Reduction of trabecular and cortical volumetric bone mineral density at the proximal femur in patients with acromegaly

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Abstract

Objective: Data on dual energy absorptiometry (DXA)-measured bone mineral density (BMD) at the level of the total hip (TH) and femoral neck (FN) in patients with acromegaly (ACRO) are conflicting. Increase in bone size associated with ACRO may limit the reliability of DXA. Our objective is to evaluate trabecular and cortical volumetric BMD (vBMD) across the proximal femur in ACRO patients.

Design: Cross sectional study in a clinical research center.

Patients: Thirty-five ACRO patients (19 males; mean age, 48 ± 7 years; BMI, 27.5 ± 4.4 kg/m²; 17 with active disease) and 35 age, gender, and BMI-matched controls.

Results: vBMD was assessed by quantitative computed tomography at the level of the TH, FN, trochanter (TR), and intertrochanteric (IT). Trabecular vBMD was lower in both total and active ACRO as compared with controls ($P < 0.01$). Cortical vBMD was lower in ACRO patients (active and controlled) vs controls at both TH and TR sites ($P < 0.05$). These findings were confirmed when only eugonadal patients were analyzed. Both total cross sectional area (CSA) and average cortical thickness (ACT) were greater in ACRO patients vs controls ($P < 0.05$). An inverse association between disease duration and trabecular vBMD at TH ($r = -0.42$, $P = 0.023$) and IT ($r = -0.41$, $P = 0.026$) was also found.

Conclusion: Both cortical and trabecular vBMD are reduced at the proximal femur in ACRO patients, regardless of gender, gonadal status, and disease activity. Disease duration is negatively associated with trabecular vBMD at the TH and IT.

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Introduction

Growth hormone/insulin-like growth factor (GH/IGF1) excess in acromegaly (ACRO) is associated with increased bone turnover, bone loss, and skeletal fragility (1). A recent meta-analysis demonstrated that while bone mineral density (BMD) measured by dual energy absorptiometry (DXA) does not differ at the lumbar spine (a site rich in trabecular bone) in patients with ACRO as compared to healthy controls, BMD at the femoral neck (FN) (where cortical bone is prevalent) tends to be higher

in ACRO (2). Increased prevalence and progression of vertebral fractures, regardless of BMD, has been observed in patients with ACRO even after biochemical control of the disease, suggesting that exposure to GH/IGF1 excess is associated with deterioration of structural and biomechanical properties of bone, which cannot be detected by DXA (3, 4). Indeed, DXA only provides two-dimensional areal BMD (aBMD), which is highly influenced by bone size and adiposity and cannot distinguish between cortical

and trabecular compartments, regions that may independently contribute to bone strength and resistance to fractures (5). Quantitative computed tomography (QCT) provides three-dimensional, volumetric BMD (vBMD) and can also separately measure cortical and trabecular bone. At present, no information is available on volumetric and biomechanical properties of bone at the level of proximal femur in patients with ACRO.

Our study is aimed at evaluating trabecular and cortical vBMD, measured through QCT, across the proximal femur in patients with ACRO, and examining potential clinical determinants of these parameters.

Subjects and methods

Subjects

We studied 35 patients with ACRO including 19 males and 16 females, with a mean age of 48 ± 7 years and BMI of 27.5 ± 4.4 kg/m² (Table 1). They were recruited successively while attending our clinic. According to the criteria established in the most recent Consensus Conference (6), 17 of them were classified as still having active disease and 18 having controlled disease, the latter defined as IGF1 concentrations within the specific age-adjusted reference

Table 1 Clinical characteristics and bone markers in 35 acromegalic patients and their sex, age, and BMI-matched control subjects.

	Acromegalics	Controls	P value
Cases	35	35	
Gender (M/F)	19/16	19/16	
Age (year)	48 ± 7	49 ± 8	0.51
BMI (kg/m ²)	27.5 ± 4.3	27.5 ± 5	0.84
Hypogonadism (%)	34%	31%	0.43
Median duration of disease (months)	144 (24–396)	–	–
Median duration of control (months)*	72 (12–312)	–	–
GH (μg/l)	4.4 ± 5.4	1.2 ± 2	<0.01
IGF1 (ng/ml)	256 ± 111	152 ± 41	<0.01
IGF1 SDS	2.7 ± 2.5	0.5 ± 0.9	<0.01
Serum calcium (mg/dl)	9.7 ± 0.3	9 ± 1.86	0.29
25(OH)D (ng/ml)	71 ± 42.5	63 ± 33	0.58
Serum PTH (pg/ml)	40.4 ± 23.8	36.2 ± 40	0.47
Osteocalcin (ng/ml)	17.8 ± 8.4	18.5 ± 6	0.72
Total P1NP (ng/ml)	45 ± 24	51 ± 21.2	0.28
CTx (ng/ml)	0.35 ± 0.21	0.38 ± 0.17	0.55
Prevalence of fragility fractures	0	0	–

*Median duration of control refers to 18 controlled patients and is defined as the lag time between the achievement of normal hormone values and the study entry. 25(OH)D, 25-hydroxyvitamin D; PTH, parathyroid hormone.

range, and in those patients who were not on GH receptor antagonist random GH concentrations were lower than 1 ng/ml. When the 75-g oral glucose load was performed, the GH values equal to or <0.4 ng/ml were considered as expression of cured disease (6). All had a GH-secreting pituitary tumor confirmed pathologically.

At study entry, 11 patients with active ACRO were taking a somatostatin analog (SSa) alone (eight patients) or in combination with a GH receptor antagonist (three patients) (median duration of treatment, 60 months (range: 12–180 months) for SSa and 12 months (range: 12–51 months) for GH receptor antagonist). One patient was on treatment with cabergoline for 18 months. Another patient was naïve to treatment, at the time of study, but subsequently had surgery. The remaining four patients had mild active disease at study entry but were not currently under medical therapy. All of them had noncurative transsphenoidal surgery (TSS) from 8 months to 17 years (median: 7 years) previously (except the treatment naïve patient), eight received postoperative radiotherapy from 1 to 17 years (median: 8 years) prior to the study entry.

Of patients with controlled disease, three were on treatment: one on SSa alone and the other two were on combination therapy with an SSa and either cabergoline or pegvisomant. All 18 controlled patients had TSS from 6 months to 25 years (median: 6 years) previously, and six of them also received radiotherapy from 9 to 24 years (median: 17 years) after unsuccessful surgery.

Five patients (14%) had secondary adrenal insufficiency and all of them were treated with stable replacement dose of hydrocortisone (between 10 and 20 mg/day). Eight patients (23%) had secondary hypothyroidism and were on adequate replacement therapy at the time of the study.

Seven males (37%) had secondary hypogonadism, defined as total testosterone levels below 300 ng/dl (10.4 nmol/l) (7). Because six of them were on stable testosterone replacement doses for more than one year, they were considered eugonadal. Five women (31%) had regular menses, whereas eleven (69%) were postmenopausal and not on hormone replacement therapy. None of the patients had GH deficiency.

Overall, 22 patients were eugonadal, including 17 normogonadal males (mean age ($\pm$ s.d.), 46.3 ± 7.5 years) and five premenopausal females (mean age ($\pm$ s.d.), 41.2 ± 2.3 years). Median duration of disease was estimated from the time elapsed between the onset of symptoms and signs of ACRO (evaluated through old photographs and clinical history) and either the date of enrollment in

this study (active patients) or the time when treatment was proven to be effective (controlled patients). Median duration of control was calculated from the lag time between the achievement of normal hormone values and the study entry.

Sex-, age-, and BMI-matched controls were recruited through advertisements at the blood donor center of our hospital. Exclusion criteria were type 2 diabetes, renal, or hepatic failure and use of anti-osteoporotic agents such as bisphosphonates, teriparatide, strontium ranelate, or denosumab. All subjects gave full informed consent and the study was approved by the local ethics committee.

Methods

Biochemical measurements

Serum IGF1 concentrations were measured by an enzyme immunoassay (Mediagnost, Reutlingen/Germany) with a sensitivity of 0.09 ng/ml. The intra- and interassay coefficients of variation (CV) were 6.7 and 6.8% respectively. In the study, IGF1 is expressed as SD score (SDS).

GH, osteocalcin, carboxy-terminal collagen crosslinks (CTX), and total procollagen type 1 amino-terminal propeptide (P1NP) were measured by electrochemiluminescent immunoassay (cobas e601; Roche Diagnostics GmbH). Imprecision for mean GH values between 0.18 and 35 µg/l was 3–3.4%, for osteocalcin concentrations between 6.11 and 160 µg/l was 2–2.3%, for β-crosslaps concentrations between 0.06 and 4.64 µg/l was 5.7–2.4%, and for total P1NP values between 14.4 and 1090 µg/l was 3.7–3.4%. Sensitivity was 0.05 µg/l for GH, 0.5 µg/l for osteocalcin, 0.01 µg/l for CTX, and 5 µg/l for P1NP. Serum 25-hydroxyvitamin D concentrations were determined using an enzyme immunoassay (IDS, Boldon, UK), with a sensitivity of 4.8 ng/ml. Imprecision for mean 25-hydroxyvitamin D values between 12 and 77.9 ng/ml was 9.9–7.7%.

Serum PTH concentrations were measured by electrochemiluminescent immunoassay (cobas e601; Roche Diagnostics GmbH). Imprecision for mean PTH values between 23.2 and 184 pg/ml was 3.4–1.7%.

Radiological imaging

aBMD was measured by DXA scanning (Hologic Discovery DXA system, HOLOGIC, Bedford, MA, USA). The CV was 1%. Patients had the proximal femur scanned. The scan acquisition and analysis were performed by a certified and experimented technician (AM) and were performed according to the ISCD standards.

(<http://www.iscd.org/documents/2015/06/2015-iscd-adult-official-positions.pdf>).

QCT was performed using a Phillips Brilliance 16 scanner. All the scans were acquired from the acetabulum directly above the femoral head down to 1 cm below the lesser trochanter (TR), resulting in 25–35 slices, with 3-mm slice thickness, over a range of 8–12 cm. All scans were performed at 120 kV and 70–200 mAs depending on height and weight of the patient, according to the Mindways technical specifications. Participants were positioned supine on the scanner table, lying on top of a solid calibration phantom (Mindways). The images were processed and analyzed using QCTpro Software Version 4.1.3 and the QCT-pro Bone Investigational Toolkit Version 2.0 (BIT, Mindways Software, Inc., Austin, TX, USA) by the same physician (JM). vBMD was obtained from the hip QCT analysis performing the following automated steps i) extraction of the proximal femur and ii) rotation and segmentation of bone voxels from soft tissue in three planes (axial, sagittal, and coronal). For each scan at each time point, a fixed threshold (450 mg/cm³) was used to discriminate cortical from trabecular compartment (8). Mechanical properties were obtained using the BIT software. A sliced narrow neck (NN) analysis was performed and the mechanical properties (buckling ratio (BR), cross sectional area (CSA; cm²) and average cortical thickness (ACT, cm) were the mean value of the NN series results. NN consists of nine slices of the FN; the average of the slice structural results was used to perform the analysis. BR is a measure of cortical elastic instability as a result of excessive cortical thinning, and it is defined as $BR = r/ct$, where r is the radius and ct is the corresponding cortical thickness. The BR was obtained from the extraction of the FN, followed by drawing of profile rays at 30° intervals and finally obtainment of outer radius and cortical thickness values from the profile rays.

Statistical analysis

The data are expressed as the mean ± s.d., except for data that were not normally distributed, in which case median values and ranges are reported. Comparisons between two groups were performed using Student's *t* (Gaussian distribution) and Mann–Whitney's *U* (non-Gaussian distribution) tests, and between three groups using ANOVA followed by Bonferroni test as a *post hoc* test or a Kruskal–Wallis *H* test, depending on the data distribution. Correlations were assessed using the Pearson's correlation coefficient or Spearman rank order depending on whether the data were normally distributed. Age, gender, disease

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duration, disease status (expressed as IGF1 SDS), hypogonadism, and 25-hydroxyvitamin D were considered as potential determinants of vBMD at each site by a stepwise multiple linear regression analysis. Tests were two-tailed, and $P < 0.05$ was considered significant.

Results

Clinical characteristics, parameters of calcium metabolism, and bone markers

The characteristics of the study population are provided in Table 1. Parameters of calcium metabolism and bone markers were not significantly different in ACRO patients as compared with healthy subjects. No differences in these values were observed when comparing active to controlled patients.

IGF1 levels were 325 ± 102 ng/ml in the active vs 199 ± 67 ng/ml in the controlled ACRO ($P < 0.001$). IGF1 SDS values were 4.2 ± 2.1 in the active vs 0.9 ± 1.1 in the controlled ACRO ($P < 0.001$).

aBMD by DXA at the proximal femur

No differences were found in the DXA parameters at the level of the proximal femur between ACRO patients and controls, as well as between active and controlled ACRO (Table 2).

vBMD and biomechanical variables by QCTpro in ACRO patients and healthy controls

QCT vBMD measures are presented in Table 2. Trabecular vBMD was significantly lower in patients with ACRO as compared with healthy controls at all the femoral sites analyzed (total hip (TH); FN; TR; intertrochanteric (IT)) ($P < 0.01$ for all the comparisons). As shown in Table 2, when active ACRO were compared with healthy subjects, the former had significantly lower trabecular vBMD at all sites ($P < 0.01$ for all the comparisons).

Cortical vBMD was significantly lower in patients with ACRO as compared with controls at both TH and TR sites ($P = 0.003$ and $P = 0.023$ respectively).

Table 2 Densitometric, volumetric, and biomechanical bone parameters in ACRO patients (total, active, and controlled) and in healthy controls.

Bone parameters	Acromegalic patients (n=35)	Control subjects (n=35)	P value*	Active acromegaly (n=17)	Controlled acromegaly (n=18)	P value**
DXA parameters						
Total hip T-score	-0.481 ± 0.96	-0.343 ± 0.951	0.38	-0.512 ± 0.823	-0.454 ± 0.998	0.67
Total hip aBMD	0.944 ± 0.143	0.963 ± 0.142	0.52	0.974 ± 0.143	0.921 ± 0.143	0.36
Femoral neck aBMD	0.831 ± 0.122	0.834 ± 0.124	0.88	0.851 ± 0.141	0.823 ± 0.121	0.83
QCT parameters						
Total hip-vBMD	299.5 ± 47	313.5 ± 48.6	0.28	297 ± 37	297 ± 56	0.4
Trabecular	123.7 ± 19.6	143 ± 23	<0.001	116.4 ± 17.3^a	128.3 ± 21	<0.001
Cortical	827 ± 58.7	870.5 ± 58.5	0.003	825.3 ± 62.8^b	831 ± 57.2^b	0.014
Femoral neck-vBMD	316 ± 41	320 ± 58	0.77	321.5 ± 39	309.8 ± 42.7	0.67
Trabecular	123 ± 27.3	145.2 ± 23.8	<0.001	113.4 ± 23^a	131.8 ± 28.2	<0.001
Cortical	828.8 ± 71	882 ± 154.2	0.10	838.6 ± 40.4	816.5 ± 90.2	0.16
Trochanteric-vBMD	224 ± 34.7	224 ± 37.5	0.99	227.7 ± 26	219 ± 40.5	0.81
Trabecular	124.3 ± 19.7	140 ± 19.8	0.001	117.8 ± 19.6^a	128.3 ± 19	<0.001
Cortical	$728.6 (612-1670.4)$	$829.3 (653.4-2297.7)$	0.023	$718.7 (612-886.4)^a$	$741.7 (655-1670.4)^b$	<0.001
Intertrochanteric-vBMD	338.7 ± 67.6	367.6 ± 58.3	0.099	329.6 ± 59.6	340 ± 76.7	0.14
Trabecular	122.6 ± 22.4	144.9 ± 27	<0.001	115 ± 20^a	126.8 ± 24	<0.001
Cortical	862.5 ± 61.6	875.3 ± 32.5	0.34	872.6 ± 65	855.8 ± 62	0.36
Biomechanical parameters						
CSA total	11.7 ± 7.5	8.8 ± 2	0.036	13.4 ± 10^b	9.9 ± 2	0.020
ACT	0.38 ± 0.10	0.33 ± 0.81	0.021	0.41 ± 0.12^b	0.35 ± 0.06	0.012
BR	5 ± 0.8	5.5 ± 1.7	0.32	5 ± 0.76	5.2 ± 0.94	0.58

*Student test comparing patients with acromegaly vs healthy controls; **ANOVA test (Kruskal-Wallis for nonparametric values) comparing healthy controls, active, and controlled acromegaly patients; DXA, dual-energy X-ray absorptiometry; QCT, quantitative computed tomography; vBMD, volumetric bone mineral density is expressed as mg/cm^3 ; ACT, average cortical thickness) is expressed as cm; BR, buckling ratio is unitless; CSA, cross-sectional area is expressed as cm^2 ; variables are expressed as mean ($\pm$ s.d.) or median (range) depending upon the distribution.

^avs controls, $P < 0.01$.

^bvs controls, $P < 0.05$.

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Cortical vBMD at the TH was also significantly lower in either active ACRO or controlled ACRO as compared with controls ($P=0.034$ and $P=0.038$ respectively) (Table 2). Cortical vBMD at the TR was significantly lower in both active ACRO and controlled ACRO as compared with controls ($P=0.008$ and $P=0.014$ respectively).

No differences in any QCT parameters were observed between active and controlled ACRO (Table 2).

Total CSA was significantly greater in both the ACRO as a whole and active ACRO as compared with controls ($P=0.036$ and $P=0.020$ respectively). ACT was also significantly higher in either ACRO group as a whole or active ACRO as compared with controls ($P=0.021$ and $P=0.012$ respectively). No differences in CSA, ACT, or BR were found in active ACRO vs controlled ACRO or in eugonadal ACRO vs eugonadal controls (Table 2).

vBMD and biomechanical variables by QCTpro in eugonadal patients

When only the 22 eugonadal patients were analyzed, all the trabecular vBMD measures were still significantly

lower in the ACRO as a whole as compared with their controls ($P<0.01$ for all the comparisons) (Table 3).

Trabecular vBMD at TH, FN, and IT was significantly lower in active ACRO as compared with healthy subjects ($P<0.01$ for all comparisons). Controlled ACRO had significantly lower trabecular vBMD at both TH and IT as compared with healthy controls ($P=0.044$ and $P=0.037$ respectively).

ACRO active patients had significantly lower cortical vBMD at the TR as compared with healthy controls ($P=0.010$).

ACRO controlled patients had significantly lower cortical vBMD at TH, TR, and IT as compared with healthy controls ($P=0.022$ for TH; $P=0.012$ for TR; $P=0.025$ for IT).

Potential determinants of vBMD

In the ACRO group as a whole, duration of ACRO was negatively associated with trabecular vBMD at the TH ($r=-0.42$, $P=0.023$) (Fig. 1A), and both total vBMD ($r=-0.39$, $P=0.036$) and trabecular vBMD ($r=-0.41$, $P=0.026$) at the IT region (Fig. 1B).

Table 3 Densitometric, volumetric, and biomechanical parameters of bone in eugonadal ACRO patients (total active, controlled) and in eugonadal healthy controls.

Bone parameters	Eugonadal acromegalic patients (n=22)	Eugonadal control subjects (n=22)	P value*	Eugonadal active patients (n=13)	Eugonadal controlled patients (n=9)	P value**
DXA parameters						
Total hip T-score	-0.35 ± 0.84	-0.11 ± 0.94	0.38	-0.2 ± 0.8	-0.5 ± 0.9	0.40
Total hip aBMD	0.99 ± 0.133	0.991 ± 0.143	0.61	0.902 ± 0.11	0.821 ± 0.09	0.38
Femoral neck aBMD	0.872 ± 0.101	0.852 ± 0.142	0.97	1.04 ± 0.131	0.902 ± 0.133	0.48
QCT parameters						
Total hip-vBMD	294.2 ± 40.5	315 ± 46	0.13	306.8 ± 35.9	278.9 ± 42.3	0.076
Trabecular	123.2 ± 17	145 ± 20	0.001	121.4 ± 17.7^a	125.5 ± 16.6^b	0.001
Cortical	814 ± 56.2	873.7 ± 61.6	0.001	822.5 ± 68.4^a	803.8 ± 37.8^b	0.012
Femoral neck-vBMD	307.7 ± 36.2	316.3 ± 56.2	0.57	314.9 ± 31.3	299 ± 41.6	0.41
Trabecular	121 ± 26.5	147.3 ± 22	0.001	116.9 ± 24.4^a	126 ± 29.5	0.001
Cortical	823.2 ± 70.8	886 ± 171	0.13	839 ± 44.9	803.8 ± 92.8	0.38
Trochanteric-vBMD	223.3 ± 28.8	223.2 ± 36.5	0.99	230.4 ± 27	214.6 ± 30.2	0.54
Trabecular	124.5 ± 17.2	141.8 ± 16.7	0.003	122.8 ± 20.7^a	126.6 ± 12.7	0.004
Cortical	$715.3 (612-886)$	$816 (695-2297)$	<0.001	$686.6 (612-886)^a$	$726.5 (655-911)^b$	0.002
Intertrochanteric-vBMD	332 ± 60.3	372.4 ± 54.3	0.017	347 ± 59.6	313.9 ± 59.3^b	0.028
Trabecular	121.3 ± 21	147.5 ± 24.4	0.001	119.8 ± 20.6^a	123.2 ± 22.4^b	0.001
Cortical	851.7 ± 60.5	875 ± 31	0.13	873.3 ± 68	825.4 ± 38.4^b	0.033
Biomechanical parameters						
CSA total	12 ± 8.2	9.3 ± 2	0.11	13.5 ± 11	10.5 ± 1.8	0.14
ACT	0.38 ± 0.09	0.34 ± 0.085	0.13	0.4 ± 0.11	0.35 ± 0.049	0.17
BR	5.2 ± 0.7	5.5 ± 1.8	0.47	5.1 ± 0.7	5.3 ± 0.83	0.76

*Student test comparing patients with acromegaly vs healthy controls; **ANOVA test (Kruskal-Wallis for nonparametric values) comparing healthy controls, active, and controlled acromegalic patients; DXA, dual-energy X-ray absorptiometry; QCT, quantitative computed tomography; vBMD, volumetric bone mineral density is expressed as mg/cm^3 ; ACT, average cortical thickness is expressed as cm; BR, buckling ratio is unitless; CSA, cross-sectional area is expressed as cm^2 ; variables are expressed as mean ($\pm$ s.d.) or median (range) depending upon the distribution.

^avs controls, $P<0.01$.

^bvs controls, $P<0.05$.

Figure 1

(A) Inverse relationship between disease duration and trabecular vBMD at total hip ($r = -0.42$, $P = 0.023$) in the ACRO patients. (B) Inverse relationship between disease duration and trabecular vBMD at intertrochanter ($r = -0.41$, $P = 0.026$) in the ACRO patients.

These relationships held true after controlling for gender, gonadal status, and disease activity.

When only controlled patients were analyzed, the duration of control was not associated with any volumetric parameter.

Discussion

This is the first study using QCT to investigate cortical and trabecular bone mass in the proximal femur in patients with acromegaly (ACRO). We have demonstrated that vBMD at trabecular compartments is decreased in ACRO patients, mainly in those with active disease and is inversely related to disease duration. Moreover, we have shown that cortical vBMD at the TH and the TR are reduced in ACRO patients as compared with healthy controls regardless of disease activity, disease duration, and gonadal status.

Several previous studies evaluating BMD at the FN in ACRO patients through DXA have provided conflicting results. While some of them have shown higher BMD in ACRO patients as compared with healthy controls (9, 10, 11, 12), others did not find any difference (5, 13, 14, 15, 16). Such discrepant results could mainly be accounted for by the use of DXA, which may not be the most appropriate technique to measure femoral bone mass in ACRO. Indeed, DXA only provides two-dimensional aBMD, a parameter highly influenced by bone size, and cannot distinguish between cortical and trabecular compartments which might be affected differentially by GH/IGF1 excess (2, 16). In contrast to DXA, QCT has a three-dimensional imaging approach which better reflects the complex structure and biomechanics of the proximal femur and can quantify the amount of cortical and trabecular bone (17).

In our study, the femoral DXA-derived aBMD did not change in ACRO patients as compared with healthy controls. Conversely, we documented a significant reduction of QCT-derived trabecular vBMD at each site analyzed in both ACRO patients as a whole and patients with active ACRO. Of note is that these results were confirmed when patients with hypogonadism were excluded from the analysis, supporting the observation by Madeira *et al.* (18) who found lower trabecular densities and impaired microarchitecture at the distal radius and distal tibia, as measured by high-resolution peripheral QCT (HR-pQCT), in eugonadal patients with either active or controlled acromegaly as compared with healthy subjects. Hypogonadism has been advocated as one of the main determinants of low DXA-derived BMD at both spine and FN in ACRO (2, 19). Battista *et al.* (20) found increased Z-value on QCT at lumbar spine in eugonadal, active ACRO as compared with their hypogonadal counterpart. Yet, we did not find any relationship between the gonadal status and trabecular vBMD at any sites of the proximal femur. Indeed, after multiple linear regressions, duration of active acromegaly was the only negative predictor of trabecular vBMD at TH and IT, suggesting that chronic exposure to GH/IGF1 negatively impacts the trabecular bone at the level of the proximal femur, counteracting the effect of the gonadal status (14, 21). No differences in trabecular vBMD were documented in controlled ACRO patients when compared to either active ACRO or healthy subjects, suggesting that the biochemical control of the disease leads to an improvement, but not normalization, of the trabecular compartment. However, it should be emphasized that the small sample size is a limitation of our study, and therefore, larger populations should be evaluated in the future to confirm our findings. In particular, although ACRO is known to be characterized by elevated bone turnover (1, 22), we did not find any differences in bone markers between ACRO patients and controls as well as between active and controlled ACRO, and this may be due to a type 2 error.

Our study has also shown that cortical vBMD at TH and TR sites is significantly reduced in both ACRO patients as a whole and active ACRO when compared to healthy controls and this difference is maintained in the whole group of ACRO patients when only subjects with normal gonadal function were included. Interestingly, cortical vBMD at the TH and TR were also lower in the controlled ACRO when compared to healthy subjects. Certainly, we found no differences in any of the volumetric parameters evaluated between active and controlled ACRO, consistent with most of the DXA studies at the level of the FN (2).

This finding may indicate that control of ACRO does not completely compensate for the detrimental effects of long lasting GH/IGF1 excess on cortical bone at the level of proximal femur. Notably, Biermasz *et al.* (23) documented a negative relationship between the duration of remission and DXA-derived BMD at FN in ACRO patients. However, the anabolic effects of GH/IGF1 are thought to be more evident on cortical bone and a recent meta-analysis showed a tendency toward higher aBMD at the FN in ACRO patients as compared with controls (2). Madeira *et al.* (18) found higher cortical density in the distal tibia in patients with active ACRO as compared with those having controlled disease, using HR-pQCT. However, this difference may be accounted for by differences between the femur and the tibia in terms of structural and geometrical characteristics (24).

Our data show different bone volumes across the proximal femur of ACRO patients, which could indicate inter-regional variance in the responsiveness to factors impacting bone mass in this condition. Moreover, it is well known that a large bone structure depends on the forces applied to the bone (25). Thus, the forces acting on normal bone are different from those applied on a bone that is growing larger and more rapidly. This could influence the bone structure and explain the differences between compartments which we have documented.

Regarding bone structural parameters, we have found that the CSA and the average thickness (ACT) are higher in ACRO patients than in controls, indicating that patients might have a stronger as well as a larger femur in the face of having lower cortical vBMD. Indeed, active patients show a trend toward higher structural properties in comparison to controlled ACRO, meaning that the pathway responsible for these changes is still active and the bone is persistently influenced by a stimulus favoring bone growth. The absence of significant differences in the BR is not surprising, since the BR is maintained even if the bone is larger and the ACT is higher. Thus, ACRO patients have lower trabecular and cortical BMD and larger bones consistent with higher activity of bone remodeling. Whereas the strength of trabecular bone at the vertebral level could be impaired, leading to fragility and vertebral fractures (3, 4), this would not occur at the level of the femur. Indeed, the structural properties of the cortical compartment, which is prevalent in the femur and mostly responsible for bone strength in normal subjects, would 'compensate' the reduction of femoral bone mass protecting ACRO patients from fractures (26). However, future studies using finite element analysis of the QCT images are needed in order to clarify if the pattern described in our

report is associated with altered strength and resistance across the proximal femur in patients exposed to GH/IGF1 excess. Of note, overexpression of GH in mice is associated with accelerated bone turnover and low cortical vBMD of the vertebrae, femur, and tibia leading to deterioration of bone mechanical strength (27). Mazziotti *et al.* (15) demonstrated that aBMD at the FN decreased in both active and controlled ACRO patients during a 3-year follow-up and this reduction significantly predicted vertebral fractures in controlled ACRO. It is to be determined if low vBMD at the TH and TR may be related to higher fracture risk in ACRO patients.

In conclusion, this study demonstrates that trabecular vBMD is reduced across the proximal femur in patients with acromegaly, regardless of gonadal status and disease activity. Moreover, the duration of the disease is significantly associated with decreased vBMD at the TH and IT region. Cortical vBMD at the TH and TR are also reduced in patients with both active and controlled disease. Further studies involving larger populations are needed to confirm our results and evaluate their potential impact on skeletal fragility in patients with GH/IGF1 excess.

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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