CLINICAL STUDY

Cortisol secretion, bone health, and bone loss: a cross-sectional and prospective study in normal nonosteoporotic women in the early postmenopausal period

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Abstract

Objective: The aim of the study was to evaluate the relationship between cortisol secretion, bone health, and bone loss in a cohort of normal women in the early postmenopausal period.

Methods: We measured lumbar and hip bone mineral density (BMD) by dual-energy X-ray absorptiometry (DXA) and heel ultrasound parameters in 82 healthy, nonosteoporotic (lumbar T-score ≥ −2.0) women (median age 52.5 years, range 42–61). These women were examined in two sessions, 1 year apart, in the early postmenopausal period (onset of menopause between 6 and 60 months). Parameters of the hypothalamic–pituitary–adrenal (HPA) axis function were morning serum cortisol, morning and midnight salivary cortisol, 24-h urinary free cortisol (UFC), serum cortisol after 0.5 and 1 mg overnight dexamethasone, and DHEA-S.

Results: In multiple regression analyses, the following significant inverse correlations were found: i) lumbar BMD and either 24-h UFC (P<0.005) or morning serum cortisol (P<0.05), ii) total femur and femoral neck BMD with morning serum cortisol (P<0.05 and P<0.05), and iii) heel ultrasound stiffness index and midnight salivary cortisol (P<0.005). The annual rate of change in lumbar and femoral BMD did not correlate with any of the above-mentioned hormonal variables. No difference was found in the parameters of HPA axis function in slow (loss of BMD <1%) vs fast (loss of BMD ≥3%) bone losers.

Conclusions: HPA axis may contribute to postmenopausal bone health, but differences in cortisol secretion do not influence the differential rate of bone loss between slow and fast bone losers in the early postmenopausal period, at least in healthy women.

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Introduction

Glucocorticoid-induced osteoporosis (GIOP) is the most common cause of secondary osteoporosis (1, 2). Patients receiving exogenous glucocorticoids have a rapid increase in the risk of fragility fractures after the initiation of their therapy (3). Patients with Cushing’s syndrome, the most apparent phenotype of endogenous glucocorticoid excess, experience such fractures in about 50% of cases (4).

Also, patients with an incidentally discovered adrenal adenoma (adrenal incidentaloma) diagnosed as having subclinical hypercortisolism have been reported to display signs of GIOP, including a reduction of bone mineral density (BMD) of the spine, altered ultrasound bone characteristics, and a number of morphometric fractures higher than those expected in the control population (5, 6, 7, 8). A number of confounding factors, however, are likely to explain inconsistencies in other reports on this issue (9, 10, 11), prompting interest for additional studies on subtle glucocorticoid excess and bone health.

In the last decade, two papers have addressed the question of whether there is an overrepresentation of subclinical hypercortisolism among patients with established osteoporosis. Kann et al. (12) have focused on impaired cortisol suppression by high-dose dexamethasone (DEX) administration in postmenopausal osteoporotic women, possibly heralding a partially autonomous glucocorticoid secretion in a number of such cases. Chiodini et al. (13) have recently reported a surprisingly high prevalence of subclinical hypercortisolism in a cohort of outpatients referred for osteoporosis. The role, if any, of the endogenous glucocorticoids in physiological bone loss after the menopause remains to be explored.

More than 30 years ago, Manolagas et al. (14) explored in 18 patients the hypothesis that the wide
variation in bone loss after surgical menopause (ovariectomy) could be attributable to differences in adrenal steroids. They found a higher urinary cortisol excretion and a paradoxically diminished cortisol response to corticotropin in those women who lost bone rapidly.

The aim of this study was to evaluate, in a cohort of healthy women without osteoporosis in the first postmenopausal period, the cross-sectional relationship between measures of function of the hypothalamic–pituitary–adrenal (HPA) axis and bone health (DXA and heel ultrasound parameters) and to prospectively determine the relationship between the HPA axis and the rate of bone loss from baseline after 1 year.

Subjects and methods

Subjects were 82 healthy, nonosteoporotic (DXA lumbar T-score $\geq -2.0$) women, aged 52.3 ± 3.6 years (mean ± S.D.), median 52.5 years, in the first postmenopausal state (onset of menopause between 6 months and 5 years). Study protocol was prepared according to the Declaration of Helsinki and subsequent relevant integrations and approved by the Local Ethics Committee. All participants gave written informed consent before enrollment. The subjects were recruited among blood donors at the Turin section of Italian Blood Donors Association. We performed clinical examination and collected data about medical and drug history, current alcohol and caffeine intake, cigarette smoking, current physical activity, and dietary calcium intake. Subjects with history of conditions or drug treatments (supplement of calcium and vitamin D included) known to interfere (in a positive or negative manner) with bone health were excluded a priori. Biochemical screening aimed to exclude potentially asymptomatic causes of bone loss (complete blood count, erythrocyte sedimentation rate, serum protein electrophoresis, serum creatinine, serum alkaline phosphatase, serum total calcium, serum calcium corrected for albumin levels, 24-h urinary calcium, serum phosphate, and TSH) was obtained in all subjects. Menopausal status was confirmed by FSH, LH, and estradiol (E$_2$) measurements. In all participants, we measured lumbar and hip BMD at baseline (first visit) and, only in the subjects recruited, also after 1 year (second visit), by DXA (Hologic QDR4500-W, Waltham, MA, USA; software version 9.03). Heel ultrasound parameters (Achilles Express, GE Healthcare, Lunar, Diegem, Belgium) were obtained in 49 subjects. Markers of the HPA axis function (morning serum cortisol, morning and midnight salivary cortisol, 24-h urinary free cortisol (UFC), serum cortisol after 0.5 mg and 1 mg overnight DEX, and DHEA-S) were measured in all subjects enrolled in the study using commercially available reagents (competitive chemiluminescent enzyme immunoassay by Immulite 2000, DPC, Los Angeles, CA, USA, for serum cortisol and DHEA-S; RIA by Radim SpA, Pomezia, Rome, Italy, for salivary cortisol and UFC).

A total of 92 women were screened; nine of them were excluded a posteriori because the basal lumbar DXA evaluation did not fulfill the inclusion criteria of a lumbar T-score $\geq -2.0$ (in one case, asymptomatic thyrotoxicosis was discovered as the main reason for reduced BMD levels) and one abandoned the study for familial reasons. For the remaining 82 women, follow-up data after 1 year were available in 80: one woman suddenly died of an acute intracerebral hemorrhage and one was excluded for the successive diagnosis of hyperthyroidism (while TSH levels were in the normal range at the time of enrollment).

Saliva samples for the determination of midnight salivary cortisol were collected at midnight in commercially available devices (Salivette) using cotton swab chewed for 2–3 min and inserted into a double-chamber plastic test tube. The saliva samples were refrigerated at 4°C and stored for at least one week. Urinary and salivary samples were collected at home, conserved in a fridge by the subjects, and delivered the day after to the hospital. Blood samples were drawn at hospital between 0800 and 0900 h and centrifuged at 1000 g for 15 min within 2 h of collection; sera were immediately frozen and stored at $-20^\circ$C until assayed. To avoid interference on cortisol secretion due to the stress of the venipuncture, an indwelling i.v. cannula was inserted in an antecubital vein, and the subjects rested inactive in a comfortable setting for at least 1 h before the withdrawal. Obviously, the 2400 h urine collections, the morning and midnight salivary samples, and the morning serum cortisol samples were obtained before those on DEX overnight suppression. Moreover, at least 1 week elapsed between the two DEX tests (0.5 and 1 mg overnight). All assays were performed in duplicate in the same test session. Intra- and interassay coefficients of variation (CV) were in any case below 8 and 12% respectively. Long-term CV for DXA instrument was 0.5% at the spine (assessed by the Hologic anthropometric spine phantom); short-term in vivo CV were 1 and 1.5% for the lumbar spine (L1–L4) and total hip respectively. Obtaining five repeated measurements in three subjects assessed the in vivo CV for ultrasound parameters. The nonstandardized CV ranged from 1.2 to 2.1%.

We performed database management and all statistical analyses using Statistica 6.0 Software (Statsoft, Inc., Tulsa, OK, USA). Normality of data was assessed by the Wilk–Shapiro test. As the majority of variables showed non-normal distribution, data are presented as median with range; differences and correlations were analyzed by two-tailed Mann–Whitney U-test and multiple linear regression analysis Spearman r coefficient respectively. For continuous variables, the parametric statistical analysis gave similar results. The level of statistical significance was set at $P < 0.05$.  

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Results

Demographic, clinical, and densitometric data from dual-energy absorptiometry (DXA) and quantitative ultrasound (QUS) are summarized in Table 1; laboratory data are reported in Table 2.

With regard to the cross-sectional evaluation at the first visit, a multiple regression analysis was initially performed including in the model each single measure of bone health separately, as dependent variable (i.e. BMD at lumbar spine, total femur, and femoral neck and heel ultrasound stiffness index) and, as independent variables, all the measures of HPA function together with age, weight, body mass index (BMI), alcohol consumption, smoking, calcium intake, years since menopause, estimated length of fertility age (from menarche to menopause), and E2 levels. Table 3 reports the correlation ‘matrix’ for any variable. The prespecified criterion of $P<0.2$ was used for selecting the variables to be included in the stepwise multivariate models. Lumbar BMD positively correlated with BMI for BMI $0.21$, $P<0.05$; $\beta$ for UFC $= -0.31$, $P<0.005$; and $\beta$ for morning serum cortisol $= -0.26$, $P<0.05$). Total femur BMD inversely correlated with morning serum cortisol ($\beta = -0.21$, $P=0.05$); also femoral neck BMD was inversely correlated with morning serum cortisol ($\beta = -0.25$, $P<0.05$: Figs 1 and 2). Finally, heel ultrasound stiffness index was inversely correlated with midnight salivary cortisol ($\beta = -0.35$, $P<0.005$). On the two graphs of Fig. 1, there is a clear outlier with an unusually elevated lumbar BMD value. There were no significant BMD differences between the four measured vertebral bodies (from L1 to L4) and DXA artifacts such as vertebral fractures, osteoarthritis, or aortic calcifications were excluded by an X-ray of the lumbar spine. However, the correlations between lumbar spine BMD and morning serum cortisol and between lumbar spine BMD and UFC remained statistically significant after removing the outlier ($\beta = -0.26$, $P<0.05$; $\beta = -0.27$, $P<0.05$ respectively). With regard to the second visit after 1-year follow-up, when considering the annual rate of change of BMD, at lumbar, total femur, and femoral neck site, no correlation was found with the hormonal variables studied, even after adjustment for age and years since menopause. The same was true for the rate of change of heel ultrasound stiffness index (data not shown).

After stratification of subjects into two different groups according to the percent year change of BMD values and using the classical definitions of ‘fast bone losers’ (annual BMD loss $\geq 3\%$) and ‘slow bone losers’ (annual BMD loss $<1\%$) (15, 16, 17), no differences were found in any considered hormonal variable. Table 4 reports evaluation of the HPA axis in the 22 fast bone losers in comparison with the 32 slow bone losers at lumbar spine. The absence of any significant difference was confirmed after analyzing the total lumbar and the femoral neck site (data not shown).

**Discussion**

This study was designed to evaluate the influence of cortisol secretion on bone health and to determine whether women who lose bone rapidly in the early postmenopausal period differ from those who do not in measures of the HPA axis. We recruited healthy women who did not change lifestyle and dietary behaviors during the year of observation, and we chose a lumbar DXA T-score of $-2.0$ or greater as the main inclusion criterion.
criterion to avoid a compelling indication to treatment (even supplementation with calcium and/or vitamin D) that could act as a confounding factor. Consequently, only women with normal or marginally decreased lumbar BMD were enrolled.

In diverse cellular models, estrogens were found to antagonize glucocorticoid action via complex interference with glucocorticoid receptor function and fate (18, 19, 20). It is likely that the estrogen fall after menopause may allow glucocorticoids to impair bone health, even if cortisol levels are within the normal range.

In agreement with this hypothesis, we found a significant inverse correlation between BMD values at the lumbar spine and femur with parameters of adrenal function, such as morning serum cortisol and UFC. Complementary to these results was the inverse correlation obtained between the heel ultrasound stiffness index and the midnight salivary cortisol levels. The measurement of cortisol in saliva has some advantages compared with serum: it avoids the stress of venipuncture and estimates more precisely the free, biologically active cortisol, not being influenced by the salivary flux (21, 22) and variations in cortisol-binding globulin levels (22). Our data suggest that variations of cortisol levels within the physiological range may impair bone mass and bone quality, at least as can be gauged by heel ultrasound (23) in the physiological menopause. The discrepancy between serum and salivary cortisol is not easily understandable. Previous data reported by Dennison et al. (24), who studied the circadian cortisol profile in 34 healthy men aged 61–72 years, are in keeping with the present findings. The negative relationship between cortisol levels and BMD at the lumbar and femoral sites disappeared in their study after menopause may allow glucocorticoids to impair bone health, even if cortisol levels are within the normal range.

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**Table 3** Correlation matrix of the multivariate analysis. In the upper lines are reported the β (r) values and in the lower lines the P values. The stepwise multivariate models (including variables selected by P<0.2) are represented in bold. Each column shows the variables remaining in the model during the stepwise analysis (i.e. the variables with a P value < 0.2).

<table>
<thead>
<tr>
<th>Lumbar BMD</th>
<th>Total femur BMD</th>
<th>Femoral neck BMD</th>
<th>QUS stiffness index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>−0.04</td>
<td>0.45</td>
<td>0.18</td>
</tr>
<tr>
<td>Weight</td>
<td>0.879</td>
<td>0.126</td>
<td>0.490</td>
</tr>
<tr>
<td>BMI</td>
<td>0.317</td>
<td>0.075</td>
<td>0.22</td>
</tr>
<tr>
<td>YSM</td>
<td>0.054</td>
<td>0.040</td>
<td>0.042</td>
</tr>
<tr>
<td>BMI</td>
<td>0.530</td>
<td>0.156</td>
<td>0.243</td>
</tr>
<tr>
<td>ELFA</td>
<td>−0.12</td>
<td>−0.38</td>
<td>−0.17</td>
</tr>
<tr>
<td>Smoking</td>
<td>0.686</td>
<td>0.177</td>
<td>0.520</td>
</tr>
<tr>
<td>Alcohol intake</td>
<td>0.05</td>
<td>0.06</td>
<td>0.16</td>
</tr>
<tr>
<td>Calcium intake</td>
<td>0.09</td>
<td>−0.23</td>
<td>−0.16</td>
</tr>
<tr>
<td>Calcium intake</td>
<td>0.466</td>
<td>0.06</td>
<td>0.117</td>
</tr>
<tr>
<td>Calcium intake</td>
<td>0.18</td>
<td>0.29</td>
<td>0.14</td>
</tr>
<tr>
<td>Calcium intake</td>
<td>0.159</td>
<td>0.318</td>
<td>0.015</td>
</tr>
<tr>
<td>Calcium intake</td>
<td>0.015</td>
<td>0.03</td>
<td>0.004</td>
</tr>
<tr>
<td>Calcium intake</td>
<td>0.015</td>
<td>0.03</td>
<td>0.004</td>
</tr>
<tr>
<td>Calcium intake</td>
<td>0.0949</td>
<td>0.02</td>
<td>0.004</td>
</tr>
<tr>
<td>Calcium intake</td>
<td>0.053</td>
<td>0.819</td>
<td>0.98</td>
</tr>
<tr>
<td>Calcium intake</td>
<td>0.511</td>
<td>0.430</td>
<td>0.546</td>
</tr>
</tbody>
</table>

YSM, years since menopause; ELFA, estimated length of fertility age; F, cortisol; UFC, urinary free cortisol; DEX, dexamethasone.
when BMI values were taken into account, while in our experience, the relationship remains significant also after adjustment for BMI. Moreover, our data are in general agreement with a number of findings in patients with adrenal incidentalomas and subclinical hypercortisolism (5, 6, 7, 8), showing that even minimal cortisol excess could exert detrimental effects on bone.

The adrenocortical secretion as a factor accelerating bone loss in the so-called fast bone losers has not received adequate attention in the literature, given the difficulties of investigating subtle differences in the HPA axis among individuals. Even if the concept of slow and fast losers appears to have lost some ground in recent decades, it was a practical way to assess whether two different groups of subjects, showing a different bone response to the fall of estrogens, are differently exposed to a detrimental effect of cortisol on postmenopausal bone loss. Age and time since menopause did not correlate in our study with the percent year change of bone loss. Age and time since menopause did not correlate in our study with the percent year change of bone loss. Age and time since menopause did not correlate in our study with the percent year change of bone loss.

The correlation lines and the dashed lines represent 95% CI. Fast bone losers and slow bone losers are indicated by open and closed triangles respectively. The remaining subjects are identified by closed circles.

Our data are in agreement with those published by Manolagas et al. (14) and do not support the view that the most abundant adrenal androgen, DHEA-S, has a protective role in the early postmenopausal bone loss. Circulating levels of DHEA-S were, in fact, superimposable in women of our study, independently of their stratification according to the rate of BMD change. At variance with the findings of Manolagas et al. (14), we did not find differences in UFC excretion between fast and slow bone losers. A possible explanation could be viewed in the different etiology of menopause (surgical vs physiological). It is conceivable that early menopause following ovariectomy is a more stressful event, in comparison with the natural cessation of ovarian function. Dennison et al. (24) also found a link between cortisol levels and the rate of bone loss in a 4-year follow-up period. The longer follow-up and the gender difference could offer an explanation for this discrepancy.

There are some limitations in our study. The sample size, even if not negligible, could have limited the statistical power of the study. The strict inclusion criteria (particularly the lumbar T-score of ≥ -2.0) could have compromised the ability to recognize associations between bone density and cortisol, leading to the exclusion of subjects more likely to have abnormal cortisol levels and be fast bone losers.

In summary, cortisol secretion seems to play a clear negative role on bone health in the first years after the menopause. Further research is worthwhile to evaluate the reasons for the subtle differences in otherwise physiological cortisol levels, possibly reflecting a different response of the HPA axis to the ‘stressful’ event of the menopause. Conversely, differences in cortisol secretion assessed do not explain the differential rate of bone loss in the early postmenopausal period, at least in healthy, nonosteoporotic women.

Table 4 Comparison of hormonal variables in fast vs slow bone losers at lumbar spine (L1–L4). Values are given as median and range. Fast bone losers, annual BMD loss ≥3%; slow bone losers, annual BMD loss <1%.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Fast BL (n=22)</th>
<th>Slow BL (n=32)</th>
<th>Significance (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinary free cortisol (µg/24 h)</td>
<td>56.3 (35.4–114.7)</td>
<td>68.7 (35.3–118.3)</td>
<td>0.102</td>
</tr>
<tr>
<td>Morning (0800 h) serum cortisol (µg/dl)</td>
<td>15.3 (7.4–21.9)</td>
<td>12.8 (9.4–21.3)</td>
<td>0.256</td>
</tr>
<tr>
<td>Midnight salivary cortisol (µg/l)</td>
<td>2.0 (1.0–5.2)</td>
<td>1.0 (1.0–5.3)</td>
<td>0.352</td>
</tr>
<tr>
<td>Serum cortisol after 0.5 mg overnight dexamethasone (µg/dl)</td>
<td>1.1 (0.5–4.3)</td>
<td>1.1 (0.5–7.5)</td>
<td>0.827</td>
</tr>
<tr>
<td>Serum cortisol after 1 mg overnight dexamethasone (µg/dl)</td>
<td>1.0 (0.5–1.9)</td>
<td>1.0 (1.0–2.1)</td>
<td>0.917</td>
</tr>
<tr>
<td>DHEA-S (µg/dl)</td>
<td>98.6 (19.3–204)</td>
<td>75.0 (27.3–164)</td>
<td>0.242</td>
</tr>
</tbody>
</table>

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Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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