Bone Involvement in Eugonadal Male Patients with Adrenal Incidentaloma and Subclinical Hypercortisolism

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Adrenal incidentalomas (AI) are not associated, by definition, with clinically evident syndromes; however, some AI patients may show biochemical indexes of subclinical hypercortisolism (SH). Previous data on female AI patients indicated that SH may lead to bone loss, at least at spine. No data are available on bone involvement in samples of only AI male patients.

We measured bone metabolism and bone mineral density at spine and femur by dual-energy x-ray absorptiometry in 38 consecutive eugonadal male AI patients and 38 healthy matched control subjects. Patients were subdivided according to the presence or absence of SH (group SH+ and group SH−, respectively). Mean Z-score levels of spinal bone mineral density measured by dual-energy x-ray absorptiometry were lower ($P < 0.05$) in group SH+ (-0.42 ± 1.62) in comparison with group SH− (0.6 ± 1.13) and controls (0.47 ± 1.06). Thus, in order for the most appropriate management to be individually tailored, bone mass evaluation is strongly indicated in AI male patients with SH, irrespective of their gonadal status. (*J Clin Endocrinol Metab* 87: 5491–5494, 2002)

I N RECENT YEARS, incidentally discovered adrenal masses [adrenal incidentalomas (AI)] have been diagnosed with increasing frequency due to the widespread use of abdominal imaging techniques (1–4). Among all such lesions, adrenal cortical adenomas are the most frequent histologic type. Although, by definition, AI patients do not show evident clinical signs, some of these subjects may show abnormalities of cortisol hypersecretion [subclinical hypercortisolism (SH); Refs.5–13], which has been defined as a mild autonomous cortisol excess detectable biochemically as functional abnormalities of the hypothalamic-pituitary-adrenal (HPA) axis (5, 6). As suggested by the Italian Study Group on Adrenal Tumors (6), to diagnose SH, at least two abnormalities of the HPA axis function should be present in the absence of evident clinical signs of hormone excess. SH may lead to clinical complications including, among others, osteoporosis, which has been reported in female patients (14–19).

Whether male AI patients may also suffer from osteoporosis is presently unknown. This lack of knowledge is not a trivial one: reduced bone mineral density (BMD) would, indeed, be relevant, when addressing the clinical management of these patients.

The aim of this cross-sectional study was to investigate data on bone turnover and mass of different skeletal sites in

38 consecutive male AI patients, compared with those obtained in 38 healthy matched controls.

Subjects and Methods

Subjects

Thirty-eight consecutive male patients with AI were enrolled from January 1999 to June 2000 in two referral Italian centers. Diagnosis of AI was based on the detection of a unilateral adrenal mass by noninvasive imaging methods of the abdomen, performed invariably for unrelated diseases (aspecific symptoms, abdominal and back pain), and the lack of overt signs and/or symptoms of hormonal hypersecretion. We enrolled only male subjects to avoid gender-related confounding effects on the skeleton (20). All patients were eugonadal, with testosterone levels above 8.7 nmol/liter, the cut-off value of international normal references (21).

Patients were divided into group SH+ (n = 13) and group SH− (n = 25). The diagnosis of SH was based on the presence of two out of the following three alterations of HPA axis: 1) increased urinary free cortisol (UFC) levels (>193.1 nmol/24 h), the cut-off of both our own and international (22) normal reference values; 2) unsuppressed serum cortisol levels after 1-mg overnight dexamethasone (Dex) suppression test (serum cortisol after Dex > 82.8 nmol/liter); and 3) low ACTH levels (<2.2 pmol/liter). Groups SH+ and SH− were not different as far as age, body mass index (BMI), and testosterone levels were concerned (Table 1).

No subject had evidence of metastatic disease. At computed tomography, all lesions were homogeneous, hypodense, and well shaped; these features are consistent with the diagnosis of adrenocortical adenoma (4). The diameter of incidentalomas was not different between group SH+ and group SH− (mean ± SD, 3.4 ± 1.1 *vs.* 2.8 ± 0.9 cm; range, 1.0–5.0 and 1.5–5.0 cm, respectively). Six patients who displayed AI diameters of at least 4 cm had previously refused surgery and were sent to our departments by surgeons from other hospitals to study their hormonal status. Pheochromocytoma and aldosteronoma were excluded by appropriate hormonal measurements (24-h urinary cat-

Abbreviations: AI, Adrenal incidentaloma(s); BGP, bone GLA protein; BMD, bone mineral density; BMI, body mass index; Cr, creatinine; Dex, dexamethasone; D-Pyr, deoxypyridinoline; DXA, dual-energy x-ray absorptiometry; FN, femoral neck; HPA, hypothalamic-pituitary-adrenal; SH, subclinical hypercortisolism; UFC, urinary free cortisol.

TABLE 1. Clinical characteristics of patients and controls

| | Controls (n = 38) | Patients | |
		Group SH− (n = 25)	Group SH+ (n = 13)
Age (yr)	56.9 ± 11.8 (26–74)	55.2 ± 12.6 (21–78)	61.4 ± 11.3 (40–75)
BMI (kg/m^2)	26.9 ± 2.6 (21.3–32.1)	27.2 ± 3.0 (21.5–32.9)	27.1 ± 3.0 (23.2–31.9)
Height (m)	1.70 ± 0.09 (1.55–1.88)	1.67 ± 0.08 (1.55–1.86)	1.69 ± 0.06 (1.58–1.77)
Testosterone (nmol/liter)	14.8 ± 3.8 (10.4–24.9)	16.7 ± 4.6 (11.4–26.0)	14.6 ± 4.4 (10.4–24.3)

Data are mean ± SD (range in *parentheses*). No statistically significant differences were found between each group of patients and controls for each parameter.

echolamines and plasma renin activity and aldosterone in recumbent position and after 3 h of upright position).

Thirty-eight healthy men recruited among the clinics' staff and matched for age, BMI, and testosterone levels served as controls (Table 1).

None of the 76 subjects were under any treatment or were affected by diseases known to interfere with skeletal or mineral metabolism. Vertebral fractures were excluded by lateral x-ray of the spine in all cases. All subjects had normal kidney and liver functions and gave their witnessed informed consent before entering the study, for which the design was approved by local Ethical Committee and in accordance with the Helsinki Declaration II.

Methods

Serum and urinary samples were collected and stored at −70 C until assayed. In all patients, ACTH (mean of three determinations at 20-min intervals) and cortisol were measured at 0800 h. Serum cortisol and UFC levels (after dichloromethanol extraction) were determined immuno-fluorimetrically by TDX-FLX kits (Abbott GmbH Diagnostika, Wiesbaden-Delkenheim, Germany); serum ACTH levels were measured by immunoradiometric analysis (BRAHMS Diagnostica GmbH, Berlin, Germany).

In all patients and controls, serum intact PTH levels were measured by a two-site immunochemiluminometric assay (Chiron Corp., East Walpole, MA), and testosterone levels were measured by RIA (Byk-Sangtec Diagnostica GmbH & Co. KG, Dietzenbach, Germany). In 23 patients and 23 controls, the following markers of bone turnover were assayed: serum bone GLA protein (BGP) by immunoradiometric analysis for the intact molecule (ELSA-OST-NAT, Cis Biointernational, Gif-sur-Yvette, France; intra- and interassay coefficients of variation, 3.8% and 4.7%, respectively), and total deoxypyridinoline on fasting spot urine corrected for creatinine excretion (D-Pyr/Cr) after reverse phase HPLC, fluorometrically by Bio-Rad Laboratories, Inc. kits (Segrate-Milano, Italy; intra- and interassay coefficients of variation, 6.6% and 12.3%, respectively).

In all patients and controls, BMD was evaluated by dual-energy x-ray absorptiometry (DXA; Norland XR-26; Norland Instruments, Fort Atkinson, WI) at the following skeletal sites: spine (DXA L2–L4, *in vivo* precision, 1.0%) and femoral neck (FN; *in vivo* precision, 2.3%). Individual BMD values were expressed as SD units (Z-values) in relation to reference population of our center (23). Osteoporosis and osteopenia were diagnosed according to World Health Organization criteria (24).

Statistical analysis

The results are expressed as mean ± SD. For each variable, normality of distribution was tested by the W statistic of Shapiro-Wilk. Data were compared by Student's *t* test, Mann-Whitney *U* test, or one-way ANOVA test and Student-Newman-Keuls test *post hoc* analysis, as appropriate. A χ^2 test was used to evaluate the difference in the ratio of osteopenic and osteoporotic patients between subgroups. The associations between variables were tested by either Pearson or Spearman correlation, as appropriate. Probability values of less than 0.05 were considered significant.

Results

Individual hormonal and BMD data are shown in Table 2. Markers of disease activity in the two groups (SH+ and SH−) are shown in Table 3.

BGP and PTH levels were similar in group SH+, group SH−, and controls (Table 3). Mean D-Pyr/Cr levels were nearly twice as high in SH+ *vs.* SH− or control subjects, but statistical significance was not reached, due to the wide variability of the values.

Mean BMD Z-score measured at lumbar spine was significantly lower in group SH+ than in group SH− and controls (Table 3). Thus, the prevalence of osteoporosis and osteopenia was significantly higher in group SH+ than in group SH− [11 of 13 (84.6%) *vs.* 9 of 25 (36.0%); $\chi^2 = 6.28$; $P = 0.01$]. Taking into account BMD at FN, mean Z-values were lower in group SH+ than in group SH− or controls, but this difference did not reach statistical significance, due to the wide range of distribution of the values (Table 3).

Finally, no correlation was found between UFC, ACTH, serum cortisol after Dex with BGP, D-Pyr/Cr, or BMD Z-values measured at each site.

Discussion

To the best of our knowledge, this is the first report of bone involvement in a sample of consecutive AI male patients. We demonstrated that patients with biochemical SH (group SH+) have reduced spinal BMD and a higher rate of osteopenia/osteoporosis compared with AI patients without SH and healthy control subjects (Table 3).

We did not observe changes in BGP levels related to the degree of cortisol secretion; this is at variance with our previous cross-sectional study on female AI patients (15). This apparent discrepancy may well be due to the milder degree of cortisol hypersecretion of male AI patients with SH in the present report compared with that of female AI patients with SH in the previous report, as reflected by mean UFC level [262.8 (range, 162.0–445.5) *vs.* 332.9 (range, 201.5–874.9) nmol/24 h].

As far as bone mass is concerned, some, but not all (16), previous data from cross-sectional studies (15, 18) and, most importantly, a longitudinal one (19), have shown that BMD of the spine is reduced in female AI patients with SH (25). The present data, showing reduced spinal BMD also in male AI patients with SH, therefore indicate that the deleterious effect of subtle cortisol hypersecretion on bone mass is not gender specific. In addition, because our male AI patients were all eugonadal, these data indicate that the effect of SH on bone mass overcomes the protective role of gonadal steroids, a finding previously reported also in female AI patients (19). In our opinion, this finding is of importance when considering that, compared with women, men are clearly at lower risk of osteoporotic fracture and have higher BMD values even when defined as osteoporotic by World Health Organization criteria (26).

TABLE 2. Biochemical parameters of adrenal function and BMD Z-scores in patients with AI

Patient no.	UFC (nmol/24 h)	ACTH (pmol/liter)	F after Dex (nmol/liter)	Z-DXA	Z-FN	SH
1	89.7	2.2	24.8	0.080	1.100	−
2	99.4	5.1	35.9	−0.020	−0.120	−
3	104.9	3.3	41.4	0.100	−0.400	−
4	106.3	3.3	60.7	0.740	0.900	−
5	118.7	4.6	52.4	1.470	3.210	−
6	123.1	3.6	55.2	1.750	1.290	−
7	124.5	2.8	35.9	2.160	−0.620	−
8	131.1	3.2	35.9	0.370	0.400	−
9	132.5	4.5	66.2	1.130	0.790	−
10	135.2	2.6	96.6	−0.700	0.940	−
11	138.0	2.0	24.8	2.090	1.980	−
12	144.1	5.1	44.2	2.610	0.440	−
13	147.7	2.3	35.9	0.670	−0.230	−
14	151.2	4.2	22.1	−1.750	−1.180	−
15	151.8	3.3	69.0	0.780	0.340	−
16	154.6	5.7	69.0	−0.960	0.280	−
17	155.1	3.3	44.2	−0.820	0.940	−
18	163.1	3.1	49.7	0.890	0.860	−
19	163.4	10.3	13.8	0.340	1.050	−
20	169.5	6.0	110.4	2.260	0.750	−
21	175.8	6.4	35.9	−0.870	−0.630	−
22	192.9	6.4	71.8	−0.270	1.360	−
23	238.7	4.3	27.6	1.500	1.350	−
24	320.2	2.4	73.1	0.890	0.740	−
25	361.8	2.2	58.0	0.550	2.100	−
26	162.0	0.7	140.8	2.760	2.660	+
27	201.5	2.0	113.2	−2.600	−1.660	+
28	203.1	0.5	91.1	−1.710	0.270	+
29	218.0	3.1	82.8	−0.700	−0.600	+
30	220.2	1.8	69.0	−1.950	0.120	+
31	248.4	1.4	58.0	1.170	0.470	+
32	258.9	1.1	132.5	1.400	−1.000	+
33	273.2	1.5	193.2	−1.200	−0.400	+
34	273.2	2.2	99.4	−1.800	−0.400	+
35	278.8	2.0	143.5	0.120	2.110	+
36	303.6	10.6	82.8	−0.600	−0.600	+
37	328.2	3.8	82.8	−1.560	−0.350	+
38	445.5	2.0	85.6	1.150	−0.340	+

UFC, Normal values less than 193.1 nmol/24 h; ACTH, mean of three determinations at 0800 h, normal values above 2.2 pmol/liter; F after Dex, serum cortisol at 0800 h after 1 mg overnight Dex, normal values less than 82.8 nmol/liter; Z-DXA L2–L4, lumbar vertebral integral spine L2–L4 BMD as Z-values; Z-FN, FN BMD as Z-values.

TABLE 3. Biochemical indexes of bone turnover and BMD Z-scores in patients and controls

	Controls (n = 38)	Patients	
		Group SH− (n = 25)	Group SH+ (n = 13)
UFC (nmol/24h)		159.7 ± 63.2 (89.7–361.8)	262.8 ± 71.4[b] (162.0–445.5)
ACTH (pmol/liter)		4.1 ± 1.87 (2.0–10.3)	2.5 ± 2.6[b] (0.5–10.6)
F after Dex (nmol/liter)		49.7 ± 23.5 (13.8–110.4)	105.7 ± 38.6[b] (58.0–193.2)
BGP (pmol/liter)	3.9 ± 1.2 (2.1–6.3)	2.8 ± 0.7 (1.6–3.9)	3.2 ± 1.0 (2.0–5.1)
Urinary D-Pyr/Cr (pmol/pmol)	12.7 ± 4.7 (5.8–22.6)	13.5 ± 5.9 (6.7–26.1)	23.2 ± 21.5 (8.2–69.8)
PTH (ng/liter)	40.9 ± 13.8 (22.4–81.2)	40.3 ± 12.3 (21–65)	38.5 ± 10.6 (20.8–61.3)
DXA L2–L4 (Z-values)	0.47 ± 1.06 (−1.00–2.47)	0.60 ± 1.13 (−1.75–2.61)	−0.42 ± 1.62[a] (−2.6–2.76)
FN BMD (Z-values)	0.49 ± 1.11 (−1.03–2.88)	0.70 ± 0.95 (−1.18–3.21)	0.02 ± 1.19 (−1.66–2.66)

Data are mean ± SD (range in *parentheses*). DXA L2–L4, Lumbar vertebral integral spine L2–L4 BMD.
[a] $P < 0.05$ *vs.* group SH− and controls.
[b] $P < 0.0001$ *vs.* group SH−.

In our series of AI male patients, bone mass at FN was reduced, although not significantly, in group SH+ when compared with group SH− and controls (Table 3). This lack of significance observed in the present study on male patients could be explained by the wide range of distribution of BMD FN values and the relatively small sample size studied.

In conclusion, our data indicate a deleterious effect of subclinical endogenous cortisol excess on bone in male AI patients, despite their normal gonadal status. Although caution is needed because of the cross-sectional design of the study, BMD evaluation is advisable in AI male patients who have evidence of subtle cortisol hypersecretion. However, further longitudinal studies are needed to better clarify this issue.

Acknowledgments

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