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

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ABSTRACT

Objective: 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 DXA and heel ultrasound parameters in 82 healthy, non osteoporotic (lumbar T-score ≥ -2.0) women (median age 52.5 years, range 42-61). These women were examined in two sessions, one year apart, in the early postmenopausal period (onset of menopause between 6 and 60 months). Parameters of HPA function were: morning serum cortisol, morning and midnight salivary cortisol, 24 hour urinary free cortisol (UFC), serum cortisol after 0.5 mg and 1 mg overnight dexamethasone, DHEA-S.
Results: In multiple regression analyses, the following significant inverse correlations were found: 1) lumbar BMD and either 24 hour UFC ($p<0.005$), or morning serum cortisol ($p<0.005$); 2) total femur and femoral neck BMD with morning serum cortisol ($p<0.005$); 3) heel ultrasound stiffness index and midnight salivary cortisol ($p<0.005$). The annual rate of change of lumbar and femoral BMD did not correlate with any of the above-mentioned hormonal variables. No difference was found in the parameters of hypothalamic pituitary adrenal (HPA)-axis function in slow (loss of BMD <1%) *vs* fast (loss of BMD ≥3%) bone losers.
Conclusions: HPA axis may contribute to the 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.

INTRODUCTION

Glucocorticoid induced osteoporosis (GIO) is the most common cause of secondary osteoporosis [1]. Patients receiving exogenous glucocorticoids have a rapid increase of the risk of fragility fractures after the start of their therapy [2]. Patients with Cushing's syndrome, the most apparent phenotype of endogenous glucocorticoid excess, experience such fractures in about 50% of cases [3].
Also patients with an incidentally discovered adrenal adenoma (adrenal incidentaloma) diagnosed as having subclinical hypercortisolism have been reported to display signs of GIO, including a reduction of bone mineral density (BMD) of the spine, altered ultrasound bone characteristics and a number of morphometric fractures higher than expected in the control population [4-7]. A number of confounding factors, however, is likely to explain inconsistencies in other reports on this issue [8-10], prompting interest for additional studies on subtle glucocorticoid excess and bone health.
In the last decade two papers have addressed the question whether there is an overrepresentation of subclinical hypercortisolism among patients with established osteoporosis. Kann et al have focused on impaired cortisol suppression by high-dose dexamethasone administration in postmenopausal osteoporotic women [11], possibly heralding a partially autonomous glucocorticoid secretion in a number of such cases. Chiodini et al have recently reported a surprisingly high prevalence of subclinical hypercortisolism in a cohort of outpatients referred for osteoporosis [12]. The role, if any, of the endogenous glucocorticoids in the physiological bone loss after the menopause remains to be explored.
More than 30 years ago, Manolagas et al 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 [13]. They found a higher urinary cortisol excretion and a paradoxically diminished cortisol response to corticotrophin in those women who lost bone rapidly.
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 determine prospectively the relationship between the HPA axis and the rate of bone loss from baseline after one year.

SUBJECTS AND METHODS

Subjects were 82 healthy, non osteoporotic (DXA lumbar T-score ≥ -2.0) women, aged 52.3 ± 3.6 years (mean ± SD), 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 Ethical 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, TSH) was obtained in all subjects. Menopausal status was confirmed by FSH, LH and estradiol measurements. In all participants we measured lumbar and hip bone mineral density (BMD), at baseline (first visit) and after 1 year (second visit), by DXA (Hologic QDR4500-W, Waltham, MA, USA; software version 9.03). Heel ultrasound parameters (GE Healthcare, Lunar Achilles Express) were obtained in 49 subjects. Markers of the HPA axis function (morning serum

cortisol, morning and midnight salivary cortisol, 24 hour urinary free cortisol (UFC), serum cortisol after 0.5 mg and 1 mg overnight dexamethasone, dehydro-epiandrosterone-sulphate (DHEA-S) were measured in all subjects using commercially available reagents (competitive chemilumiscent enzyme immunoassay by Immulite 2000 DPC, Los Angeles – USA, for serum cortisol and DHEA-S; radioimmunoassay by RADIM S.p.A., Pomezia (RM) – Italy, for salivary cortisol and UFC).
A total of 92 women were screened: 9 of them were excluded *a posteriori* because the basal lumbar DXA evaluation not fulfilled the inclusion criteria of a lumbar T-score ≥-2.0 (in one case asymptomatic thyrotoxicosis was discovered as the main reason of 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 died suddenly for 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 can be refrigerated at 4°C and stored at least for a week. Urinary and salivary samples were collected at home, conserved in fridge by the subjects, and delivered, the day after, at hospital. Blood samples were drawn at hospital between 0800 and 0900 a.m. and centrifuged at 1000 *g* for 15 min within 2 h of collection; sera were immediately frozen and stored at -20° until assayed. To avoid interference on cortisol secretion due to the stress of the venipuncture, an indwelling iv cannula was inserted in an antecubital vein, and the subjects rested inactive in a comfortable setting, 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 dexamethasone overnight suppression. Moreover, at least one week of time elapsed between the two dexamethasone tests (0.5 and 1 mg overnight). All assays were performed in duplicate in the same test session. Intra- and inter-assay coefficients of variation were in any case below 8% and 12%, respectively. Long term coefficient of variation 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 5 repeated measurements in 3 subjects assessed the in vivo CVs for ultrasound parameters. The non-standardized CVs 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. Since the majority of variables showed non-normal distribution, data are presented as median with range; differences and correlations were analyzed by 2-tailed Mann-Whitney U-test and by multiple linear regression analysis Spearman R coefficient, respectively. For continuous variables we checked that parametric statistical analysis gave similar results. Level of statistical significance was set at $p<0.05$.

RESULTS

Demographic, clinical and data from DXA and 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; heel ultrasound stiffness index) and, as independent variables, all the measures of HPA function together with age, BMI, alcohol consumption, smoking, calcium intake, years since menopause, estimated length of fertility age (from menarche to menopause) and estradiol levels. Among all the variables processed, only BMI and some measures of cortisol secretion (UFC, morning serum cortisol and midnight salivary cortisol) remained in the final models. When considering basal BMD as the dependent variable and BMI together with hormonal variables, as the independent ones, inverse

correlations were found between lumbar BMD and either 24 hour UFC and morning serum cortisol (R of the model =0.48, R^2 =0.23, p<0.005; β for UFC=-0.32, p<0.005 and β for morning serum cortisol =-0.32, p<0.005). Total femur BMD resulted inversely correlated with morning serum cortisol (R of the model =0.47, R^2 =0.22, p<0.005; β =-0.34, p<0.005); also femoral neck BMD was inversely correlated with morning serum cortisol (R of the model =0.42, R^2 =0.17, p<0.05, β=-0.36 p<0.005) (Figures 1 and 2). Finally, heel ultrasound stiffness index was inversely correlated with midnight salivary cortisol (R=0.46, R^2 =0.21, p<0.05; β=-0.38, p<0.005). The strength of the above mentioned correlations decreases slightly removing BMI from the models, but remains statistically significant (Table 3).
With regard to the second visit after one-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. The same was true for the rate of change of heel ultrasound stiffness index (data not shown).
After stratification of subjects into 2 different groups according to the percent year change of BMD values and using the classical definitions of "fast bone losers" (annual BMD loss ≥3%) and "slow bone losers" (annual BMD loss <1%) [14-16], 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 when analyzing the total femur and the femoral neck (data not shown).

DISCUSSION

The present 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 post-menopausal 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 criterium to avoid a compelling indication to treatment that may have compounded our results. 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 [17-19]. It is likely that the estrogen fall after menopause may allow that glucocorticoids 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 urinary free cortisol. Complementary to this results, was the inverse correlation obtained between the heel ultrasound stiffness index and midnight salivary cortisol levels. The measurement of cortisol in saliva has some advantages compared to serum: it avoids the stress of venipuncture and estimates more precisely the free, biologically active cortisol, not being influenced by the salivary flux [20, 21] and variations in cortisol binding globulin (CBG) levels [21]. Our data suggest that variations of cortisol levels within the physiological range may impair bone mass and bone quality in the physiological menopause. The discrepancy between serum and salivary cortisol is not easily understandable. Previous data reported by Dennison et al [22], 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 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 [4-7] 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 group of subjects, showing a different bone response to the fall of estrogens, are differently exposed to a detrimental effect of cortisol on post-menopausal bone loss. Age and time since menopause did not correlate in our study with the percent year change of BMD; in other words, the chance of being labeled as fast or slow loser was not simply related to time since the cessation of ovarian function. On the other hand, our data do not provide evidence that in healthy women the yearly rate of bone loss in the first post-menopausal period has any relationship with a number of tests exploring the HPA function. In our cohort, fast bone losers and slow bone losers had the same levels of the examined adrenal steroids in blood, saliva and urine, and the same sensitivity to dexamethasone inhibition. The period of one year was appropriate for stratifying the subjects in two groups of women who loose bone rapidly or not. Taking into account the in vitro and in vivo coefficient of variation for our DXA at the lumbar spine (0.5 and 1%, respectively) the least significant change ranged between 1,38% and 2,77%. So, also the short time interval elapsed between the 2 DXA measurements (just one year) can be considered adequate for detecting a BMD percent change ≥ 3%, that reflects a real biological change.

Our data are in agreement with those published by Manolagas et al [13] 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 our women, independently of their stratification according to the rate of BMD change. At variance with the findings of Manolagas et al [13], we did not find differences in urinary free cortisol 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 [22] also found a link between cortisol levels and the rate of bone loss in a 4-yr 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 of 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 post-menopausal period, at least in healthy, non-osteoporotic women.

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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FIGURE LEGENDS

Figure 1. Linear correlations between lumbar bone mineral density and morning serum cortisol, on the left panel and between lumbar bone mineral density and 24 hour urinary cortisol (UFC), on the right panel.

Figure 2. Linear correlations between total femur bone mineral density and morning serum cortisol, on the left panel and between femoral neck bone mineral density and morning serum cortisol, on the right panel.

Table 1

Main characteristics of the subjects: demographic, clinical and densitometric data.	
Age, yrs	52.5 (42;61)
Menopausal age , yrs	50 (40;59)
Menopausal duration, yrs	2.1 (0.5;5)
Body mass index, Kg/m^2	26.5 (19.4;38.2)
Cigarette smoking: never / previous / current	39(47)/31(38)/12(15)
Moderate alcohol consumption*: yes/no	53(65)/29(35)
Moderate caffeine consumption**: yes/no	76(93)/6(7)
Lifestyle: active/sedentary	39(48)/43(52)
Estimated calcium dietary intake, mg/daily	812 (350;1555)
Lumbar spine BMD (L_1-L_4), g/cm^2	0.938 (0.821;1.443)
Lumbar spine T-score (L_1-L_4)	-1.0 (-2.0;3.6)
Lumbar spine Z-score (L_1-L_4)	-0.1 (-1.5;4.4)
Lumbar spine % change/yr	-1.3 (-7.8;4.1)
Total hip BMD, g/cm^2	0.859 (0.659;1.108)
Total hip T-score	-0.7 (-2.3;0.7)
Total hip Z-score	-0.1 (-1.7;2.3)
Total hip % change/yr	-1.2 (-5.5;5.3)
Femoral neck BMD, g/cm^2	0.733 (0.591;0.940)
Femoral neck T-score	-1.0 (-2.3;0.8)
Femoral neck Z-score	-0.2 (-1.5;1.6)
Femoral neck % change/yr	-1.1 (-12;9.9)
Heel ultrasound stiffness index (in 49 subjects)	84.5 (52;138)
Heel ultrasound T-score (in 49 subjects)	-1.2 (-3.7;2.9)
Heel ultrasound Z-score (in 49 subjects)	0.1 (-2.4;4.3)
Values are given as number and percentage of subjects or median and range. BMD: bone mineral density. *Moderate alcohol consumption: no more than one drink a day. **Moderate caffeine consumption: no more than 300 mg of caffeine a day.	

Table 2

Main characteristics of the subjects: laboratory data.	
Serum total calcium, mmol/L	2.47 (2.20-2.60)
Serum corrected calcium, mmol/L	2.43 (2.02-2.70)
Urinary calcium, mmol/24h	4.8 (1.9-10.2)
Serum alkaline phoshatase, IU/L	76 (33-162)
TSH, µIU/ml	1.76 (0.41-5.18)
DHEA-S, µg/dl	78.9 (19.3-306)
Morning serum cortisol (h 8.00), µg/dl	13.8 (3.7-23.9)
Urinary free cortisol, µg/24h	65 (35-202)
Morning salivary cortisol (h 8.00), µg/L	10.1 (4.5-25.7)
Midnight salivary cortisol (h 24.00), µg/L	1.0 (1.0-5.5)
Serum cortisol after 1 mg overnight dexamethasone, µg/dl	1.0 (0.5-2.1)
Serum cortisol after 0.5 mg overnight dexamethasone, µg/dl	1.0 (0.5-7.5)
Values are given as median (range).	

Table 3	**Lumbar BMD**			**Total femur BMD**				**Femoral neck BMD**				**QUS Stiffness index**		
Age	*-0.04* *0.879*			*0.45* *0.126*	*0.18* *0.490*			*0.40* *0.211*				*-0.21* *0.575*		
Weight	*-0.37* *0.317*			*-0.56* *0.104*	*-0.10* *0.713*			*-0.29* *0.433*				*-0.09* *0.808*		
BMI	*0.75* *0.054*	*0.22* *0.040*	*0.21* *0.042*	*0.87* *0.017*	*0.36* *0.223*			*0.54* *0.162*	*0.14* *0.219*			*0.19* *0.641*		
YSM	*-0.10* *0.530*			*-0.22* *0.156*	*-0.16* *0.243*			*-0.11* *0.497*				*0.12* *0.528*		
ELFA	*-0.12* *0.686*			*-0.38* *0.177*	*-0.17* *0.520*			*-0.41* *0.183*	*-0.09* *0.437*			*0.08* *0.801*		
Smoking	*-0.05* *0.682*			*0.06* *0.609*				*0.16* *0.210*				*-0.16* *0.271*		
Alcohol intake	*-0.09* *0.466*			*-0.23* *0.06*	*-0.16* *0.117*	*-0.17* *0.117*		*0.05* *0.685*				*-0.29* *0.063*	*-0.22* *0.432*	
Calcium intake	*0.18* *0.159*	*0.10* *0.318*		*0.29* *0.015*	*0.14* *0.210*			*0.29* *0.024*	*0.17* *0.114*	*0.14* *0.190*		*-0.21* *0.157*	*-0.19* *0.236*	
DHEA-S	*0.21* *0.088*	*0.10* *0.338*		*0.02* *0.803*				*0.06* *0.634*				*0.07* *0.633*		
Serum F 8.00	*-0.29* *0.030*	*-0.27* *0.012*	*-0.26* *0.014*	*-0.39* *0.002*	*-0.26* *0.017*	*-0.25* *0.023*	*-0.21* *0.054*	*-0.34* *0.011*	*-0.28* *0.012*	*-0.24* *0.023*	*-0.25* *0.023*	*-0.05* *0.744*		
UFC	*-0.32* *0.015*	*-0.32* *0.03*	*-0.31* *0.004*	*-0.25* *0.039*	*-0.17* *0.121*	*-0.14* *0.199*		*-0.18* *0.170*	*-0.12* *0.274*			*-0.14* *0.374*		
Salivary F 8.00	*-0.01* *0.949*			*0.03* *0.774*				*-0.05* *0.707*				*-0.15* *0.369*		
Salivary F 24.00	*-0.13* *0.290*			*-0.12* *0.286*				*-0.16* *0.207*				*-0.34* *0.023*	*-0.39* *0.001*	*-0.35* *0.004*
F after DEX 1 mg	*-0.01* *0.953*			*0.02* *0.819*				*-0.01* *0.918*				*-0.05* *0.739*		
E2	*-0.08* *0.511*			*-0.09* *0.430*				*-0.07* *0.546*				*0.01* *0.950*		

Correlation matrix of the multivariate analysis. In upper lines are reported the β (r) values and in lower lines the p values. The step-wise multivariate models (including variables selected by the pre-specified criterion of $p<0.2$) are highlighted by a darker background. BMI=Body Mass Index; YSM=Years Since Menopause; ELFA=Estimated Lenght of Fertility Age; DHEA-S= dehydro-epiandrosterone-sulphate; F=cortisol; UFC=Urinary Free Cortisol; DEX=Dexamethasone; E2=Estradiol.

Table 4

Comparison of hormonal variables in fast vs slow bone losers at lumbar spine (L1-L4)			
	Fast BL (N°=22)	**Slow BL (N°=32)**	**Significance (*p*)**
Urinary free cortisol (µg/24h)	**56.3 (35.4-114.7)**	**68.7 (35.3-118.3)**	**0.102**
Morning (h 8.00) serum cortisol (µg/dl)	**15.3 (7.4-21.9)**	**12.8 (9.4-21.3)**	**0.256**
Midnight salivary cortisol (µg/L)	**2.0 (1.0-5.2)**	**1.0 (1.0-5.3)**	**0.352**
Serum cortisol after 0.5 mg overnight dexamethasone (µg/dl)	**1.1 (0.5-4.3)**	**1.1 (0.5-7.5)**	**0.827**
Serum cortisol after 1 mg overnight dexamethasone (µg/dl)	**1.0 (0.5-1.9)**	**1.0 (1.0-2.1)**	**0.917**
DHEA-S (µg/dl)	**98.6 (19.3-204)**	**75.0 (27.3-164)**	**0.242**

Fast bone losers: annual BMD loss ≥3%; Slow bone losers: annual BMD loss <1%.
Values are given as median and range