Effects of sex steroids on bone in women with subclinical or overt endogenous hypercortisolism

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Abstract

Objective: Glucocorticoid-induced osteoporosis is the most frequent cause of secondary osteoporosis. Nevertheless, limited data are available on bone status in patients with endogenous cortisol excess. This study is aimed at investigating the role of sex steroids and severity of hypercortisolism on bone mineral density (BMD) and prevalence of vertebral fractures in female patients.

Design: Cross-sectional, case–control study.

Patients: Seventy-one consecutive women were enrolled: 36 with overt hypercortisolism (26 with ACTH-secreting pituitary adenoma and 10 with cortisol-secreting adrenal tumor) and 35 with subclinical hypercortisolism due to adrenal incidentalomas. They were compared with 71 matched controls.

Methods: At diagnosis, we measured serum cortisol, FSH, LH, estradiol, testosterone, androstenedione and DHEAS, and urinary cortisol excretion. BMD was determined by dual energy X-ray absorptiometry at the lumbar spine and femoral neck. Vertebral fractures were investigated by a semiquantitative scoring method.

Results: Between women with overt and subclinical hypercortisolism BMD values and prevalence of any vertebral (69 vs 57%, \( P = 0.56 \)), clinical (28 vs 11.4%, \( P = 0.22 \)), and multiple vertebral fractures (36 vs 31%, \( P = 0.92 \)) did not differ. Among patients with subclinical hypercortisolism, amenorrhoic women had a lower BMD (\( P = 0.035 \)) and more frequent vertebral fractures (80 vs 40%, \( P = 0.043 \)) when compared with the eumenorrhoic ones. Among women with overt hypercortisolism, there was no difference in lumbar BMD (\( P = 0.37 \)) and prevalence of fractures (81 vs 60%, \( P = 0.26 \)) between those amenorrhoic and eumenorrhoic. By logistic regression analysis, lumbar spine BMD values and cortisol-to-DHEAS ratio were the best predictors of vertebral fractures (\( P < 0.01 \)).

Conclusions: Vertebral fractures are very common in women with endogenous cortisol excess, regardless of its severity. The deleterious effects of hypercortisolism on the spine may be partly counterbalanced by DHEAS increase at any degree of cortisol excess, and by preserved menstrual cycles in women with subclinical but not in those with overt hypercortisolism.

Introduction

Glucocorticoid-induced osteoporosis is the most frequent cause of secondary osteoporosis and bone fractures are the most worrisome and frequent events due to osteoporosis in patients on glucocorticoid treatments. Increased incidence of fractures has been described in patients on daily doses as low as 2.5 mg prednisolone equivalents, although the fracture risk is known to be dose dependent (1–3). However, to date the prevalence of fractures at any site has been poorly analyzed in patients with different degrees of severity of endogenous hypercortisolism.

Vertebral and costal fractures have been reported as a presenting symptom of Cushing’s syndrome by several authors (4, 5), but few studies performed a systematic evaluation of fractures in this particular population (6–8). A high incidence of non-traumatic fractures in the period preceding the diagnosis of Cushing’s syndrome was described in a retrospective study by Vestergaard et al. (6). Chiodini et al. (7) reported vertebral fractures in more than half of women with subclinical hypercortisolism due to adrenal incidentaloma. Similarly, we have recently described vertebral fractures in 76% of a mixed male and female population, regardless of the etiology of the patients’ hypercortisolism; clinically evident fractures were observed in 52% of these subjects (8).

Cortisol exerts direct and indirect effects on bone, enhancing its resorption first and then inhibiting its...
formation (9–13). Direct effects of glucocorticoid excess include inhibited differentiation of pluripotent mesenchymal cells toward the osteoblastic lineage and enhancement of their differentiation into the adipocytic pathway (13). Cortisol also reduces the apoptosis of osteoclasts and promotes that of osteoblasts and osteocytes (14). Cortisol excess reduces the production of growth factors and bone matrix proteins, and decreases calcium absorption (15). All these effects contribute to reduced bone resistance and quality.

Further negative effects of cortisol excess on bone consist of its inhibition of the gonadal axis function in both genders (11, 12). It is important to consider, however, that androgen production is increased in adrenocorticotropic (ACTH)-dependent and in some adrenal-dependent hypercortisolism. The relationship between hypercortisolism and sex steroids may be complex. Androgen excess exerted protective effects on bone mass in women with polycystic ovary syndrome, counterbalancing the harmful effects of amenorrhea (20, 21). Considering the effects of estrogens, both exogenous and endogenous cortisol excess have been shown to cause more severe bone damage in postmenopausal than reproductive-aged women (22, 23). Estrogens are frequently reduced and amenorrhea occurs in women with overt cortisol excess (18, 19). Nevertheless, it is still unclear to which extent the androgens and estrogens can counterbalance the negative bony effects of endogenous cortisol excess.

This study aimed at investigating the effects of sex steroids on bone density and vertebral fractures in eumenorrhoic and amenorrhoic women with different degree of severity of endogenous hypercortisolism.

Subjects and methods

Study design

In this cross-sectional case–control study, we analyzed bone mineral density (BMD) and vertebral fractures in women with subclinical and overt hypercortisolism due to ACTH-dependent Cushings disease (CD) or adrenal adenoma. We took into consideration body mass index (BMI), menstrual history, estimated disease duration, levels of serum cortisol (F), testosterone (T), androstenedione, DHEAS, and 17-β-estradiol (E2), and the ratios between serum F, androgens, and E2. All determinations were performed at the diagnosis of hypercortisolism. The study was performed according to the procedures indicated by the Helsinki II Declaration. All enrolled subjects gave their written informed consent to participate in the study.

Subjects

Seventy-one consecutive female patients with endogenous hypercortisolism were enrolled: 26 of them had CD, while 10 women had overt and 35 subclinical hypercortisolism due to adrenal adenoma. The diagnosis of overt hypercortisolism was made on the basis of the typical clinical signs and symptoms and inappropriately elevated values of serum F and urinary free F (UFF) excretion. The diagnosis of subclinical Cushings syndrome was based on the lack of typical clinical features (striae rubrae, moon face, gibbous, visceral obesity, and peripheral muscular atrophy) in the presence of adrenal mass and at least two abnormalities in functional tests of the hypothalamic–pituitary–adrenal axis (HPA) (24–26). The incomplete inhibition of serum cortisol levels (> 30 ng/ml) after an overnight 1 mg dexamethasone (DXM) suppression test was the abnormality that was always required, since it has a high specificity to detect autonomous adrenal function (27). Other abnormalities of the HPA axis function included: baseline serum F or plasma ACTH at 0800 h (mean of at least two samples taken on different days); 24-h UFF excretion; daily cortisol average, calculated as (F 0800+F 1600+F 2400 h)/3 or cortisol 24:00/08:00 percent ratio expressing F circadian rhythm abnormalities, calculated as (F 24:00/F 08:00) × 100.

In all patients, we measured UFF and circulating levels of F (at 0800, 1600, and 2400 h), ACTH, follicle-stimulating hormone (FSH), luteinizing hormone (LH), and prolactin. A low-dose 2 mg DXM suppression test was performed in all cases, while a high-dose 8 mg DXM suppression test was carried out in all patients with suspected ACTH-dependent hypercortisolism. Pituitary magnetic resonance imaging or adrenal computed tomography was performed as appropriate. Histology confirmed the diagnosis of pituitary ACTH-secreting adenoma in all 26 women (microadenoma in 21 and macroadenoma in 5). Adrenal adenoma was the diagnosis in all women with adrenal lesions.

Disease duration was estimated by the interval between the symptom onset mentioned by the patients when specifically asked (i.e. weight gain, increase in blood pressure, appearance of striae rubrae, etc.) and the diagnosis. In patients with subclinical hypercortisolism, the period of onset or worsening of underlying hypertension, increase in serum glucose levels, and weight gain were considered for the estimation of the duration of mild hypercortisolism (27). Women were considered amenorrhoic when menstrual cycles did not occur for at least 6 months. Eight post-menopausal women were included in the group of amenorrhoic ones (three in the group with overt and five in the group with subclinical hypercortisolism).

For each patient enrolled, a control subject matched for age and BMI was included in the data analysis; 71 women with eucortisolism were recruited among patients with euthyroid goiter (thyrotropin: 1–2.5 U/l). Age was matched to within 1 year of the birth date and BMI was matched within ± 1.5 kg/m². None of the 142 women had previously taken calcium or vitamin D supplements, oral contraceptives, hormonal
replacement therapy, or drugs known to interfere with bone metabolism, nor used to drink more than four cups of coffee per day and/or two alcohol beverages per day.

Twenty-two women with overt hypercortisolism and 14 women with subclinical hypercortisolism due to adrenal adenoma who were included in this study had also been included in previous studies (reference nos (8) and (28) respectively).

Assessment of bone density and turnover

Serum calcium (Ca), phosphorus (P), creatinine, alkaline phosphatase (ALP), albumin, intact molecule parathyroid hormone (iPTH), type I collagen C-telopeptide (sCTX), and osteocalcin were determined after at least 8-h fasting, while urinary calcium was measured in a 24-h urine collection, and corrected for creatinine excretion. BMD at the lumbar spine (LS; L1–L4, in anterior-posterior scan) and femoral neck (FN) was determined by dual energy X-ray absorptiometry (DEXA), using Hologic QDR 1000 densitometer (Hologic Inc., Waltham, MA, USA). Due to the age difference of the women, individual BMD values were expressed as Z-scores. The coefficient of variation for the DEXA technique was <1.7% for LS and <1.9% for FN. The reference population adopted in this study was the international pooled sample provided by the manufacturer; their data, however, did not differ significantly from those obtained on a local sample in a study performed when the device was set up (29). Quality control was maintained by daily scanning of an anthropomorphic spine phantom. A systematic review of each BMD was performed and artifacts from fractured vertebral bodies, kyphosis, and scoliosis were excluded from the evaluation. No patient had embedded metal at the lumbar vertebrae or severe joint disease at the L1–L4 segments.

Assessment of vertebral fractures

All women were evaluated for height decrease and back pain as clinical symptoms of vertebral fractures. Potential asymptomatic vertebral compression fractures were investigated in all women by standard spinal radiographs in anterior–posterior and lateral positions of the vertebrae Th4–L4. A prevalent fracture was defined, in accordance with previous studies, as at least 20% difference in anterior-posterior, middle–posterior or posterior–posterior adjacent ratio (30, 31). Since most patients had a very severe demineralization of the vertebral bodies, it was impossible to use the digitalized technique for vertebral body measurement. An analysis of radiographic films was independently performed in chronological order by two operators: an orthopedician (C R) and an endocrinologist trained in osteoporosis management (L T); they were blind to the status of case/control. The validity of each finding was established in concordance by both operators for the detection of presence and number of fractures; the inter-observer agreement was calculated using k statistics and resulted well (k=0.88).

Assays

Hormone determinations were performed with the same commercial kits for the whole study period: F, T, E2, and DHEAS, by Immulite, solid-phase chemiluminescent enzyme immunoassay (DPC, Los Angeles, CA, USA), and androstenedione by RIA Diagnostic Systems Laboratories (Webster, TX, USA). iPTH was measured by RIA and serum osteocalcin levels were measured by IRMA (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA). Serum CTX was measured with the CrossLaps assay (Osteometer Biotech, Herlev, Denmark). Intra- and inter-assay coefficients of variation were <8.5 and <10% for F; <5 and <6.4% for E2; <6 and 9% for DHEAS; <11 and 13% for T; and <6.7 and <12% for androstenedione respectively. For bone turnover determinations, they resulted <5.2 and <6.7% for osteocalcin and <3 and <10% for sCTX.

Blood chemistry profile, including levels of Ca, P, ALP, 24-h urinary Ca excretion, and creatinine, was analyzed using a standard autoanalyzer.

Statistical analysis

Statistical analysis was performed by the SPSS Inc. (Chicago, Ill, USA) package (release 13.0) and StatsDirect statistical software (Altrincham, UK; release 2.5.2). Data distribution was analyzed by the Kolmogorov–Smirnov test. To compare data for continuous variables, ANOVA followed by the Bonferroni test as a post hoc test or Kruskal–Wallis H test followed by Dwass–Steel–Chirchlow–Fligner test were used, according to the data distribution. Categorical variables were compared by the Pearson’s $\chi^2$ test. The data were compared between patients and controls, between patients with overt and subclinical cortisol excess, and between patients with and without fractures. Age, gonadal status, BMI, estimated disease duration, urinary F excretion, and circulating levels of F, androgens, E2 and F-to-androgen, and F-to-E2 ratios were considered as potential predictive factors for BMD by the linear regression analysis. All these parameters plus BMD were included in the evaluation of risk factors for vertebral fractures by the logistic regression. The stepwise forward selection was used for both procedures. Statistical significance was set at 5%.

Results

Anthropometric and endocrine profiles of women with overt and subclinical Cushing’s

When comparing the women with overt and subclinical hypercortisolism, the age, BMI, the ratio of amenorrhoic versus eumenorrhoic women, and amenorrhea duration
were similar in both groups. As expected, in patients with overt hypercortisolism, the estimated disease duration was shorter ($P < 0.05$ versus subclinical), whereas morning and midnight serum cortisol levels ($P < 0.05$) and urinary cortisol excretion ($P < 0.01$) were higher. Post-DMX cortisol was also higher in subjects with overt hypercortisolism as well as were DHEAS, androstenedione, and testosterone ($P < 0.05$; Table 1).

When comparing cases with controls, both groups of patients had significantly higher levels of morning, midnight, and post-DMX cortisol, whereas UFF, DHEAS, androstenedione, and T levels were significantly higher only in women with overt, but not in those with subclinical hypercortisolism. Values of E$_2$, prolactin, FSH, and LH widely overlapped in all three groups, without significant differences between the groups.

When comparing women with pituitary and adrenal overt hypercortisolism, their age, estimated disease duration, and the ratio of amenorrhoic versus eumenorrhoic women were similar. DHEAS was higher in women with pituitary adenomas than in those with adrenal adenomas ($8.4 \pm 3.6$ vs $3.3 \pm 3$ $\mu$mol/l, $P < 0.0001$). Serum F ($737 \pm 320$ vs $616 \pm 194$ nmol/l) and UFF ($1997 \pm 1930$ vs $1520 \pm 1241$ nmol/l) were also higher but without reaching statistical significance. Androstenedione, T, and E$_2$ levels were similar in both groups.

### Assessment of bone density, bone turnover, and vertebral fractures

Serum Ca, P, PTH, and Vitamin D values did not differ between controls and patients with subclinical or overt hypercortisolism. Osteocalcin was lower and CV was higher in patients than in controls and in women with overt hypercortisolism ($P < 0.05$ versus subclinical; Table 2).

BMD values at LS and FN were lower in patients than in controls, but did not differ between the groups with overt or subclinical hypercortisolism. According to the World Health Organization criteria, osteoporosis at LS was found in 14 (42%) women with overt and 10 (29%) with subclinical hypercortisolism; osteoporosis at FN was revealed in 9 (25%) and 7 (20%) women respectively. Osteopenia was diagnosed in 20 (55%) patients with overt and in 10 (29%) with subclinical Cushing's.

In the group affected by overt hypercortisolism, vertebral fractures were revealed in 25 (69%) of the women and were multiple in 13 (36%) of them. Clinically symptomatic collapsed vertebral were found in 10 (28%) of women; they were associated with pain, functional limitation, and height shortening by 3–7 cm of final stature. There was no significant difference between BMD values (LS $Z$-score: $-1.79 \pm 1.3$ vs $-1.40 \pm 1.0$; FN $Z$-score: $-0.96 \pm 0.89$ vs $-1.07 \pm 1.2$) and fracture rates (14/36 vs 11/35) between patients with pituitary or adrenal overt hypercortisolism.

In the group of women with subclinical hypercortisolism, vertebral fractures were observed in 20 (57%) of them, being multiple in 11 (31%). Clinically symptomatic collapsed vertebral were revealed in four (11%) patients. Only one post-menopausal woman in the control group had one vertebral fracture, therefore, this prevalence (1.4%) was significantly different from that observed in patients ($P < 0.01$).

The fractures were more frequent at the thoracic spine; involvement of the thoracic vertebral bodies alone was seen in 52% of patients, the lumbar vertebrae only were fractured in 23%, and both thoracic and lumbar bodies were involved in 25% of cases.

When comparing the whole group of patients with fractures to those without fractures, the first group had increased F values ($743 \pm 300$ vs $364.6 \pm 102$ nmol/l; $P < 0.001$) and F-to-DHEAS ratio ($0.49 \pm 0.3$ vs $0.24 \pm 0.2$; $P < 0.001$), and decreased LS BMD ($0.86 \pm 0.1$ vs $1.05 \pm 0.15$ $g/cm^2$; $P < 0.001$), while both groups had similar age, BMI, and other endocrine and biochemical parameters. Other fractures included four rib fractures (three in overt and one in subclinical hypercortisolism) and two foot fractures (one per group); all fractures

### Table 1 Demographic and endocrine parameters according to the degree of hypercortisolism.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Overt</th>
<th>Subclinical</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of women</td>
<td>36</td>
<td>35</td>
<td>71</td>
</tr>
<tr>
<td>Age (years)</td>
<td>42 (28–66)</td>
<td>46 (30–68)</td>
<td>44 (28–68)</td>
</tr>
<tr>
<td>Body mass index (kg/m$^2$)</td>
<td>30.3 (21–40)</td>
<td>29 (23–36)</td>
<td>29 (22–39)</td>
</tr>
<tr>
<td>Estimated disease duration (months.)</td>
<td>9 (6–14)*</td>
<td>21 (7–25)</td>
<td>–</td>
</tr>
<tr>
<td>No. of amenorrhoic women</td>
<td>16</td>
<td>15</td>
<td>30</td>
</tr>
<tr>
<td>Cortisol 0800 (nmol/l)</td>
<td>596 (276–1343)*</td>
<td>548 (288–926)</td>
<td>285 (180–530)$^\dagger$</td>
</tr>
<tr>
<td>Cortisol 2400 (nmol/l)</td>
<td>310 (80–670)*</td>
<td>280 (230–590)</td>
<td>115 (78–240)$^2$</td>
</tr>
<tr>
<td>Urinary free cortisol excretion (nmol/24h)</td>
<td>1156 (223–928)$^1$</td>
<td>410 (210–838)</td>
<td>390 (80–232)$^9$</td>
</tr>
<tr>
<td>Post-DMX cortisol (nmol/l)</td>
<td>326 (210–1320)*</td>
<td>133 (120–502)</td>
<td>35 (20–46)$^5$</td>
</tr>
<tr>
<td>DHEAS (µmol/l)$^a$</td>
<td>5.9 (0.33–99)$^9$</td>
<td>2.5 (0.5–4.5)</td>
<td>2.4 (1.3–6)$^6$</td>
</tr>
<tr>
<td>Androstenedione (nmol/l)$^a$</td>
<td>9 (0.34–58)$^8$</td>
<td>3.6 (0.4–7)</td>
<td>3.8 (0.5–3)$^3$</td>
</tr>
<tr>
<td>Testosterone (nmol/l)$^a$</td>
<td>4.33 (0.35–52)$^a$</td>
<td>2.3 (0.37–3.5)</td>
<td>2.42 (0.3–3)$^5$</td>
</tr>
<tr>
<td>17α-estradiol (pmol/l)$^a$</td>
<td>110 (40–220)</td>
<td>130 (55–235)</td>
<td>150 (70–240)</td>
</tr>
</tbody>
</table>

Data are expressed as median and range; *$P < 0.05$ and $^1P < 0.01$ versus subclinical hypercortisolism; $^2P < 0.05$ versus both groups of patients and $^3P < 0.05$ versus overt hypercortisolism.

$^a$In eumenorrhoic women determined in early follicular phase (days 3–6 of menstrual cycles).
occurred 6–18 months before being admitted to our department.

Effects of sex steroids and the severity of hypercortisolism on BMD and fractures

The prevalence of fractures and BMD values did not differ significantly between patients with overt or subclinical hypercortisolism (Table 3).

Among patients with overt hypercortisolism, there was no difference in lumbar BMD Z-score and prevalence of fractures between amenorrhoic and eumenorrhoic women. Their F, DHEAS, and E2 levels did not differ significantly, whereas androstenedione and T were higher in amenorrhoic women.

On the other hand, in the group with subclinical hypercortisolism, eumenorrhoic women had lower BMD (P = 0.035) and more frequent vertebral fractures (P = 0.043) when compared to those who were eumenorrhoic. Their E2 and androgen levels did not differ significantly, while serum F and UFF were lower in women without cycles.

When considering the patients altogether, lumbar BMD correlated with total T (r = 0.39, P = 0.03). Femoral BMD correlated with T (r = 0.6; P = 0.01) and DHEAS (r = 0.5; P = 0.04). There was no correlation between BMD, cortisol, and E2 values. A significant correlation was also found between femoral BMD and BMI (r = 0.4; P = 0.02).

Logistic regression analysis showed that morning F levels (odd ratio (OR) 3.9; P = 0.025) and high F-to-DHEAS ratio (OR 4.6; P = 0.006) were significant predictors of the fractures in all patients, while in the subgroup with subclinical Cushing’s, the gonadal status was also a significant predictor (OR 6.3; 95% confidence interval (CI): 1.73–23.4; P = 0.009).

Table 2 Parameters of bone turnover, bone density, and fractures in patients according to the degree of hypercortisolism.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Overt</th>
<th>Subclinical</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of women</td>
<td>36</td>
<td>35</td>
<td>71</td>
</tr>
<tr>
<td>Serum Ca (mmol/l)</td>
<td>2.36 ± 0.13</td>
<td>2.34 ± 0.14</td>
<td>2.33 ± 0.11</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>3.9 ± 0.44</td>
<td>3.86 ± 0.5</td>
<td>4.1 ± 0.3</td>
</tr>
<tr>
<td>Urinary Ca excretion (mg/24 h)</td>
<td>182 ± 95</td>
<td>171 ± 44</td>
<td>166 ± 73</td>
</tr>
<tr>
<td>Intact parathyroid hormone (ng/l)</td>
<td>43 ± 12</td>
<td>38 ± 15</td>
<td>40 ± 16</td>
</tr>
<tr>
<td>Osteocalcin (ng/ml)</td>
<td>1.9 ± 0.5*</td>
<td>3.4 ± 0.9</td>
<td>8.9 ± 2.4*</td>
</tr>
<tr>
<td>Alkaline phosphatase (U/l)</td>
<td>164 ± 52</td>
<td>175 ± 61</td>
<td>180 ± 58</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>88 ± 9</td>
<td>82 ± 10</td>
<td></td>
</tr>
<tr>
<td>Serum CTX (ng/ml)</td>
<td>0.69 ± 0.24*</td>
<td>0.56 ± 0.22</td>
<td>0.34 ± 0.18†</td>
</tr>
<tr>
<td>Lumbar Z-score (s.d.)</td>
<td>−1.8 (−5.1 to −0.37)</td>
<td>−1.1 (−4.2 to −0.35)</td>
<td>−0.02 (−0.7 to −1.1)†</td>
</tr>
<tr>
<td>Femoral Z-score (s.d.)</td>
<td>−0.9 (−2.35 to −0.29)</td>
<td>−0.6 (−1.8 to −0.25)</td>
<td>0.05 (−0.3 to −0.4)‡</td>
</tr>
</tbody>
</table>

Prevalence (%) of Any vertebral fracture 25 (69%) 20 (57%) 1.4‡ Clinical fractures 10 (28%) 4 (11.4%) 0 Food multiples 13 (36%) 11 (31%) 0 Other fractures (rib, foot) 4 (11%) 3 (8.5%) 0

Data are expressed as mean ± s.d. or median and range, as appropriate, according to data distribution. *P < 0.05 versus subclinical hypercortisolism; †P < 0.05 and ‡P < 0.01 versus both groups of patients.

Table 3 Endocrine parameters, bone density, and prevalence of fractures in patients with overt or subclinical hypercortisolism according to gonadal status.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Overt hypercortisolism</th>
<th>Subclinical hypercortisolism</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Eumenorrhoic</td>
<td>Amenorrhoic</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>16</td>
</tr>
<tr>
<td>Age (years)</td>
<td>37 (28–47)†</td>
<td>48 (34–66)</td>
</tr>
<tr>
<td>Cortisol 0800 (nmol/l)</td>
<td>520 (276–1100)</td>
<td>635 (359–1343)</td>
</tr>
<tr>
<td>Urinary free cortisol excretion (nmol/24h)</td>
<td>1425 (223–7930)</td>
<td>1730 (310–9287)</td>
</tr>
<tr>
<td>DHEAS (µmol/l)</td>
<td>5.1 (0.33–56)</td>
<td>6.3 (0.83–99)</td>
</tr>
<tr>
<td>Androstenedione (nmol/l)³</td>
<td>3.2 (0.34–39)†</td>
<td>10 (1.4–58)</td>
</tr>
<tr>
<td>Testosterone (nmol/l)</td>
<td>3.4 (0.35–39)†</td>
<td>4.9 (1.3–52)</td>
</tr>
<tr>
<td>17-α-Estradiol (nmol/l)</td>
<td>134 (68–220)</td>
<td>78 (40–200)</td>
</tr>
<tr>
<td>Femoral Z-score (s.d.)</td>
<td>−1.7 (−4.4 to −0.37)</td>
<td>−1.93 (−5.1 to −0.5)</td>
</tr>
<tr>
<td>Prevalence (% of any vertebral fracture)</td>
<td>12 (60%)</td>
<td>13 (81%)</td>
</tr>
</tbody>
</table>

Data are expressed as median and range; *P < 0.05 versus amenorrhoic women with subclinical hypercortisolism; †P < 0.05 versus amenorrhoic women with overt hypercortisolism.

*In eumenorrhoic women determined in early follicular phase (days 3–6 of menstrual cycles).
Discussion

In this study, we evaluated the effects of sex steroids and the degree of hypercortisolism on BMD and vertebral fractures in a large female population. We chose the female population with endogenous hypercortisolism, since it represents an experimental model of pure effects of cortisol excess on bone, unlike exogenous corticosteroid treatments that are administered due to the underlying diseases, which often have their own negative influence on bone status. Limited data are available regarding predictive factors for bone damage in endogenous cortisol excess.

As expected, cortisol and androgen levels, BMD values, and fracture prevalence were significantly different between patients and controls.

Although some authors described more severe bone loss in patients with adrenal than pituitary Cushing’s (32, 33), we failed to find any significant difference in BMD values and the prevalence of vertebral fractures between patients (males and females) with pituitary or adrenal origin of hypercortisolism in a previous study (8). This study confirmed the lack of any difference in BMD values and fracture rates between adrenal and pituitary overt hypercortisolism in a female population: only DHEAS was significantly higher in women with pituitary Cushing’s. Nevertheless, this study was not aimed at looking for such differences, and women with pituitary and adrenal overt hypercortisolism were pooled together. Two groups of women were considered, according to the severity of their cortisol excess: overt versus subclinical hypercortisolism. Women with overt hypercortisolism had typical clinical symptoms of cortisol excess and more severe biochemical hypercortisolism; women with subclinical hypercortisolism had no typical symptoms of cortisol excess and milder biochemical abnormalities. Subclinical hypercortisolism is much more frequent in adrenal lesions than the overt one and the progression toward the overt form is very rare (34).

Serum androgens were higher in women with overt hypercortisolism; the reason can be related to the large proportion of women with pituitary Cushing’s in this group, while only women with adrenal mass were in the group with subclinical hypercortisolism. Estradiol levels widely overlapped among women with subclinical and overt disorder.

Higher femoral BMD values were observed in subjects with higher BMI, most likely due to a major mechanical loading. Testosterone had T positive effects on BMD at either LS or FN. DHEAS also positively influenced femoral BMD. Our observation on a significant influence of androgens on BMD is in line with our previous experience in a mixed population (8) and a study by Minetto et al. who reported a positive correlation between DHEAS and BMD at both LS and FN in endogenous hypercortisolism (32). Ohmori et al. did not find a direct correlation between BMD, cortisol, and androgens; however, they suggested a possible role of DHEAS increase in counterbalancing the negative bone effects of endogenous hypercortisolism (33).

Greater bone loss was also found in women on replacement therapy for 21-hydroxylase deficit and lower androgen levels (34), supporting the hypothesis on the importance of cortisol-to-androgen ratio in BMD maintenance (8, 32, 33). Finally, a strong correlation was observed between DHEAS and lumbar/femoral BMD values in healthy women (36).

Since cortisol, androgen, and E2 levels overlapped among women with overt or subclinical hypercortisolism, we tried to search predictive factors for bone status in the whole population of women. The ratios of cortisol-to-androgens and cortisol-to-estradiol were also considered. By applying logistic regression, only two predictive factors for vertebral fractures were found: cortisol levels and F-to-DHEAS ratio, whereas gonadal status (eumenorrhoic versus amenorrhoic) was a significant predictor only in the subgroup with subclinical Cushing’s syndrome. Amenorrhoic women with subclinical hypercortisolism had higher urinary cortisol excretion. This observation suggests that more severe hypercortisolism may more easily lead to amenorrhea, and both factors can contribute to bone loss in the subclinical setting. Moreover, protective effects of preserved menstrual cycles were observed in this group. This finding is in line with a previous report by Chiodini et al. who found better BMD values and fewer fractures in pre- than post-menopausal women with adrenal incidentaloma, with or without subclinical Cushing’s syndrome (7).

Women with overt hypercortisolism had similar bone density and prevalence of fractures, regardless of their gonadal status: this can be partly explained by protective effects of higher androgen levels in the group with amenorrhea. Indeed, the lack of any relationship between E2, amenorrhea duration, BMD, and fractures in overt hypercortisolism are likely to be due to strong negative bony effects of very high cortisol levels that overwhelm the positive effects of preserved menstrual cycles. In contrast to our finding’s, Karavitaki et al. documented reduced forearm BMD in 16 post-menopausal but not in 13 pre-menopausal women with Cushing’s syndrome (23): nevertheless, the peripheral and axial skeleton may have a different behavior during the cortisol excess.

For the first time, a significantly higher F-to-DHEAS ratio was found to be associated with vertebral fractures, suggesting that variations in the cortisol-to-androgen ratio also influenced the resistance of trabecular bone.

A high prevalence of vertebral fractures was revealed by this study among women affected by both overt (69%) and subclinical (57%) hypercortisolism. These numbers are, however, similar to those recently described by ourselves in overt hypercortisolism (8) and by Chiodini et al. (7), in women with subclinical...
hypercortisolism. In that study, the prevalence of vertebral fractures resulted 43% in pre-menopausal and 78% in post-menopausal patients. Thoracic vertebrae were more frequently fractured than the lumbar ones in experience. Although the reason for this observation is unclear, the smaller size of thoracic vertebrae can represent one plausible explanation.

This study confirms that patients with cortisol excess are at risk for bone loss and vertebral fractures, regardless of its severity and origin (exogenous or endogenous) (1, 7, 8, 37–41). Back pain and stature shortening should be investigated in all patients with endogenous hypercortisolism, and a radiogram of the spine is required in order to assess morphology of vertebral bodies also in non-symptomatic patients. The particular predisposition to bone fractures in patients with hypercortisolism is likely to be related to the nature of cortisol effects on the bone, which are multiple, both direct and indirect on either mineralization or bone matrix (38). The high prevalence of vertebral fractures observed at the diagnosis of endogenous cortisol excess and their irreversibility highlights the necessity of a very precocious preventive treatment to reduce the detrimental effects of cortisol excess on the bone. The lack of difference between BMD values and fracture prevalence between subjects with overt and subclinical hypercortisolism can be explained by a longer exposure to cortisol excess, although mild, in the group with subclinical disorder. Indeed, the meta-analysis of effects on bone of glucocorticoid therapy by van Staa et al. (1) showed that cumulative rather than daily doses of glucocorticoids correlated with BMD values. The cumulative dose depends on both daily dose and the duration of the therapy. By analogy, endogenous hypercortisolism may influence bone by both its severity and duration.

In conclusion, this study confirms and extends previous observations on the negative effects of endogenous hypercortisolism on bone density, bone turnover, and fracture incidence (7, 8, 40, 41). Our data suggest that bone demineralization and spinal fractures are frequent at any degree of cortisol excess, being partly counterbalanced by increased BMI and androgen levels, whereas preserved menstrual cycles had protective effects only in women with less severe, i.e. subclinical disorder. Therefore, women affected by either overt or subclinical hypercortisolism should receive adequate evaluation of bone status and appropriate treatment at the moment of diagnosis of cortisol excess.

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