whether it is due to changes in BMC or area. We then further investigate whether premenopausal bone loss is related to the two biological factors of body weight and sex hormone levels.

Abbreviations: BMC, Bone mineral content; BMD, bone mineral density; CV, coefficient of variation; DHEA, dehydroepiandrosterone; E1, estrone; P, progesterone.

Subjects and Methods

Source of data

We studied a cohort of 130 non-Hispanic white women, aged 31–50 yr. Study subjects were recruited from advertising in various media. To be eligible, they had to be healthy normal subjects who had intact uterus and ovaries and no abnormalities in a health screen. They did not have any metabolic bone disease and had not taken drugs that affected bone metabolism, such as steroids and thyroid medication or other bone-active drugs. These women were followed every 6 months until July 1997, when the interval between visits increased to 1 yr. The women were considered premenopausal because they all reported regular menstrual cycles and no menopausal symptoms such as hot flushes. For the women who became perimenopausal during the study, their data were deleted starting from 1 yr before their first reports of any signs or symptoms of menopause or the use of hormone replacement. Written, informed consent was obtained from all subjects, and the study was approved by the Indiana University Institutional Review Board. The age distribution and follow-up information on the Indiana cohort of 130 premenopausal white women are presented in Table 1. Seventeen of these women had their last visit deleted, and 24 of them had 2 or more visits deleted because of onset of perimenopausal symptoms or hormone replacement.

Bone mass measurements

Bone area, BMC, and BMD were measured at the lumbar spine (L2–L4) and proximal femur (total hip and femoral neck) using dual-energy x-ray absorptiometry on a Hologic model 1000W (Hologic, Inc., Waltham, MA).

Quality control of the bone mass measurements included regular scans of external phantoms to monitor for machine drift. The manufacturer's spine phantom was scanned daily, the hip phantom weekly, and a custom-designed step-wedge monthly. Analysis of the hip scans was performed using the Compare feature to match the region of interest of the subject's previous scan. In our laboratory, the short-term coefficient of variation (CV) at the spine was less than 1% for both BMD and BMC, and the long-term variation (including both measurement error and biological variation from linear change) was 0.016 g/cm^2 (1.5%) for BMD and 1.1 g (2.3%) for BMC. The CV at the femoral neck was 1% for BMD and 1.6% for BMC, and the long-term variation was 0.02 g/cm^2 (2.5%) for BMD and 0.14 g (3.3%) for BMC. The CV at the total hip was less than 1% for BMD and 2% for BMC, and the long-term variation was 0.013 g/cm^2 (1.4%) for BMD and 0.67 g (2.2%) for BMC.

Sex hormones

Each visit was scheduled such that blood was drawn during the early follicular phase (days 3–7 of the menstrual cycle) for premenopausal women in the morning insofar as possible. All serum specimens were frozen and run in batches. SHBG capacity was measured using the filter disc method of Mickelson and Petra (21) as described (22). The inter- and intra-assay CVs were 10.9 and 8.0%, respectively. Free E2 and free T were measured using a modification of the method described by Hammond *et al.* (23). The inter- and intra-assay CVs were 13.3 and 4.7%, respectively, for free T and 11.1 and 6.1% for free E2. As previously described (22, 24), measurements were made for serum levels of LH, FSH, estrone (E1), E2, E1 sulfate, progesterone (P), T, androstenedione, dehydroepiandrosterone (DHEA), and its sulfate (DHEAS). The non-SHBG bound T and E2 were calculated using the method of Södergard *et al.* (25) as described (26, 27).

Table 1. Age and follow-up information of Indiana cohort

	Mean	SD	Minimum	Maximum
Age at entry (yr)	40.4	4.2	31	50
No. of visits	7.3	3.4	3	17
Length of time on study (yr)	3.9	1.9	0.9	9.3
Mean spine BMD (g/cm^2)	1.10	0.14	0.76	1.47
Mean spine BMC (g)	49.40	8.78	29.87	72.34
Mean spine area (cm^2)	44.73	4.34	29.31	56.41
Mean total hip BMD (g/cm^2)	0.91	0.13	0.66	1.25
Mean total hip BMC (g)	29.79	5.63	19.81	49.13
Mean total hip area (cm^2)	32.51	3.07	26.18	42.81
Mean femoral neck BMD (g/cm^2)	0.83	0.13	0.57	1.23
Mean femoral neck BMC (g)	4.21	0.78	2.81	6.62
Mean femoral neck area (cm^2)	5.08	0.44	3.89	6.90

Statistical analysis

Analyses were performed separately on BMC, BMD, and area at each skeletal site. We fitted a random coefficients model to the longitudinal data to estimate the overall rate of change and to test whether it was significantly different from zero. From this model, we could also decompose the variability of the observed rates of change across individuals into two components, the variability of the true rates and the variability due to measurement errors. All rates of change are reported as annual rates, *i.e.* change per year.

Because BMD showed a significant overall decline only at the femoral neck but not at the spine or total hip, individual rates of change were estimated only for femoral neck BMD for further investigation. Potential factors that we investigated include both the mean body weight and the rate of change in weight. The longitudinal measurements of the sex hormones, however, were too variable to obtain reliable rates of change, so only the mean hormone levels during the study period were calculated for each individual. We estimated Pearson's correlation between the rate of change in femoral neck BMD and each of the potential factors. In addition, to allow for possible nonlinear relationships and for more explicit display of the data, we divided the individual rates of change in BMD into three groups by the percentage change per year, *i.e.* the nonlosers (>0% per year), slow losers (−1 to 0% per year), and fast losers (less than −1% per year). The mean levels of each factor were compared among the three groups using χ^2 test for trend.

To investigate the possibility that, despite having regular cycles, some of the premenopausal women might have declining ovarian function, we divided women into those with high FSH (>20 IU/liter) at any visit and those with low FSH (≤20 IU/liter) at all visits. We then examined the rates of bone loss separately in the two different groups. We also investigated whether the associations between factors with bone loss in the whole group were valid even in women with no sign of ovarian slowdown, *i.e.* the subgroup with consistently low FSH.

Multiple regression analysis was used to examine the concomitant effects of multiple predictors of bone loss.

Results

Table 1 displays the age distribution of the participants at baseline as well as the amount of follow-up in total number of visits and time interval. It also contains the distributions of BMD, BMC, and area at the spine (L2–L4) and hip (total hip and femoral neck) at the baseline visit for each participant.

From the mixed model fitted to the longitudinal data, we estimated the overall rates of change of the various measurements over time within individuals, together with the SD of individual true rates, *i.e.* after statistically removing the variance due to measurement errors (Table 2). In the spine, we can see that both BMC and area increase significantly over time, as does BMD. However, if we normalize the BMC by bone volume, then the volumetric density does not change with age (overall rate, 0.0001 g/cm^3 per year; $P = 0.3$). No significant change with age can be detected at the total hip, whether it is area, BMC, or BMD. The average rate of loss in femoral neck BMD is about 0.0036 g/cm^2 per year. This loss can be attributed to an annual loss of 0.0092 g in BMC and a gain of 0.011 cm^2 in area. Thus, the loss in BMD in these premenopausal women is not simply an artifact of bone expansion, but there is a loss of BMC as well.

When we explored the effects of sex hormone and body weight on changes in femoral neck BMD, we found that individual rates of change in BMD had significant positive

Table 2. Rate of change for 130 premenopausal women at the spine (L2–L4), femoral neck, and total hip

	Mean rate of change	SD of rates in population	% Change	*P* value
Spine L2–L4 BMD (g/cm^2 · yr)	0.00209	0.0050	0.19	0.001
Spine L2–L4 BMC (g/yr)	0.2011	0.3086	0.41	<0.001
Spine L2–L4 area (cm^2/yr)	0.09586	0.2214	0.21	0.001
Total hip BMD (g/cm^2 · yr)	−0.00054	0.0039	−0.06	0.268
Total hip BMC (g/yr)	−0.03318	0.1682	−0.11	0.148
Total hip area (cm^2/yr)	−0.01623	0.1128	−0.05	0.330
Femoral neck BMD (g/cm^2 · yr)	−0.00357	0.0025	−0.43	<0.001
Femoral neck BMC (g/yr)	−0.00918	0.0266	−0.22	0.025
Femoral neck area (cm^2/yr)	0.01113	0.0119	0.22	<0.001

Table 3. Means of characteristics and sex steroid levels of fast losers and nonlosers

	Fast losers <−1.0% n = 30	Slow losers −1.0–0.0% n = 62	Nonlosers >0.0% n = 38
Age (yr)	41.3	42.6	42.2
Height (cm)	164.7	164.9	165.4
Weight change (kg/yr)	0.39	1.01	1.39[a]
Weight (kg)	66.6	68.2	68.1
BMD at initial visit	0.84	0.84	0.81
LH (IU/liter)	5.4	4.7	3.9[b]
FSH (IU/liter)	13.3	11.5	9.9[c]
E1 (pmol/liter)	167.5	168.9	184.7
E1 sulfate (pmol/liter)	1,195.6	1,191.3	1,520.1[c]
E2 (pmol/liter)	198.6	224.0	244.1[c]
Non-SHBG bound E2 (pmol/liter)	60.6	74.7	64.9
Free E2 (pmol/liter)	2.7	3.5	3.4
P (nmol/liter)	2.0	1.8	1.6
T (nmol/liter)	0.9	0.8	0.9
Non-SHBG bound T (nmol/liter)	0.24	0.22	0.23
Free T (nmol/liter)	0.01	0.02	0.01
Androstenedione (nmol/liter)	4.5	4.3	4.2
DHEA (nmol/liter)	9.4	8.2	9.6
DHEAS (μmol/liter)	5.2	4.7	4.7
SHBG (nmol/liter)	54.5	58.5	64.7

[a] *P* value for weight change is 0.058. The correlation between weight change and percentage loss BMD is 0.27 with a *P* value of 0.001.
[b] $P < 0.01$, test for linear trend from fast losers to nonlosers.
[c] $P < 0.05$, test for linear trend from fast losers to nonlosers.

correlations with weight change, E1 sulfate, and E2, and significant negative correlations with LH and FSH (data not shown). To further examine these relationships, we classified these women by their rate of change in BMD into three groups: nonlosers with no loss, slow losers with less than 1% annual loss, and fast losers with more than 1% annual loss. Table 3 shows the mean values of all the factors we examined. It shows that the three groups were similar in age, body size, and mean BMD. However, the rate of change in body weight shows a marginally significant trend, with less bone loss associated with more weight gain. Among the sex hormones, significant trends were detected in LH and FSH, which were increasingly higher from the nonlosers to fast losers. For estrogens, E1, E1 sulfate, and E2 were highest in the nonlosers. We could find no clear-cut relationship between non-SHBG bound E2, free E2, non-SHBG bound T, and free T with the rate of change in BMD, although the fast losers had lower concentrations of these hormonal elements, in general, than the slow losers or nonlosers. These results all agree with the correlation analysis.

When we separated the women by their FSH levels, we found 85 women who always had FSH under 20 IU/liter, whereas the FSH in the remaining 45 women had risen above 20 IU/liter at least once. Although the overlap in age range was substantial between the low FSH group (31–48 yr) and the high FSH group (33–50 yr), the mean age of the low FSH group was significantly lower by 3.8 yr ($P < 0.0001$) (Table 4). Both groups had significant decrease in BMD. However, the decrease was due more to a loss in BMC in the high FSH group, although it was due more to an expansion in bone area in the low FSH group. In the low FSH group, the correlations of the individual rates of change in BMD with the various factors were slightly attenuated relative to the correlations in the combined groups, but most remained in the same direction (except for FSH, as expected). These correlations were no longer significant, however, partially because of the smaller sample size.

To investigate whether the positive effect of weight gain on BMD change was a direct result of greater weight-bearing or whether it was mediated through higher estrogen levels resulting from aromatization of adipose tissue, we fitted different models to predict BMD change using weight gain alone and weight gain with various measures of estrogen. Table 5 shows that the regression coefficient of weight gain in predicting BMD change is only minimally changed when either E1 sulfate or E2 is added. This result suggests that the

positive association between changes in weight and BMD is not entirely mediated through a higher estrogen level. Including the present or prior use of birth control pills in the model did not change the results.

Discussion

In this study, we examined longitudinal changes in premenopausal white women. We found that the increase in vertebral BMC, area, and BMD with age that was previously observed in adolescents (4, 28) continues through adulthood in white women. However, volumetric density at the spine, which increases in adolescents, showed no significant change with age in our sample of premenopausal women.

In the total hip, we could not detect any significant change in the BMC, area, or BMD. Mazess and Barden (8) suggest not using the total hip for follow-up because the trochanter, a major component of total hip, varies substantially with age. Furthermore, in less experienced hands, inconsistent repositioning of the hip or failure to match regions of interest in the analysis of the scans could lead to larger errors in the total hip. Even though the long-term precision in our laboratory was better at the total hip than at the femoral neck, it is possible that an increase in one component of the total hip balances the decrease in other components so there appears to be no overall change. Whatever the reason, we could not detect any changes in the total hip of premenopausal women.

In contrast, we found in the same women an age-related decrease in femoral neck BMD due to both a decrease in BMC and an increase in bone area. Between ages 30 and 50 yr, BMD decreases at an average rate of about 0.4% per year, with an annual loss of about 0.2% in BMC and an annual gain of 0.2% in the area of the femoral neck. This agrees with the cross-sectional data from the Third Health and Nutrition Examination and Survey (NHANES III) based on a large nationally representative sample (29). The bone loss also corresponds roughly to histological data from biopsies (30). There is, however, substantial variation of rates of loss in BMD among individuals in a population as shown in Table 2. In Table 3, we note that the rate of loss in femoral neck BMD does not increase or decrease systematically with age in the premenopausal years. The mean age is very similar across the three groups defined by their rates of bone loss; in fact the fast losers have the lowest mean age. Thus, the grouping does not simply reflect an age-related onset of bone loss in premenopausal women.

Because of the size of the measurement error of BMD relative to the small magnitude of change in premenopause, most of the estimated individual rates of change in BMD are very unreliable, making it difficult to detect correlations with other factors. Nevertheless, we were able to find that more rapid weight gain is associated with slower bone loss. This association has previously been noted in older and postmenopausal women (19, 20). In the premenopausal women in this study, weight change was not correlated with LH, FSH, or E2, but it was positively correlated with E1 sulfate. In postmenopausal women, the benefits of body weight on bone mass are often attributed to the aromatase activity in adipose tissue (31, 32), which results in more estrogen formed from circulating androgens (33). Because E1 sulfate is formed from both E1 and E2 (34, 35), its level reflects the total estrogenic influence better than either E1 or E2 alone. Hence, some of the beneficial effects of weight change could be due to the increased estrogenic activity. However, when we added E1 sulfate to the model predicting bone loss, it did not change the estimated effect of weight change. Thus, we believe that in premenopausal women the change in body weight has direct effects on bone mass, although it might also partially act through estrogens.

When we compared the average sex-hormone levels among the fast losers, slow losers, and nonlosers of femoral neck BMD, we found that higher levels of FSH and LH, and lower levels of E1 sulfate and E2 were associated with faster bone loss. This suggests that some premenopausal women may have suboptimal levels of sex steroids, but not to an extent that would precipitate perimenopausal symptoms. In an earlier publication (16), we reported lower T levels in fast losers based on a subset of the women in the present study. With more subjects and longer follow-up, we no longer observe any T effects on change in BMD, but the estrogens have emerged as a factor. Although many publications have reported that low estrogen levels are associated with bone loss in peri- and postmenopausal women (17, 18), this relationship has not been thoroughly investigated in longitudinal studies of premenopausal women. We were unable to show any relationship between P concentration and bone density. However, it should be noted that our women were studied in the early follicular phase of the cycle when P concentrations would be low.

To further investigate whether premenopausal bone loss occurs only in the subset of women whose ovarian functions

Table 4. Comparisons of rates of change at the femoral neck between subjects with low FSH (consistently <20 IU/liter) and those with high FSH (at least one FSH >20 IU/liter)

Rate of change at femoral neck	Low FSH (n = 85)	High FSH (n = 45)
BMD (g/cm^2·yr)	−0.00324[b]	−0.00420[b]
BMC	−0.00434	−0.01706[a]
Area	0.01307[b]	0.00958[c]

[a] $P < 0.05$ testing whether within-group change is different from zero.

[b] $P < 0.01$ testing whether within-group change is different from zero.

[c] $0.05 < P < 0.1$ testing whether within-group change is different from zero.

Table 5. Weight change regression coefficients for rate of changes in femoral neck BMD

Predictors of rate of change in femoral neck BMD	Estimated effect of weight change on change in femoral neck BMD (g/cm^2 · kg)	SE of the estimated effect
Weight change only	0.00133	0.0004
Weight change and E2	0.00121	0.0004
Weight change and E1 sulfate	0.00114	0.0004

have slowed down, we separated out the women in whom we observed at least one FSH spike. Generally, the women with high FSH have faster rates of bone loss, which agrees with the Michigan study (2). But even those women who consistently had FSH below 20 IU/liter in our study had on average a significant decrease in BMD, although it is due more to an expansion in bone area and less to a decrease in BMC.

In summary, we observed an average of 0.4% annual loss in femoral neck BMD in white premenopausal women over age 30 yr. If this loss is sustained from age 30 through 50, a woman can lose more than 0.5 SD population before she becomes menopausal. More weight gain is associated with less bone loss. Moreover, this loss in BMD is greater in women with reduced estrogen levels and ovarian function, although they are asymptomatic. If apparently premenopausal women with suboptimal levels of sex hormones are detected early, perhaps prophylactic measures may prevent early bone loss, especially among women with excessive weight loss.

Acknowledgments

Received June 29, 2001. Accepted January 4, 2002.

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This work was partially supported by NIH Grants AG04518, AG05793, AG18397, and M01 RR750.

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