Bone Mineral Density, Prevalence of Vertebral Fractures, and Bone Quality in Patients with Adrenal Incidentalomas with and without Subclinical Hypercortisolism: An Italian Multicenter Study

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Context: In patients with adrenal incidentalomas and subclinical hypercortisolism (SH), the factors influencing bone and the prevalence of vertebral fractures are debated. Spinal deformity index (SDI), which reflects bone quality, has never been evaluated.

Objective: The objective of the study was to investigate in these patients SDI and factors influencing the prevalence of fractures.

Design: This was a retrospective, multicenter study.

Setting: The study was conducted on an in- and outpatient basis.

Patients: Patients included 287 adrenal incidentaloma patients (111 eugonadal males, 31 premenopausal, 145 postmenopausal females) and 194 controls (90 eugonadal males, 29 premenopausal, 75 postmenopausal females).

Main Outcome Measure: Bone mineral density (BMD) was measured by dual X-ray absorptiometry at lumbar spine and femoral neck. By radiograph each vertebra was assessed as intact (grade 0) or grade 1 (20–25%), 2 (25–40%), or 3 (>40%) deformity; SDI was calculated by summing the grade of deformity for each vertebra. SH was diagnosed in the presence of at least two of the following: urinary free cortisol greater than 70 μg per 24 h (193.1 nmol/liter), cortisol after 1-mg dexamethasone test greater than 3.0 μg/dl (>82.8 nmol/liter), ACTH less than 10 pg/ml (<2.2 pmol/liter).

Results: BMD was significantly lower in SH+ than SH− patients and controls (lumbar spine -0.73 ± 1.43, 0.17 ± 1.33, 0.12 ± 1.21, respectively; femoral neck -0.37 ± 1.06, 0.07 ± 1.09, 0.17 ± 1.02). Patients with SH had higher fracture prevalence and SDI than those without SH and controls (70.6, 22.2, 21.8%, respectively, $P < 0.0001$; 0.31 ± 0.68, 0.39 ± 0.93, 1.35 ± 1.27, respectively, $P < 0.0001$). Fractures and SDI were associated with SH (odds ratio 7.27, 95% confidence interval 3.94–13.41, $P = 0.0001$; $\beta = 0.352$, $t = 6.241$, $P = 0.0001$, respectively) regardless of age, BMD, menopause, and gender.

Conclusion: SH is associated with low BMD, high fracture prevalence, and reduced bone quality as measured by SDI. (***J Clin Endocrinol Metab* 94: 3207–3214, 2009**)

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Abbreviations: AI, Adrenal incidentaloma; BMD, bone mineral density; BMI, body mass index; DST, dexamethasone suppression test; FN, femoral neck; LS, lumbar spine; SDI, spinal deformity index; SH, subclinical hypercortisolism; SQ, semiquantitative visual assessment; UFC, urinary free cortisol.

Subclinical hypercortisolism (SH) is characterized by the presence of alterations of the hypothalamic-pituitary-adrenal function in the absence of the classical signs or symptoms of overt hypercortisolism (1). This condition has been described in up to the 30% of patients bearing an incidentally discovered adrenal mass [adrenal incidentaloma (AI)] and associated with an increased prevalence of metabolic syndrome, osteoporosis, and vertebral fractures (2, 3). However, available data regarding bone involvement are not fully concordant and are derived from relatively small studies (4–10). To date, only two controlled studies were specifically designed to investigate the prevalence of vertebral fractures in patients with and without SH (11, 12). In addition, due to the relatively small sample size of the previous studies, no clear information is available regarding the possible factors, which, together with SH, may contribute to fracture's risk (13).

Spinal deformity index (SDI) is a semiquantitative (Fig. 1) method that integrates the number and the severity of vertebral fractures and is considered an accurate prognostic tool to assess the vertebral fracture risk over time (14, 15). Recent data suggest that the severity of vertebral fractures may be a surrogate index of bone microarchitecture, a component of bone quality (16), thus reinforcing the possible usefulness of SDI. In particular, in patients with overt glucocorticoid excess, a reduction in bone quality could explain why in these patients the risk of fractures is increased for a given level of bone mineral density (BMD) (17). In keeping with this, previous data suggested that also in patients with SH vertebral fracture prevalence was increased regardless for BMD (11, 12) and that in more than 40% of these patients, fractures occurred despite a normal or only osteopenic BMD (12). Thus, it can be hypothesized that in subtle hypercortisolism, as in overt cortisol excess, not only bone mass but also bone quality can be affected. To date no studies investigated bone quality, as reflected by SDI, in patients with SH.

The present study was designed to evaluate in a large group of AI patients with and without SH the factors influencing BMD, SDI, and the prevalence of vertebral fractures.

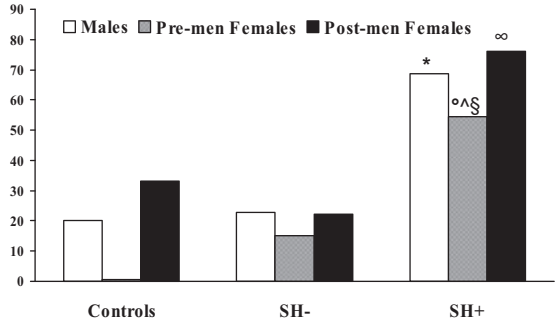

FIG. 1. Comparison of the prevalence of fractures among male, premenopausal (Pre-men) females, and postmenopausal (Post-men) females in the different groups: control subjects, patients with adrenal incidentalomas without subclinical hypercortisolism (SH−), patients with adrenal incidentalomas with subclinical hypercortisolism (SH+). The prevalence of vertebral fracture is significantly higher in SH+ than SH− patients and control subjects irrespective of gender and menopausal status. Interestingly, the prevalence of vertebral fractures is higher in premenopausal SH+ female patients than postmenopausal SH− patients (see text). *, SH+ male patients *vs.* SH− male patients and controls: $P = 0.0001$; °, premenopausal SH+ female patients *vs.* premenopausal SH− female patients: $P = 0.001$; ^, premenopausal SH+ female patients *vs.* premenopausal controls: $P = 0.0001$; §, premenopausal SH+ female patients *vs.* postmenopausal SH− female patients: $P = 0.0026$; ∞, postmenopausal SH+ female patients *vs.* postmenopausal SH− patients and controls: $P = 0.0001$.

Patients and Methods

Patients

The study was performed in four referral Italian endocrinology units: Casa Sollievo della Sofferenza, Instituto di Ricovero e Cura a Carattere Scientifico in San Giovanni Rotondo; San Giuseppe Hospital in Milan; Fondazione Ospedale Maggiore Policlinico, Mangiagalli e Regina Elena, Instituto di Ricovero e Cura a Carattere Scientifico in Milan; and Policlinico San Donato Instituto di Ricovero e Cura a Carattere Scientifico in Milan. We retrospectively evaluated data from 287 patients affected with AI and enrolled in our centers from January 1997 to December 2008.

Diagnosis of AI was based on the detection of a unilateral adrenal mass by noninvasive imaging methods of the abdomen, performed for unrelated disease. The exclusion criteria were: 1) past or current history of hypogonadism (in males testosterone levels <3.0 ng/ml, 9.4 nmol/liter; in premenopausal females less than six menstrual cycles per year) and other diseases known to affect bone metabolism (*i.e.* thyrotoxicosis, bowel diseases, chronic renal failure, chronic hepatic disease, depression, alcoholism, eating disorders, rheumatological or hematological diseases); 2) administration of drugs influencing bone, cortisol and dexamethasone metabolism, or cortisol secretion; and 3) signs or symptoms specific of cortisol excess (moon facies, striae rubrae, skin atrophy, buffalo hump).

No patient had evidence of metastatic diseases. At computed tomography all adrenal masses were homogeneous, hypodense, and well shaped, features consistent with the diagnosis of adrenocortical adenomas.

In all patients, the diagnosis of pheochromocytoma and aldosteronoma was excluded by appropriate hormonal determinations (24 h urinary catecholamines and plasma renin activity and aldosterone).

The diagnosis of SH was based on the presence of at least two the following three alterations of hypothalamus-pituitary-adrenal axis secretion: 1) increased urinary free cortisol (UFC) levels greater than 70 μg per 24 h (193 nmol/liter); 2) serum cortisol levels after 1-mg dexamethasone suppression test (DST) greater than 3.0 μg/dl (83 nmol/liter); and 3) low ACTH levels (<10 pg/ml, 2.2 pmol/liter). On the basis of these criteria, 85 patients were diagnosed as having SH (group SH+) and 202 as not having SH (group SH−).

Patients from group SH+ showed a higher mean diameter of adrenal masses than those from group SH− (mean ± SD, 2.9 ±

1.2 *vs.* 2.4 ± 1.0 cm, $P = 0.001$; range 1.0–5.5 and 1.0–8.0 cm, respectively). Twenty-seven patients were affected by AI larger than 4.0 cm. Among these, 20 had previously refused surgery (and were sent to our departments to assess the possible presence of hormonal hypersecretion) and seven were studied before surgical operations.

One hundred ninety-four subjects served as controls. These subjects were enrolled during the same period of time on the basis of the above-mentioned inclusion criteria from our outpatient clinics at which they were referred for unrelated diseases (multinodular goiter with normal thyroid function). Age, body mass index (BMI), and gender were similar compared with the patient group. Testosterone levels were comparable between male AI patients and control subjects. All control subjects affected with osteoporosis underwent 1-mg DST and none of the included subjects showed 1-mg DST greater than 1.8 μg/dl (50 nmol/liter).

All subjects gave their witnessed informed consent before entering the study, which was approved by local ethical committees and in accordance with Helsinki Declaration II.

Methods

In 231 AI patients (120 females, 111 males), the presence of the metabolic syndrome was assessed, following the Adult Treatment Panel III criteria (18).

Serum and urinary samples were collected and stored at −20 C until assayed. In each patient serum ACTH levels (mean of three determinations at 20 min intervals) were measured by immunoradiometric assay (BRAHMS Diagnostica GmbH, Berlin, Germany), serum cortisol, and UFC levels (after dichloromethane extraction) were determined immunofluorimetrically by TDX-FLX kits (Abbott GmbH Diagnostika, Wiesbaden-Delkenheim, Germany) at the study entry. The intra- and interassay coefficients of variation were less than 15% for ACTH and less than 10% for all other assays.

In all patients, BMD was measured by dual-energy x-ray absorptiometry (Hologic Discovery, Waltham, MA) at lumbar spine (LS; *in vivo* precision 1.0%) and femoral neck (FN; *in vivo* precision 1.8%). Individual BMD values were expressed as SD units (Z-values) in relation to our reference population (19). Fractured vertebrae were excluded from BMD measurement.

To ensure the precision of these measurements, an anthropomorphic phantom composed of four hydroxyapatite vertebrae embedded in a tissue mimicking epoxy-resin block 17.5 cm thick was used (Hologic spine phantom). When measuring this test object, the standard Hologic protocols for spine scan acquisition and analysis were used, following the Shewhart charts combined with visual inspection (20). The phantom was measured on each machine over a 2-yr period using an identical automated software program. The short- and long-term precision was estimated from the consistency of these measurements and the SD was expressed in terms of coefficient of variation in percent. Short- and long-term precision was good, and we reported calibration within 0.5% of average Hologic scanner calibration. No significant drift of the scanner *vs.* time were observed.

Conventional spinal radiographs in lateral (T4-L4) and anteroposterior projection (L1-L4) were obtained in all subjects with standardized technique. Two trained radiologists, who were blinded to BMD and hormonal data, independently reviewed the radiographs. The radiologists discussed questionable cases to agree on a diagnosis; the interrater reliability between the two radiologists was good (k = 0.82). Vertebral fractures were diagnosed on visual inspection using the semiquantitative visual assessment (SQ), previously described by Genant *et al.* (21). According to this technique, fractures assessed on lateral thoracolumbar spine radiographs were defined as reductions of more than 20% in anterior, middle, or posterior vertebral height. From lateral spine radiographs, each vertebra is visually assessed as intact (SQ grade 0) or as having approximately mild (20–25% compression), moderate (25–40% compression), or severe (>40% compression) deformity (SQ grades 1, 2, and 3, respectively). Subsequently for each subject the SDI was calculated by summing the SQ grade for each of the 13 vertebrae from T4 to L4 ($SDI = SQ_{T4} + \ldots + SQ_{T12} + SQ_{L1} + \ldots + SQ_{L4}$) (15).

Statistical analysis

Statistical analysis was performed by SPSS version 12.0 statistical package (SPSS Inc., Chicago, IL). The results are expressed as mean ± SD.

Comparison of continuous variables among the different groups was performed using one-way ANOVA and Bonferroni *post hoc* analysis. Categorical variables were compared by χ^2 test. The associations among the different indexes of cortisol secretion and between these indexes and BMD were tested by either Pearson product moment correlation or Spearman correlation as appropriate.

In all patients multivariate linear regression analysis was used to evaluate the influence of the presence of SH on BMD or SDI after adjusting for age, BMI, gonadal status, gender, and spinal BMD (the latter only for SDI).

In all patients, logistic regression analysis assessed the association between the presence of fractures (dependent variable, expressed as categorical variable) and independent variables: age, BMI, gender, gonadal status, presence of SH, and spinal BMD (expressed as Z-score).

Univariate general linear modeling and Bonferroni *post hoc* analysis have been used to compare SDI values between the two groups of patients and controls to adjust for age.

On the basis of the results of logistic regression analysis, which showed that only SH, age, and spinal BMD were significantly associated with vertebral deformities, receiver operating characteristic curve analysis was performed to assess the best cutoff of age and spinal BMD Z-score for predicting vertebral fractures. The best cutoff for age and BMD Z-score levels was chosen on the basis of the best diagnostic accuracy, which corresponds to the ratio between the sum of true positive plus true negative findings and the whole number of patients (*i.e.* the highest number of patients correctly predicted as fractured or not fractured by the chosen cutoff of age and BMD Z-score).

$P < 0.05$ was considered significant.

Results

Clinical characteristics

Clinical characteristics of patients with and without SH and controls are reported in Table 1. Age, BMI, and prevalence of male and female subjects were comparable among patients with SH, without SH, and controls. Male patients with and without SH and control subjects showed similar testosterone levels (data not shown). As expected, 1-mg DST and UFC levels were higher, whereas ACTH

TABLE 1. Clinical characteristics of patients affected with AIs with and without SH and control subjects

	Control subjects (n = 194)	SH− patients (n = 202)	SH+ patients (n = 85)
Age (yr)	61.1 ± 13.7 (21–79)	61.2 ± 11.4 (21–81)	62.9 ± 9.9 (34–79)
BMI (kg/m^2)	28.7 ± 4.9 (19.5–40.6)	28.6 ± 4.9 (19.5–40.6)	29.2 ± 4.7 (21.3–40.9)
Females/males	104/90	123/79	53/32
LS BMD (Z-score)	0.12 ± 1.21 (−2.34–3.04)	0.17 ± 1.33 (−2.80 to 3.61)	−0.73 ± 1.43 (−4.5–3.08)[a,b]
FN BMD (Z-score)	0.17 ± 1.02 (−2.28 to 3.65)	0.07 ± 1.09 (−2.80 to 5.33)	−0.37 ± 1.06 (−2.50 to 2.19)[a,c]
No. of fractured patients (%)	43 (22.2)	44 (21.8)	60 (70.6)[a,b]
No. of fractured patients with T-score BMD greater than −2.5 (%)	11 (25.0)	15 (34.9)	35 (58.3)[d]
SDI	0.31 ± 0.68 (0–4)	0.39 ± 0.93 (0–7)	1.35 ± 1.27 (0–6)[a]
ACTH (pg/ml)		15.3 ± 8.1 (3.6–48.3)	7.4 ± 2.8 (0.7–16.2)[b]
1-mg DST (μg/dl)		1.8 ± 1.1 (0.5–11.9)	4.3 ± 2.8 (0.5–12.0)[b]
UFC (μg per 24 h)		43.4 ± 24.5 (10.0–169.1)	65.1 ± 31.5 (10.0–163.0)[b]
Diameter of adenoma (cm)		2.4 ± 1.1 (0.8–8.0)	2.9 ± 1.2 (0.9–5.5)[c]

Data are mean ± SD with range in parentheses or absolute number with percentage in parentheses. Subclinical hypercortisolism was diagnosed in presence of two of the following: 1-mg DST greater than 3.0 μg/dl (82.7 nmol/liter), UFC greater than 70 μg per 24 h (193.0 nmol per 24 h), or ACTH less than 10 pg/ml (2.2 pmol/liter).

[a] $P = 0.0001$ *vs.* controls.

[b] $P = 0.0001$ *vs.* SH− patients.

[c] $P = 0.001$ *vs.* SH− patients.

[d] $P = 0.001$ *vs.* controls.

levels lower in SH+ than SH− patients. Patients with SH had lower BMD at LS and FN and higher prevalence of vertebral fractures and SDI values compared with those without SH and controls. Interestingly, the number of fractured patients with T-score BMD at LS and FN higher than −2.5 was higher in SH+ than SH− and control groups. The prevalence of metabolic syndrome was higher in SH+ (60.0%) than SH− subjects (29.8%, $P < 0.001$).

Clinical characteristics of subjects subdivided on the basis of gender, presence of SH, and prior postmenopausal status for females are reported in Table 2.

Male SH+ patients showed lower LS and FN BMD Z-scores, higher prevalence of vertebral fractures, and higher SDI than their SH− counterparts and controls. They showed lower LS and FN BMD compared with premenopausal SH+ female patients after adjusting for age by general linear modeling but similar fracture prevalence.

TABLE 2. Clinical characteristics of AI patients and control subjects subdivided according to gender, gonadal status, and presence of SH

	Controls			Patients
	Eugonadal males (n = 90)	Premenopausal females (n = 29)	Postmenopausal females (n = 75)	Eugonadal SH− males (n = 79)
Age (yr)	63.9 ± 13.8 (24–79)	43.0 ± 9.3 (21–57)	64.7 ± 7.7 (46–78)	61.8 ± 12.2 (21–79)
BMI (kg/m^2)	27.7 ± 3.2 (24.0–79.0)	27.3 ± 5.5 (19.5–40.0)	30.4 ± 5.7 (19.5–40.6)	28.0 ± 3.0 (22.9–38.1)
Years since menopause			15.4 ± 8.6 (2–34)	
LS BMD (Z-score)	0.20 ± 1.28 (−2.28 to 2.88)	0.23 ± 1.32 (1.95–3.04)	−0.03 ± 1.10 (−2.34 to 2.20)	0.15 ± 1.34 (−2.80 to 3.60)
FN BMD (Z-score)	0.26 ± 1.06 (−2.34 to 2.86)	0.36 ± 1.26 (−1.56 to 3.65)	−0.04 ± 0.89 (−1.87 to 1.76)	−0.03 ± 1.00 (−2.8 to −2.7)
Number of fractured patients (%)	18 (20.0)	0 (0.0)	25 (33.3)	18 (22.8)
SDI	0.50 ± 0.16 (0–7)	0.0 ± 0.0 (0–0)	0.30 ± 0.10 (0–2)	0.50 ± 0.10 (0–7)
ACTH (pg/ml)				17.5 ± 8.0 (5.0–47.0)
1-mg DST (mg/dl)				1.7 ± 0.9 (0.5–6.3)
UFC (mg per 24 h)				47.3 ± 22.4 (10.0–118.5)
Diameter (cm)				2.5 ± 1.0 (0.8–6.4)

Data are mean ± SD with range in parentheses or absolute number with percentage in parentheses. SDI mean are age adjusted by general linear modeling and expressed as mean ± ES. Subclinical hypercortisolism was diagnosed in presence of two of the following: 1-mg DST greater than 3.0 μg/dl (82.7 nmol/liter), UFC greater than 70 μg per 24 h (193.0 nmol per 24 h), ACTH less than 10 pg/ml (2.2 pmol/liter). Diameter, Diameter of adenoma.

[a] $P = 0.0001$ *vs.* SH− and SH+ males; [b] $P < 0.05$ *vs.* premenopausal controls; [c] $P < 0.05$ *vs.* postmenopausal controls; [d] $P = 0.0001$ *vs.* males controls; [e] $P < 0.05$ *vs.* SH− males; [f] $P < 0.05$ *vs.* premenopausal SH+ females (after adjusting for age by general linear modelling); [h] $P = 0.01$ *vs.* premenopausal SH− females; [i] $P = 0.01$ *vs.* postmenopausal controls; [j] $P < 0.05$ *vs.* post-menopausal SH− females.

In male SH+ patients, LS and FN BMD was similar to that of postmenopausal SH+ female patients.

Premenopausal SH+ female patients had higher prevalence of vertebral fractures as compared with premenopausal controls and premenopausal SH− patients (even without reaching the statistical significance for SDI *vs.* this latter group, $P = 0.069$). Interestingly, they showed higher SDI than postmenopausal SH− patients but not different BMD, expressed as Z-value.

Postmenopausal SH+ female patients had lower BMD and higher prevalence of fractures and SDI values as compared with postmenopausal SH− female patients and control subjects (Fig. 1).

Factors influencing BMD, prevalence of vertebral fractures, and SDI values

Bivariate analyses showed that in AI patients LS and FN BMD was directly associated with BMI (R = 0.24, $P = 0.0001$ and R = 0.29, $P = 0.0001$, respectively). Lumbar spine BMD was inversely associated with UFC levels (R = −0.15, $P = 0.011$) and 1-mg DST (R = −0.15, $P = 0.013$). Multivariate linear regression analysis confirmed that LS BMD was inversely associated with UFC levels and 1-mg DST after adjustment for age, gender, BMI, and presence of postmenopausal status ($\beta = -0.16$, $P = 0.007$ and $\beta = -0.17$, $P = 0.005$, respectively). The same analysis showed that LS BMD was independently and significantly predicted by the presence of SH, BMI, gender, and menopausal status (Table 3) and FN BMD by the presence of SH and BMI (Table 4).

The prevalence of vertebral fractures was significantly associated with the presence of SH, age, and LS BMD but not BMI, postmenopausal status, and gender as shown by logistic regression analysis (Table 5). Receiver operating characteristic curve analysis showed that age 64 yr and LS Z-values less than 0.0 were the cutoffs best associated with the presence of vertebral fractures (area under the curve 0.608 and 0.649, respectively). The combination of SH, age older than 64 yr, and LS BMD Z-values less than 0.0 was associated with a 14.7-fold higher risk to have vertebral fractures (odds ratio 14.7, 95% confidence interval 5.9–36.8, $P = 0.0001$) after adjusting for BMI and gender. Indeed, 36 of 40 patients showing this combination were fractured.

Bivariate analyses showed that SDI was inversely associated with LS and FN BMD (R = −0.29, $P = 0.0001$ and R = −0.24, $P = 0.0001$, respectively) and directly with age (R = 0.19, $P = 0.001$), UFC (R = 0.13, $P = 0.024$), and 1-mg DST (R = 0.22, $P = 0.0001$). Multivariate regression analysis showed that SDI was independently and significantly predicted by the presence of SH, age and LS BMD but not by gender, BMI, and postmenopausal status (Table 6).

Discussion

The present study shows that patients with adrenal incidentalomas and subclinical hypercortisolism have a reduced bone density, a possible architectural deterioration

TABLE 2. Continued

Patients				
Eugonadal SH+ males (n = 32)	**Premenopausal SH− females (n = 20)**	**Premenopausal SH+ females (n = 11)**	**Postmenopausal SH− females (n = 103)**	**Postmenopausal SH+ females (n = 42)**
66.0 ± 9.1 (42–77)	43.0 ± 9.2 (26–59)[a]	48.2 ± 5.9 (34–56)	64.3 ± 7.1 (48–81)	64.4 ± 7.8 (46–79)
28.3 ± 3.6 (22.0–37.1)	30.4 ± 5.8 (26.0–59.0)[b]	29.9 ± 5.6 (21.5–39.2)	28.7 ± 5.5 (19.5–40.6)[c]	29.6 ± 5.2 (21.3–40.9)
			15.2 ± 9.4 (2–35)	15.4 ± 8.1 (4–32)
−1.14 ± 1.49 (−4.50 to 2.26)[d,e,f]	0.55 ± 1.56 (−2.70 to 2.96)	0.21 ± 0.94 (−1.48 to 1.50)	0.11 ± 1.27 (−2.80 to 3.52)	−0.65 ± 1.38 (−3.07 to 3.08)[h,i]
−0.53 ± 1.0 (−2.50 to 1.50)[d,e,f]	0.46 ± 1.65 (−1.70 to 5.33)	0.25 ± 1.15 (−1.48 to 1.50)	0.08 ± 1.02 (−2.50 to 2.14)	−0.42 ± 1.05 (−2.50 to 1.60)[h,i]
22 (68.8)[e]	3 (15.0)	6 (54.5)[b,h,j]	23 (22.3)	32 (76.2)[c,j]
1.35 ± 0.16 (0–4)[d,e]	0.34 ± 0.21 (0–1)	0.90 ± 0.27 (0–2)[b,j]	0.26 ± 0.09 (0–3)	1.44 ± 0.14 (0–6)[c,j]
7.6 ± 3.4 (2.3–16.2)	14.1 ± 6.7 (5.8–29.0)	6.7 ± 2.5 (0.7–9.8)	13.9 ± 8.1 (3.6–48.3)	7.5 ± 2.5 (3.0–14.8)
4.7 ± 2.7 (1.4–12.0)	1.5 ± 1.1 (0.5–5.7)	4.5 ± 3.3 (1–12.0)	1.8 ± 1.3 (0.5–11.9)	4.0 ± 2.7 (0.5–12.0)
74.7 ± 33.9 (27.0–163.0)	46.2 ± 20.3 (10.0–102.8)	64.2 ± 24.8 (27.3–113.0)	39.9 ± 26.4 (10.0–169.1)	58.1 ± 29.7 (10.0–144.0)
2.9 ± 1.1 (1.0–5.0)	2.5 ± 1.6 (0.8–8.0)	2.8 ± 1.4 (0.9–5.0)	2.4 ± 1.0 (0.8–5.5)	2.9 ± 1.2 (1.0–5.5)

TABLE 3. Factors influencing LS BMD by multivariate linear regression analysis

	β	t	P
Age (1 yr increase)	0.100	−2.850	0.005
BMI (1 kg/m^2 increase)	0.232	4.220	0.0001
Male gender	−0.279	−2.161	0.009
Postmenopausal status (absence *vs.* presence)	−0.273	−2.506	0.013
SH (presence *vs.* absence)	−0.315	−5.737	0.0001

SH was diagnosed in presence of two of the following: 1-mg DST greater than 3.0 μg/dl (82.7 nmol/liter), UFC greater than 70 μg per 24 h (193.0 nmol per 24 h), ACTH less than 10 pg/ml (2.2 pmol/liter). BMD was measured by dual x-ray absorptiometry.

of trabecular bone, as reflected by increased spinal deformity index, and an increased prevalence of vertebral fractures. Together with SH, increasing age and decreasing BMD independently contribute to the enhanced risk of fracture, regardless of gender and gonadal status. However, in more than half of cases, fractures occurred in the presence of normal or only osteopenic bone density.

Data about the possible negative effect of subclinical hypercortisolism on bone mass, particularly regarding cortical bone, are not conclusive (4, 10, 13, 22, 23). This may be due to the relative small sample size of the previous studies (10, 22, 23) and the differences in the diagnostic criteria for SH, the proportion of male/female, and the gonadal status (4, 13). The present study, performed on a large sample of AI patients with and without SH, suggests that SH exerts a deleterious effect on bone at both trabecular (spine) and cortical (femoral neck) sites, similarly to what has already been shown in patients with overt cortisol excess (24, 25).

It is well known that vertebral fractures are the classic hallmark of osteoporosis and that their prevalence increases the risk of both new vertebral and nonvertebral osteoporotic fractures (26–28). Diagnosing vertebral fractures, even asymptomatic, is therefore of great importance in patients affected with diseases that can affect bone. The present data confirm on a large sample the results of previous studies demonstrating an increased prevalence of vertebral fractures in patients with adrenal incidentalomas and SH (11, 12). This negative effect of SH is present in both males and females and overcomes the protective effect of androgen/estrogens independently of age, as suggested by the finding that premenopausal females with SH have higher prevalence of vertebral fractures than postmenopausal females without SH.

An important result of our study is that we were able to identify a combination of risk factors (SH, age >64 yr, and LS BMD Z-values <0.0) associated with a 14.7-fold higher risk to have vertebral fractures; an individual showing this combination has a 85% probability of being fractured. This may be an additional clinical tool to select AI subjects to be screened for vertebral fractures.

The severity of prevalent vertebral fracture is a strong clinical marker of disease progression, predicts future fractures independently of BMD, and has been suggested as indicative of significant architectural deterioration of

TABLE 4. Factors influencing FN BMD by multivariate linear regression analysis

	β	t	P
Age (1 yr increase)	−0.001	−0.015	0.988
BMI (1 kg/m^2 increase)	0.267	4.750	0.0001
Male gender	−0.201	−1.850	0.065
Postmenopausal status (absence *vs.* presence)	−0.182	−1.635	0.103
SH (presence *vs.* absence)	−0.206	3.665	0.0001

SH was diagnosed in presence of two of the following: 1-mg DST greater than 3.0 μg/dl (82.7 nmol/liter), UFC greater than 70 μg per 24 h (193.0 nmol per 24 h), ACTH less than 10 pg/ml (2.2 pmol/liter). BMD was measured by dual x-ray absorptiometry.

TABLE 5. Odd ratios for vertebral fractures for potential risk factors using logistic regression analysis

	Odds ratio	P value	95% CI
Age (1 yr increase)	1.05	0.008	1.01–1.08
BMI (1 kg/m^2 increase)	1.01	0.773	0.93–1.08
Female *vs.* male gender	1.55	0.487	0.46–5.00
Postmenopausal status (absence *vs.* presence)	1.33	0.617	0.43–4.16
LS BMD (1 Z-score unit decrease)	1.33	0.011	1.06–1.66
SH (presence *vs.* absence)	7.27	0.0001	3.94–13.41

SH was diagnosed in presence of two of the following: 1-mg DST greater than 3.0 μg/dl (82.7 nmol/liter), UFC greater than 70 μg per 24 h (193.0 nmol per 24 h), ACTH less than 10 pg/ml (2.2 pmol/liter). BMD was measured by dual x-ray absorptiometry. The logistic regression analysis assessed the association between the presence of fractures (dependent variables, expressed as categorical variable) and independent variables: age, BMI, gender, menopausal status, LS BMD (expressed as Z-score), and presence of SH. CI, Confidence interval.

TABLE 6. Factors influencing SDI by multivariate linear regression analysis

	β	t	P
Age (1 yr increase)	0.151	2.386	0.018
BMI (1 kg/m^2 increase)	0.000	0.030	0.998
Male gender	0.062	0.588	0.557
Postmenopausal status (absence *vs.* presence)	0.007	0.064	0.949
LS BMD (1 Z-score unit increase)	−0.157	−2.719	0.007
SH (presence *vs.* absence)	0.352	6.241	0.0001

SH was diagnosed in presence of two of the following: 1-mg DST greater than 3.0 μg/dl (82.7 nmol/liter), UFC greater than 70 μg per 24 h (193.0 nmol per 24 h), ACTH less than 10 pg/ml (2.2 pmol/liter). BMD was measured by dual x-ray absorptiometry.

bone (14–16). Bone microarchitecture is considered a component of bone quality (29), whose deterioration has been associated with vertebral fractures regardless for bone density. Unfortunately, bone microarchitecture can be assessed directly only by histomorphometric or microcomputed tomography analysis of invasively obtained bone biopsy sample or by microcomputed tomography systems (30, 31).

The SDI integrates both the number and severity of vertebral fractures into a single measure, reflecting the total burden of the spine (15), and has recently been proposed as a surrogate marker of bone microarchitecture (16).

The present study for the first time evaluates SDI in patients with subclinical hypercortisolism. Our finding of an increased SDI in patients with SH independent of age, BMI, gender, and gonadal status suggests that the presence of a subtle cortisol excess may affect bone microarchitecture and quality, similarly to what happens in patients affected with overt cortisol excess (17). The decrease in bone quality, indeed, is considered a typical consequence of overt hypercortisolism, explaining why in these patients the fracture threshold is lowered and fractures occur despite the presence of normal or only osteopenic BMD (17). In keeping with this, our finding of a higher number of fractured subjects with normal or only osteopenic bone density in patients with SH than in those without SH and controls reinforces the hypothesis that a reduced bone quality is present in subclinical as well as in overt hypercortisolism.

Although our data strongly suggest that SH has a negative effect on bone mass and quality and it represents a risk factor for vertebral fractures, it must be considered that the present study is retrospective and conducted in referral centers, and therefore, the results might have been biased. Moreover, the cross-sectional design allows us to investigate only associations and not causality between the presence of SH and bone damage. Only longitudinal observational or eventually intervention studies, in fact, could clearly ascertain the effect of SH on fracture risk. Unfortunately, scarce data are available regarding the effect on bone tissue of the recovery from SH (32). On the other hand, the hypothesis of a negative effect of SH on bone is reinforced by literature data showing that SH is more prevalent in patients with osteoporosis (33), and patients with SH have an increased rate of bone loss as reported by a previous longitudinal study (7). However, it is important to note that the characteristics of the disease *per se* also may influence the results of these studies. Indeed, because there are no gold standard tests for diagnosing SH, and probably cortisol secretion in AI patients is a continuum trait, the definition of SH may be controversial (34). However, because of the lack of widely accepted guidelines (35), a combination of three criteria, as we used in the present study, is generally accepted for diagnosing SH (36). Moreover, it is known that in AI patients cortisol secretion may fluctuate with time, and some patients may be alternatively defined as having SH or not. Unfortunately, due to its cross-sectional design, the present study could not evaluate the cortisol secretion over time. On the other hand, to date no studies evaluated the *a posteriori* occurrence of cortisol insufficiency after surgical removal of AI, which might be the best diagnostic criteria for SH.

In conclusion our study demonstrates on a large sample of AI patients that: 1) SH is associated with a reduced cortical and trabecular bone density and with an increased prevalence of vertebral fractures and spinal deformity index, a surrogate marker of bone microarchitecture; and 2) SH, age, and bone density independently predict the decrease of bone quality as evaluated by SDI and the higher prevalence of fractures.

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