Associations between Low Levels of Serum Estradiol, Bone Density, and Fractures among Elderly Women: The Study of Osteoporotic Fractures*

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ABSTRACT
To evaluate the skeletal effects of endogenous serum estradiol, we measured bone mineral density (BMD) at the calcaneus and radius (single photon absorptiometry) and at the hip and spine (dual x-ray absorptiometry) in 274 women aged 65 yr or more who participated in the Study of Osteoporotic Fractures. Lateral radiographs of the thoracic and lumbar spine were also taken, and serum was assayed for estradiol. Those who had estradiol levels from 10–25 pg/mL had 4.9%, 9.6%, 7.3%, and 6.8% greater BMD at total hip, calcaneus, proximal radius, and spine than those with levels below 5 pg/mL. After multiple adjustments, BMD differences remained statistically significant and corresponded to about 0.4 SD. Vertebral deformities were less prevalent among women whose estradiol level exceeded 5 pg/mL; the multiple adjusted odds ratio was 0.4 (95% confidence interval, 0.2–0.8). We conclude that physiologically low estradiol has a salutary effect on the skeleton in elderly women, possibly by reducing skeletal remodeling. (J Clin Endocrinol Metab 83: 2239–2243, 1998)

IN WOMEN, serum estradiol is an important determinant of bone loss; when ovarian estrogen production decreases and serum levels fall into the postmenopausal range (<30 pg/mL), accelerated bone loss ensues (1). Estrogen replacement therapy typically elevates serum estradiol levels to the range of 40–60 pg/mL (2), and these levels are considered the minimum level sufficient to prevent or retard bone loss (3).

Recently, Cummings and co-workers1 found a strong inverse relation between endogenous serum estradiol and risk of hip and vertebral fracture among elderly women in the Study of Osteoporotic Fractures (SOF) cohort. Using a case-cohort design and excluding women currently using estrogen, they found that women with undetectable estradiol levels (<5 pg/mL) were about 2.5 times more likely to suffer hip or vertebral fracture than women with detectable levels (5–25 pg/mL). This apparent protective effect of serum estradiol in the range of 5–25 pg/mL persisted after multiple adjustment was made for fracture risk factors, including measurements of body weight and bone mineral density (BMD).

Subsequently, Stone and co-workers (5) found an inverse association between serum estradiol level and femoral bone loss among randomly chosen women (not using estrogen) from the same cohort. They also found an inverse association between serum estradiol and bone loss at the calcaneus. These new data challenged the widely held belief that levels of endogenous estradiol below 20–30 pg/mL have little or no physiological role and therefore do not exert any important effect on skeletal metabolism. Because of the potential implications of this finding, we performed additional analyses to further evaluate the skeletal effects of low endogenous serum estradiol levels. Our hypotheses were that women whose serum estradiol levels were between 5–25 pg/mL would have higher baseline BMD and a lower prevalence of baseline vertebral deformities than women with lower serum estradiol levels and that these salutary effects would be moderated by reduced bone turnover. We tested these hypotheses in the same population-based sample studied by Cummings et al. (see Footnote 1) and Stone et al. (5) and validated these findings in a second sample of women randomly chosen from the cohort.

Subjects and Methods
SOF subjects and clinic examinations
Subjects in this analysis were participants in the SOF, which has been described in detail previously (6). White women, aged 65 yr or more, were recruited for SOF from population-based listings at four clinical centers (Portland, Minneapolis, Monongahela Valley near Pittsburgh, and Baltimore). The study was approved by the appropriate committees.

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on human research, and all the women provided written informed consent. We excluded black women (because of their low incidence of hip fracture), women who were unable to walk without assistance from another person, and women with a history of bilateral hip replacement.

All 9704 SOF participants were interviewed and examined at 1 of the clinical centers during the baseline visit in 1986–1988. At that visit, detailed data about physician-diagnosed medical conditions and past medications were collected. We obtained standardized assessments of height, weight, and strength (6). We measured BMD of the calcaneus and proximal third of the radius by using single photon absorptionmetry (Osteoanalyzer, Siemens-Osteon, Wahiawa, HI). Lateral radiographs of the thoracic and lumbar spine were obtained. Serum samples were collected from each participant.

Participants completed questionnaires annually and had three bennial follow-up visits to the clinic. At the first follow-up visit (conducted during 1988–1990), we measured BMD of the proximal end of the femur using dual energy x-ray absorptiometry (QDR 1000, Hologic, Waltham, MA). Details of these measurement methods and quality control procedures for densitometry have been published previously (7).

Serum samples and biochemical tests

All participants were instructed to adhere to a fat-free diet overnight and to minimize lipemia on the morning of the examination, which would interfere with assays. Blood was drawn between 0800–1400 h, and serum was frozen at −20 C. Within 2 weeks, all samples were shipped to the Biomedical Research Institute (Rockville, MD), where they were stored in liquid nitrogen (−190 C). The long term stability of these samples was determined by comparing estradiol results obtained after 2-week storage at −20 C with those obtained after 3.5 yr storage at −190 C; for 51 samples, the correlation coefficient was 0.94, and the mean ± sd was 11.8 ± 9.0 after 2 weeks and 10.9 ± 9.0 after 3.5 yr.

In the initial cohort studied, biochemical tests using baseline serum were available from 400 randomly selected participants. For this analysis, we excluded women for whom we did not have measures of serum estradiol (n = 134) and those who reported current use of systemic estrogen therapy (n = 39); 247 women remained available for the current analysis.

The following biochemical analyses were performed for each subject: calcitropic factors included calcium, 25-hydroxyvitamin D, 1,25-dihydroxyvitamin D, and PTH; growth factors included insulin-like growth factor I and insulin-like growth factor-binding protein-3; bone formation markers included bone-specific alkaline phosphatase and osteocalcin; sex and adrenal hormones included estradiol, estrone, testosterone (total and free), sex hormone-binding globulin (SHBG), dehydroepiandrosterone (DHEA) sulfate; other endocrine tests included TSH. Methods for these biochemical analyses have been described previously (3). The immunoassays for estradiol were performed by Endocrine Sciences Laboratory (Tarzana, CA), which used a highly sensitive assay with a 5 pg/mL lower limit of detection.

To validate our findings in an independent cohort, we measured baseline serum estradiol from a second random sample of 222 SOF participants who satisfied the same inclusion and exclusion criteria as the initial analysis sample. Serum estradiol measurements in the second (validation) sample (n = 222), we used the same serum estradiol cut-off points; the proportions of subjects in these strata were 25.2%, 27.3%, 24.8%, and 22.5%.

We first examined outcomes adjusting for subjects’ weight and age. Because weight and body mass index are highly correlated and because weight shows the stronger relation with BMD (9), we selected weight for inclusion in subsequent models. We then evaluated the possible contribution of covariates by first examining variables we considered to be potential predictors of bone density or osteoporotic fracture. If any potential confounder showed a consistent trend (ANOVA with test for linear trend) across the strata of estradiol, we further evaluated its effects in multivariate models for which outcomes were bone density at the four skeletal sites of interest. These models included age, clinic site, and serum estradiol as well as the candidate predictor variable. If the candidate variable was retained in most of these models, it was considered a confounder. We also added to the model grip strength and current smoking because these variables proved in our prior studies to be predictors of BMD (9) and fracture (6).

Using ANOVA, we calculated the adjusted mean BMD for each stratum of estradiol. We used Dunnett’s test to determine the statistical significance of difference between the adjusted BMD of the lowest stratum (undetectable estradiol) vs. each of the three higher strata. The logistic regression model was used to calculate the multiple adjusted odds ratio (OR) and the 95% confidence intervals for risk of prevalent vertebral deformity for each of the three higher strata of estradiol vs. the lowest stratum. Analyses were performed using the Statistical Analysis System (SAS Institute, Cary, NC).

Results

Subject characteristics by estradiol level

On the average, the women in this study were about 72 yr of age (Table 1). Mean estradiol did vary with age; those aged 65–74 yr had a mean (±sd) estradiol level of 5.8 (±5.1) pg/mL compared with 6.3 (±4.9) pg/mL for those 80 yr or older. Height did not differ across estradiol strata. Body weight was statistically significantly higher in women in the highest estradiol stratum. Biochemical tests that did not show large or consistent differences across estradiol strata included calcitropic factors, growth factors, bone-specific alkaline phosphatase, and DHEA sulfate (results not shown). Estrone and total testosterone were about 2-fold higher in women with the highest estradiol levels compared with those with undetectable estradiol. Several variables, including those relating to body weight, showed striking and consistent differences across estradiol strata. Osteocalcin tended to be lower at higher levels of estradiol; the highest stratum was 10.9% lower than the lowest (undetectable) stratum of estradiol. Because they also showed difference across estradiol strata and fairly consistent relations to the four BMD outcomes, weight and SHBG were included in the model along with age, grip strength, and current smoking.

Estradiol and BMD

The age- and weight-adjusted baseline BMD of all four skeletal sites showed a similar trend to be higher with higher levels of estradiol (Fig. 1). Compared with women with serum estradiol levels below 5 pg/mL, those with levels between 10–25 pg/mL had statistically significantly greater mean BMD at all skeletal sites; the differences were 4.6%,...
6.1%, 5.8%, and 7.1% for total hip, calcaneus, proximal radius, and spine (P < 0.05 for each comparison). After multiple adjustment, the difference remained statistically significant: 5.7%, 6.3%, 6.5%, and 6.9% for total hip, calcaneus, proximal radius, and spine.

Validation study

The women in the validation study showed characteristics similar to those of the women in our initial study, including serum estradiol levels. Similar associations between serum estradiol level and BMD were found in the validation cohort. Age- and weight-adjusted BMD at all four skeletal sites showed a trend similar to the original cohort (Fig. 2). Compared with women who had less than 5 pg/mL serum estradiol, those with levels between 10–25 pg/mL had 4.9%, 9.6%, 7.3%, and 6.8% greater BMD at total hip, calcaneus, proximal radius, and spine. After multiple adjustment, the difference remained statistically significant: 3.8%, 7.0%, 5.4%, and 6.9% greater BMD at total hip, calcaneus, proximal radius, and spine.

Estradiol and vertebral deformity

Of women in the lowest estradiol stratum (<5 pg/mL), 30% had one or more prevalent vertebral deformities, in contrast to the lower prevalence in the other three strata, which ranged from 7–19%. After age and weight adjustment,
prevalent vertebral deformities were 60% less likely among women with estradiol levels between 5–25 pg/mL than in those who had undetectable estradiol levels (OR = 0.4; 95% confidence interval = 0.2–0.8); this ratio was minimally affected by multiple adjustment (OR = 0.4; confidence interval = 0.2–0.7). In the validation cohort, we did not find any trend toward an association between estradiol level and prevalence of vertebral deformity; prevalence was similar across all strata and ranged from 15–19%.

Discussion

We have extended and validated the findings of Cummings and co-workers that serum estradiol levels below 5 pg/mL are detrimental to skeletal health in older women (see Footnote 1). Their original results were limited to incident hip fracture and incident vertebral deformities. Their case-cohort analysis of women with incident vertebral fracture vs. controls without fracture yielded an OR of 0.4 for estradiol levels of 5 pg/mL or more vs. levels below 5 pg/mL. Similarly, for incident hip fracture, they found a relative risk (RR) of 0.4. We suspect that part of this effect is mediated by BMD; after age and weight adjustment, we found that women with estradiol levels below 5 pg/mL had substantially less BMD at all skeletal sites. Additionally, we found that osteocalcin, an indicator of bone turnover, tends to be higher in women with lower serum estradiol levels. Thus, we hypothesize that low levels of estradiol exert clinically important effects on the skeleton of an elderly woman. A likely explanation is that estradiol, when present in low concentrations, reduces skeletal remodeling, allows for both better quality and mass of bone, and thereby reduces fracture rates.

The difference in BMD that we observed was substantial and corresponded to about 0.4 sd even after adjusting for multiple factors. This difference would be expected to reduce the risk of hip fracture by 23% (assuming 1.0 sd = 0.17 g/cm² and 1.0 sd difference = RR of 2.1). Because Cummings et al. found that estradiol’s association with reduced risk of fracture persisted after adjustment for BMD (see Footnote 1), estradiol’s effect on fracture risk may be mediated by mechanisms other than reduced BMD.

Estrone has been widely examined as a predictor of skeletal health among postmenopausal women. In an earlier ancillary study (10), BMD was found to be cross-sectionally related to serum estrone levels in both white and black women. However, this study was limited because estradiol levels were below the limit of detection in more than half of the women. Stone et al. found that higher estrone levels were associated with lower rates of BMD loss at the calcaneus (5). However, Cummings et al. found that higher estrone levels were associated with increased risk of incident vertebral fractures (see Footnote 1). Estrone was not predictive of incident hip fractures. In postmenopausal women, estrone is quantitatively the predominant estrogen and is produced mainly from the conversion of adrenal androstenedione. Estradiol is produced through the reduction of estrone and the aromatization of ovarian and adrenal testosterone, which is derived from the conversion of androstenedione and DHEA (10). We found a relatively high correlation between serum levels of estrone and estradiol in both the initial sample (r = 0.65) and the validation sample (r = 0.78). Estradiol, not estrone, is believed to be the effector hormone at the nuclear receptor (11). Estradiol is also 4–10 times more potent than estrone. That estradiol was the major sex steroid hormone that had a strong, consistent, and positive relation to skeletal outcome could therefore be expected.

Estradiol could produce beneficial skeletal effects through several possible mechanisms; it reduces activation of bone metabolic units (12), it antagonizes PTH’s stimulation of bone resorption (13), it may enhance the survival of osteo-

Fig. 2. Age- and weight-adjusted mean BMD among 222 women grouped by level of estradiol in the validation sample. *, P < 0.05.
Conclusion

We have found a protective effect of low levels of estradiol against low bone mass and fracture; this effect is consistent because it can be observed for bone density at several skeletal sites, for bone loss at the hip and calcaneus, and for spine fracture risk. We have been able to reproduce our initial findings by using a validation sample of randomly selected women and by using a different laboratory’s highly sensitive estradiol assay. We hypothesize that estradiol’s beneficial skeletal effects in the elderly may occur at levels that have been previously believed to have no physiological impact. Serum estradiol appears to exert its effect on the skeleton across a wide range of values, and an “all or none” threshold for estradiol’s protection against osteoporosis does not seem to exist. Future studies will be aided by sensitive and precise estradiol measurements and should focus on the potential benefits of low dosage estrogen replacement or methods of enhancing better endogenous estrogen production in the elderly.

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References

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