Bone microarchitecture and estimated bone strength in men with active acromegaly

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Abstract

Context: Both acromegaly and adult growth hormone deficiency (GHD) are associated with increased fracture risk. Sufficient data are lacking regarding cortical bone microarchitecture and bone strength, as assessed by microfinite element analysis (μ FEA).

Objective: To elucidate both cortical and trabecular bone microarchitecture and estimated bone strength in men with active acromegaly or GHD compared to healthy controls.

Design and subjects: Cross-sectional study at a clinical research center, including 48 men (16 with acromegaly, 16 with GHD and 16 healthy controls).

Outcome measures: Areal bone mineral density (aBMD), cortical and trabecular bone microarchitecture and estimated bone strength (μ FEA) at the radius and tibia.

Results: aBMD was not different between the 3 groups at any skeletal site. At the radius, patients with acromegaly had greater cortical area ($P < 0.0001$), cortical thickness ($P = 0.0038$), cortical pore volume ($P < 0.0001$) and cortical porosity ($P = 0.0008$), but lower trabecular bone density ($P = 0.0010$) compared to controls. At the tibia, patients with acromegaly had lower trabecular bone density ($P = 0.0082$), but no differences in cortical bone microstructure. Compressive strength and failure load did not significantly differ between groups. These findings persisted after excluding patients with hypogonadism. Bone microarchitecture was not deficient in patients with GHD.

Conclusions: Both cortical and trabecular microarchitecture are altered in men with acromegaly. Our data indicate that GH excess is associated with distinct effects in cortical vs trabecular bone compartments. Our observations also affirm the limitations of aBMD testing in the evaluation of patients with acromegaly.

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Introduction

Growth hormone (GH) activates both bone formation and resorption via direct and indirect effects on the skeleton (1). Insulin-like growth factor 1 (IGF-I), secreted in response to GH stimulation, mediates many of the effects of GH on bone, acting in an endocrine and paracrine manner (1). A host of other factors, including parathyroid hormone (PTH) whose secretion is modulated by GH, regulate IGF-I expression in osteoblasts (1).

Patients with acromegaly are at increased risk of vertebral fractures (2, 3, 4). Of note, the findings of a meta-analysis have identified men with acromegaly as being at higher risk for fracture than women, but did not include any data on bone microarchitecture (2). Among patients with acromegaly, those with hypogonadism also appear to be at higher fracture risk than those who are eugonadal (2). Published data on areal bone mineral density (aBMD)

Clinical Study	P P B Silva, F G Amlashi and others	Bone microarchitecture in acromegaly	177:5	410
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in patients with acromegaly have been conflicting. Indeed, different studies have variably reported normal, increased or decreased aBMD in patients with acromegaly (1, 2). These findings have been confounded by the presence of hypogonadism in a subgroup of these patients, which may independently contribute to bone loss (1, 2). There appears to be no correlation between aBMD and fracture risk in acromegaly, suggesting that aBMD is not adequate in the skeletal assessment of these patients (2). Patients with acromegaly have been reported to have impaired trabecular bone microarchitecture (5). However, this study did not include any data on the estimated bone strength in this population and analyzed data in patients of both genders in aggregate. In addition, adequate data on detailed cortical microarchitecture in patients with acromegaly are lacking, based on available reports.

Hypopituitary adults with GH deficiency (GHD) are also at increased fracture risk (1, 6, 7). Some, but not all, studies of hypopituitary adults with unreplaced GHD have found that their aBMD is decreased in the central and appendicular skeleton in comparison with age- and gender-matched controls (8, 9). Of note, GH replacement increases aBMD in adults with GHD who have been treated for longer than 18–24 months (10). However, there appears to be no association between aBMD and fracture risk in adults with GH deficiency (1). There are no data on microarchitecture or the mechanical properties of bone in adults with GHD. In aggregate, available data do not adequately explain the structural basis for increased bone fragility in adult patients with GH excess or deficiency.

To further elucidate the basis for the increased fracture risk associated with GH excess or deficiency, we studied the bone microarchitecture and estimated bone strength in adults with acromegaly or adult GHD in comparison with healthy controls. In the present study, we hypothesized that deficiencies in trabecular and/or cortical compartments are present in patients with chronic GH excess or deficiency. To avoid the potential confounding effects of menopause-related bone loss and gender-dimorphic variations in bone microarchitecture, we limited enrollment to men in the present study.

Patients and methods

Inclusion–exclusion criteria

Adult men, aged 20–55 years, were eligible to participate in the study, if they had active acromegaly (including elevated serum IGF-I levels at study entry) or unreplaced GHD as a consequence of hypopituitarism, or if they

were generally healthy (serving as healthy controls). The diagnosis of acromegaly was abstracted from the medical record and was based on typical symptoms, elevated serum IGF-I levels and radiographic evidence of a pituitary adenoma. Patients with acromegaly were eligible to participate regardless of whether they had a history of previous pituitary surgery, provided that persistent GH excess was present postoperatively through the time of study. The diagnosis of acromegaly was supported by histologic evidence of a GH-secreting adenoma in patients who had undergone pituitary surgery before study entry. Adult patients with hypopituitarism, caused by sellar masses or traumatic brain injury, were considered to have GHD if they met the diagnostic criteria of the GH Research Society (11). These criteria include one of the following: peak growth hormone <3 ng/mL on glucagon or insulin stimulation testing or body mass index (BMI)-adjusted GH cutpoints on GH-releasing hormone (GHRH)-arginine stimulation testing as follows: if BMI <25 kg/m², peak growth hormone <11.5 ng/mL; if BMI: 25–30 kg/m², peak growth hormone <8 ng/mL; if BMI >30 kg/m², peak growth hormone <4 ng/mL or low age-adjusted serum IGF-I levels in the presence of ≥3 additional pituitary hormone deficiencies. Data on the results of GH stimulation testing were abstracted from the patients' medical record. Healthy controls were also recruited, whose chronologic age was within 5 years from the age of individual patients with acromegaly or GHD.

Subjects were excluded if they met any of the following criteria: onset of pituitary disease before age 18 years, history of Cushing's syndrome, history of treated acromegaly (in the GH deficient group), primary hyperparathyroidism, GH replacement within the past 12 months (or during childhood/adolescence), bisphosphonate therapy, chronic liver or kidney disease, supraphysiologic glucocorticoid therapy within the past year (prednisone >5 mg/daily or hydrocortisone >25 mg/daily), use of other medications that may have negative effects on bone mass, history of organ transplant, gastrectomy or gastric bypass surgery, immobility, regular endurance exercise (running >20 miles/week), eating disorder; BMI <18 kg/m² or body weight >159 kg (350 lb).

Study procedures

The study was approved by the Partners HealthCare Institutional Review Board. Patients were recruited through outpatient MGH clinics or local advertisements. Every effort was made to consecutively enroll all eligible

patients. However, one eligible patient with acromegaly and two patients with GHD declined to participate for personal reasons. Written informed consent was obtained from eligible study subjects after the nature of the study and related procedures were fully reviewed and discussed. Study subjects were evaluated as outpatients at the MGH Harvard Catalyst Clinical Research Center and the MGH High Resolution Peripheral Quantitative Computed Tomography Core facility.

Information regarding prevalent fracture was obtained from the medical records as well as through patient interview. Data on calcium intake, smoking, alcohol use and physical activity were obtained using standardized, validated questionnaires (12).

Endocrine testing

Serum IGF-I was measured by liquid chromatography–tandem mass spectrometry (LC/MS–MS) with a detection limit of 4 ng/mL and intra-assay coefficient of variation (CV) of up to 3.3% (13). Serum calcium, 25 hydroxy-vitamin D, PTH, free T4, prolactin, (morning) testosterone, estradiol, sex hormone-binding globulin (SHBG), osteocalcin and C-terminal peptide of type 1 collagen (CTX) were assayed using laboratory immunoassays at a commercial laboratory (LabCorp, Burlington, NC, USA). Immunoassays used were as follows: 25 hydroxy-vitamin D was measured with a competitive immunoassay (DiaSorin, Stillwater, MN, USA) with a detection limit of 9.9 nmol/L and CV between 2.9 and 5.5%; PTH was measured with a two-site immunoassay (Elecsys, Roche Diagnostics North America) with a detection limit of 0.13 pmol/L and CV between 1.5 and 2.7%; free T4 was measured with a competition immunoassay (Cobas, Roche Diagnostics North America) with a detection limit of 0.6 pmol/L and CV between 0.9 and 4.0%; prolactin was measured with a two-site immunoassay (Elecsys, Roche Diagnostics North America) with a detection limit of 2.0 pmol/L and CV between 1.8 and 4.0%; (total) testosterone was measured with a competition immunoassay (Cobas, Roche Diagnostics North America) with a detection limit of 0.087 nmol/L and CV between 1.4 and 4.7%; estradiol was measured with a competition immunoassay (Cobas, Roche Diagnostics North America) with a detection limit of 18.4 pmol/L and CV between 1.6 and 5.7%; SHBG was measured with a two-site immunoassay (Cobas, Roche Diagnostics North America) with a detection limit of 0.35 nmol/L and CV between 2.1 and 2.7%; osteocalcin was measured with an enzyme immunoassay (Immunodiagnostic Systems, Inc,

Fountain Hills, AZ, USA) with a detection limit of 0.5 µg/L and CV between 1.3 and 2.2% and CTX was measured with a two-site immunoassay (Immunodiagnostic Systems, Inc) with a detection limit of 0.02 µg/L and CV between 2.1 and 4.9%. Serum calcium was measured using a photometric method (Cobas, Roche Diagnostics North America) with a detection limit of 0.2 mmol/L and CV between 1.8 and 2.9%.

Bone mineral density and body composition

Areal BMD and body composition were assessed by dual-energy X-ray absorptiometry (DXA), including scans of the posterior–anterior lumbar spine, total hip, one-third distal radius and total body (Hologic 4500A, Hologic Inc, Waltham, MA, USA). The CV for DXA parameters ranged between 0.8 and 2.1% (with the lowest figures pertaining to the lumbar spine and the highest figures for the hip). Estimated radiation exposure for a DXA scan was 31 µSv.

Bone microarchitecture and estimated bone strength

Cortical and trabecular bone density and microarchitecture were examined at the distal radius and tibia using high-resolution peripheral quantitative computed tomography (HR-pQCT, XtremeCT, Scanco Medical AG, Brüttisellen, Switzerland). During image acquisition, the non-dominant forearm or leg of the study subject was placed in an anatomically formed carbon fiber shell and an anteroposterior scout view was used in order to manually place a reference line at the endplate of the radius or tibia. The first CT slice was acquired 9.5 mm proximal to the reference line of the distal radius and 22.5 mm proximal to the reference line of the distal tibia. Scans were acquired at 82 µm isotropic voxel size. At each site, 110 CT slices were acquired extending ~9 mm axially. The effective radiation dose was <5 µSv per measurement.

Bone microarchitecture parameters were determined using semi-automated software. The short-term reproducibility ranged between 0.2 and 1.7% for density data and 0.7–8.6% for microarchitecture data, as previously described (14). Microfinite element analysis (µFEA) was performed using the HR-pQCT images to estimate radius and tibia strength, as previously described (15). Using dedicated software, each bone voxel was converted to a hexahedral finite element with linear elastic and isotropic material behavior, having Young's modulus of 10 GPa and Poisson's ratio of 0.3. Failure load was estimated by scaling the resultant load from 1% apparent compressive

Clinical Study	P P B Silva, F G Amlashi and others	Bone microarchitecture in acromegaly	177:5	412
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strain until 2% of all elements reached an effective strain exceeding 7000 μ strain (16). The (intra-subject) CV was 2.7% (failure load) and 3.0% (compressive stiffness).

Statistical analysis

Statistical testing was conducted using JMP Pro 13.0.0 (SAS Institute, Inc, Cary, NC, USA, 2016). The following power calculations were conducted (based on data on bone microarchitecture in another study population) (17): with 48 patients, we estimated a 95% probability of detecting a difference in trabecular bone density of 19 mgHA/cm³ between the study groups at a significance (alpha) level of 0.05 with a standard deviation of 33 mgHA/cm³. The Shapiro–Wilk test was used to examine whether distributions were normal. Continuous data that were normally distributed were analyzed using analysis of variance (ANOVA) followed by the Tukey–Kramer test for pairwise testing, if overall significance testing showed $P < 0.05$. The Wilcoxon test was used to analyze data that were not normally distributed. Additional adjustments for multiple comparisons were not indicated, since a preliminary test of overall (global) significance was employed between the 3 groups (18). Nominal data were analyzed using the chi square or Fisher's exact test (as appropriate). Further adjustments for multiple comparisons were not performed, since bone outcomes were likely highly intercorrelated, making such adjustments overly conservative. Regression analyses, including mixed models and standard least square regression models were used to adjust for potential confounders. Multivariate, forward stepwise linear regression analyses were used to identify predictors of bone microarchitecture. Data are presented as mean values and standard error of the mean (S.E.M.) or median and range (as appropriate). Data on aBMD are presented as absolute values and Z scores; data on bone microarchitecture are presented as absolute values. P values < 0.05 are considered as statistically significant.

Results

Demographic, clinical and endocrine data

General characteristics of the study population are shown in Table 1. A total of 48 male subjects were recruited and completed the study, including 16 patients in each group (acromegaly, GH deficiency, healthy controls). There was no difference in chronologic age, age at diagnosis

of pituitary disease (when applicable), body weight or height, prevalent fracture, daily estimated calcium intake, smoking history, alcohol use or exercise between the groups. All fractures were considered to be traumatic.

As a consequence of study design, the interval between the diagnosis of abnormal GH secretion and study visit was shorter in patients with (active) acromegaly than in those with GHD. However, the duration of exposure to GH excess cannot be ascertained based on our data; it is likely that patients with acromegaly had GH excess for several years before enrollment. Nine patients with acromegaly had pituitary surgery before enrollment, but all had persistent GH excess postoperatively. Patients with acromegaly had higher BMI than those in the other 2 groups. One patient with acromegaly had type 2 diabetes mellitus. Patients with acromegaly had higher serum levels of IGF-I, calcium and biomarkers of bone turnover (osteocalcin and CTX), but lower serum levels of testosterone and SHBG. All calcium levels were within the normal range. Three patients with acromegaly had central hypogonadism and were not receiving testosterone replacement at study enrollment (in consultation with their treating physician).

Patients with GHD were more likely than those with acromegaly to have central hypogonadism, hypothyroidism or hypoadrenalism or to have received pituitary radiotherapy. Ten out of 16 patients with GH deficiency had previously received GH replacement for 41.9 ± 11.9 months, but had been off GH replacement for 59.6 ± 23.2 months before study entry.

Areal bone mineral density and body composition

Data obtained by DXA are shown in Table 2. There was no difference in aBMD at any of the skeletal sites measured between the groups. The fat free (lean) body mass was 26.5% higher in patients with acromegaly in comparison with controls.

Bone microarchitecture and μ FEA of the radius

Data obtained by HR-pQCT of the radius are shown in Fig. 1 and Table 3. Patients with acromegaly had more favorable cortical morphology, including higher cortical area (+35.8%), cortical perimeter (+8.5%) and cortical thickness (+23.6%). On the other hand, patients with acromegaly also had cortical pore volume (+152.1%) and cortical porosity (+70.8%) in comparison with controls. The ratio of cortical area to total cross-sectional area

Clinical Study	P P B Silva, F G Amlashi and others	Bone microarchitecture in acromegaly	177:5	413
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Table 1 Demographic, clinical and endocrine characteristics of the study population.

Variable	Acromegaly (n=16) mean $\pm$ S.E.M.	GH deficiency* (n=16) mean $\pm$ S.E.M.	Control (n=16) mean $\pm$ S.E.M.	Overall P value	Pairwise P value		
					(acromegaly vs control)	(GH deficiency vs control)	(acromegaly vs GH deficiency)
Age at study (year)	40.7 $\pm$ 2.8	47.4 $\pm$ 2.3	43.8 $\pm$ 2.6	NS	NA	NA	NA
Age at diagnosis of pituitary disease (year)	40.0 $\pm$ 2.7	37.1 $\pm$ 2.0	NA	NS	NA	NA	NA
Interval between diagnosis of GH excess/deficiency and study visit (months)	7.9 $\pm$ 5.3	90.1 $\pm$ 17.4	NA	<0.0001	NA	NA	<0.0001
Body weight (kg)	106.9 $\pm$ 5.1	95.8 $\pm$ 3.5	91.4 $\pm$ 7.5	NS	NA	NA	NA
Height (m)	1.81 $\pm$ 0.02	1.78 $\pm$ 0.02	1.76 $\pm$ 0.02	NS	NA	NA	NA
BMI (kg/m ²)	32.7 $\pm$ 1.5	30.3 $\pm$ 1.1	27.4 $\pm$ 0.9	0.013	0.009	NS	NS
Pituitary surgery (%)	9 (56%)	10 (62%)	0 (0%)	0.0002	NA	NA	NA
Pituitary radiotherapy (%)	0 (0%)	9 (56%)	0 (0%)	<0.0001	NA	NA	NA
Hypogonadism (%)	3 (19%)	14 (87%)	0 (0%)	<0.0001	NA	NA	NA
Testosterone replacement (%)	0 (0%)	14 (87%)	0 (0%)	<0.0001	NA	NA	NA
Glucocorticoid replacement (%)	1 (6%)	9 (56%)	0 (0%)	0.0008	NA	NA	NA
Levothyroxine replacement (%)	0 (0%)	12 (75%)	0 (0%)	<0.0001	NA	NA	NA
Prevalent fracture (%)	6 (37%)	2 (12%)	3 (19%)	NS	NA	NA	NA
Calcium intake (mg/day)	1175 $\pm$ 149	940 $\pm$ 128	932 $\pm$ 104	NS	NA	NA	NA
Smoking (%)	5 (31%)	1 (6%)	2 (12%)	NS	NA	NA	NA
Alcohol use (%)	10 (62%)	4 (25%)	4 (25%)	NS	NA	NA	NA
Drinks per week (n)	5 $\pm$ 1.6	3 $\pm$ 0.5	2 $\pm$ 0.8	NS	NA	NA	NA
Light activity (h/week)	78 $\pm$ 8.8	96 $\pm$ 3.5	89 $\pm$ 5.4	NS	NA	NA	NA
Moderate exercise (h/week)	22 $\pm$ 3.9	16 $\pm$ 3.6	21 $\pm$ 4.5	NS	NA	NA	NA
Vigorous exercise (h/week)	11 $\pm$ 3.9	7 $\pm$ 3.4	9 $\pm$ 1.7	NS	NA	NA	NA
IGF-I (nmol/L)	94.4 $\pm$ 9.9	12.9 $\pm$ 1.3	22.0 $\pm$ 1.7	<0.0001	<0.0001	NS	<0.0001
IGF-I Z score	3.9 $\pm$ 0.3	-0.9 $\pm$ 0.2	0.2 $\pm$ 0.2	<0.0001	<0.0001	0.008	<0.0001
Peak GH response on stimulation testing** (μ g/L)	NA	1.5 $\pm$ 0.2	NA	NA	NA	NA	NA
Calcium, serum (mmol/L)	2.4 $\pm$ 0.03	2.3 $\pm$ 0.03	2.3 $\pm$ 0.03	0.0380	NS	NS	0.0307
25 hydroxy-vitamin D (nmol/L)	67.4 $\pm$ 6.9	67.4 $\pm$ 7.5	72.4 $\pm$ 7.9	NS	NA	NA	NA
PTH (ng/L)	32 $\pm$ 3.0	40 $\pm$ 3.9	42 $\pm$ 7.7	NS	NA	NA	NA
Prolactin (pmol/L)	565.2 $\pm$ 200.0	695.6 $\pm$ 239.1	391.3 $\pm$ 34.8	NS	NA	NA	NA
Free T4 (pmol/L)	15.4 $\pm$ 1.3	14.2 $\pm$ 1.3	14.2 $\pm$ 1.3	NS	NA	NA	NA
Testosterone (nmol/L)	8.6 $\pm$ 1.1	18.9 $\pm$ 3.5	16.7 $\pm$ 1.6	0.0097	0.0543	NS	0.0100
Estradiol (pmol/L)	80.8 $\pm$ 9.5	143.2 $\pm$ 36.7	88.1 $\pm$ 7.3	NS	NA	NA	NA
SHBG (nmol/L)	21 $\pm$ 2.4	26 $\pm$ 2.3	35 $\pm$ 2.9	0.0014	0.0011	0.0488	NS
Osteocalcin (μ g/L)	42 $\pm$ 6.3	15 $\pm$ 2.7	16 $\pm$ 1.9	<0.0001	0.0001	NS	0.0001
CTX (μ g/L)***	1.0 (0.7, 1.4)	0.4 (0.2, 14.8)	0.5 (0.2, 0.6)	0.0141	0.0017	NS	0.0771

*Causes of hypopituitarism include: non-functioning pituitary adenoma (6 patients); pituitary apoplexy (1 patient); prolactinoma (4 patients); craniopharyngioma (2 patients); germinoma (1 patient); traumatic brain injury (2 patients); **the following tests were used to diagnose GH deficiency: glucagon stimulation (4 patients); insulin tolerance (1 patient); GHRH-arginine (8 patients); low serum IGF-I and 3 or more additional pituitary deficiencies (3 patients); ***values represent median (25th percentile, 75th percentile).

BMI, body mass index; CTX, C-terminal peptide of type 1 collagen; GH, growth hormone; IGF-I, insulin-like growth factor I; NA, not applicable; NS, not significant; PTH, parathyroid hormone; SHBG, sex hormone-binding globulin; T4, thyroxine.

was significantly higher ($P=0.0072$) in patients with acromegaly (0.26 ± 0.02) in comparison with patients with GHD (0.19 ± 0.01) and controls (0.21 ± 0.01). In contrast, trabecular bone density and trabecular thickness were, respectively, 21.4% and 16.5% lower in patients with acromegaly in comparison with controls. There were no significant differences between patients with GHD and controls. There were no differences between patients with GHD who had previously received GH replacement and those who had not (data not shown).

Representative images of the left (non-dominant) radius of 3 study subjects (1 from each group) are shown in Fig. 2. The compressive stiffness and failure load were not significantly different between the groups. These findings persisted in additional analyses after either excluding men with hypogonadism or after statistical adjustments were made for serum testosterone levels or BMI (Table 3). Sensitivity analyses that excluded 1 patient with diabetes mellitus did not influence these findings (data not shown).

Table 2 Areal bone mineral density and fat free mass of the study population. Data are presented as mean $\pm$ S.E.M.

Variable	Acromegaly* (n=16)	GH deficiency (n=16)	Control* (n=16)	Overall P value†	Pairwise P value		
					(Acromegaly vs control)†	(GH deficiency vs control)	(Acromegaly vs GH deficiency)
Lumbar spine aBMD (g/cm ²)	1.081 $\pm$ 0.049	1.098 $\pm$ 0.028	1.088 $\pm$ 0.036	NS	NA	NA	NA
Lumbar spine aBMD Z score	0.1 $\pm$ 0.4	0.4 $\pm$ 0.3	0.1 $\pm$ 0.3	NS	NA	NA	NA
Total hip aBMD (g/cm ²)	1.093 $\pm$ 0.042	1.090 $\pm$ 0.028	1.064 $\pm$ 0.030	NS	NA	NA	NA
Total hip aBMD Z score	0.6 $\pm$ 0.3	0.7 $\pm$ 0.2	0.4 $\pm$ 0.2	NS	NA	NA	NA
Femoral neck aBMD (g/cm ²)	0.929 $\pm$ 0.053	0.874 $\pm$ 0.031	0.878 $\pm$ 0.033	NS	NA	NA	NA
Femoral neck aBMD Z score	0.5 $\pm$ 0.3	0.2 $\pm$ 0.2	0.1 $\pm$ 0.2	NS	NA	NA	NA
1/3 Radius aBMD (g/cm ²)	0.754 $\pm$ 0.050	0.788 $\pm$ 0.022	0.799 $\pm$ 0.016	NS	NA	NA	NA
1/3 Radius aBMD Z score	-0.2 $\pm$ 0.4	0.2 $\pm$ 0.3	0.1 $\pm$ 0.3	NS	NA	NA	NA
Total body aBMD (g/cm ²)	1.115 $\pm$ 0.032	1.162 $\pm$ 0.020	1.163 $\pm$ 0.027	NS	NA	NA	NA
Total body BMC (g)	2821 $\pm$ 140	4086 $\pm$ 1271	2686 $\pm$ 79	NS	NA	NA	NA
Fat free (lean) mass (g)	74872 $\pm$ 2913	64504 $\pm$ 2234	59177 $\pm$ 1903	0.0001 (0.0001); 0.0004	<0.0001 (0.0001)	NS	0.0099

*aBMD, areal bone mineral density; NA, not applicable; NS, not significant.

Bone microarchitecture and μ FEA of the tibia

Data obtained by HR-pQCT of the tibia are shown in Table 4. Overall, there were fewer significant differences in bone microarchitecture between patients with acromegaly and controls at this skeletal site. Trabecular bone density was 17.1% lower in patients with acromegaly in comparison with controls. There were no significant differences between patients with GHD and controls. There were no differences between patients with GHD who had previously received GH replacement and those who had not (data not shown). The compressive stiffness and failure load were not significantly different between the groups. These findings persisted in additional analyses after either excluding men with hypogonadism or after statistical adjustments were made for serum testosterone levels or BMI (Table 4). Sensitivity analyses that excluded 1 patient with diabetes mellitus did not influence these findings (data not shown).

Associations between bone microarchitecture and hormone levels

On multivariate analysis, serum IGF-I level was a (positive) predictor of cortical area ($\beta=0.0355$, $P=0.0019$), cortical perimeter ($\beta=0.0107$, $P=0.0035$), cortical thickness ($\beta=0.0003$, $P=0.0229$), cortical pore volume ($\beta=0.0393$, $P=0.0001$), cortical porosity ($\beta=0.0035$, $P<0.0001$) at the radius, after adjusting for age, BMI and serum testosterone levels. Serum IGF-I level was a (negative) predictor of trabecular bone density ($\beta=-0.0405$, $P=0.0322$) at the radius, after adjusting for age, BMI and serum testosterone levels. Serum IGF-I level was a (negative) predictor of trabecular bone density ($\beta=-0.0391$, $P=0.0264$) at the tibia, after adjusting for age, BMI and serum testosterone levels.

Discussion

In the present study, we identified significant alterations in both cortical (increased cortical porosity and increased cortical thickness) and trabecular (decreased trabecular density and thickness) bone microarchitecture and presented novel data on HSA and microfinite element analysis of the radius of men with acromegaly studied in comparison with healthy controls. Our findings on cortical microarchitecture, HSA and estimated strength have not been previously reported.

Impairments in bone microarchitecture likely translated into impaired trabecular bone strength.

Figure 1

Box plots of data on cortical area (panel A), cortical thickness (panel B), cortical pore volume (panel C), cortical porosity (panel D) and trabecular bone density (panel E) of the non-dominant radius of men with acromegaly, growth hormone deficiency and healthy controls. Patients with acromegaly had higher cortical area, cortical thickness, cortical pore volume and cortical porosity, but lower trabecular bone density compared to the other two groups. Exclusion of the 2 outliers in panel C and panel D did not affect the statistical significance of the findings. Statistically significant differences ($P < 0.05$) are shown by asterisks (*). There were no differences between patients with growth hormone deficiency and healthy controls.

Our findings persisted after adjusting for BMI, consistent with the hypothesis that GH excess may influence trabecular bone microarchitecture. Of note, there were fewer differences in bone microarchitecture in the tibia than the radius. It is possible that weight bearing (in the tibia) may mitigate the deficits in bone microarchitecture identified in the radius of patients with acromegaly. In aggregate, these novel findings help elucidate the abnormalities in the structure and mechanical properties of bone in patients with acromegaly. Our observations also affirm the limitations of aBMD testing, a technique that does not assess cortical and trabecular compartments separately, in the evaluation of patients with GH excess.

Growth hormone and IGF-I increase both bone formation and resorption, as evidenced by the increased bone biomarkers in patients with acromegaly studied in the present report as well as in previous studies (1). However, the effects of GH excess are not uniform across skeletal compartments. Our findings indicate that GH excess is associated with an increase in cortical area and thickness but also an increase in cortical porosity, likely reflecting increased bone turnover. Indeed, the increase in cortical perimeter with comparable trabecular area suggests increased periosteal apposition in acromegaly.

On the other hand, the increase in cortical porosity also indicates increased bone resorption in cortical bone. In contrast, the lack of difference in trabecular number suggests that there is no increase in bone resorption in the trabecular compartment in acromegaly.

Despite the increase in cortical porosity, the estimated compressive stiffness and failure load were not significantly different between patients with acromegaly and healthy subjects, suggesting that the increase in cortical area and thickness may have compensated for the increase in cortical porosity and decreased trabecular bone density. However, it is conceivable that bones with a higher proportion of trabecular bone (such as vertebrae) are structurally deficient in patients with acromegaly as a consequence of decreased trabecular density and possibly decreased trabecular number. This would explain the increased risk for vertebral (but not appendicular) fractures reported in patients with acromegaly (2). More research is needed to fully elucidate this issue.

Previously published data have suggested that adults with acromegaly have impaired trabecular bone microarchitecture in the appendicular skeleton, but did not include detailed cortical analysis, HSA or microfinite element analysis (5). In another study, both cortical and

Table 3 Bone microarchitecture and estimated bone strength of the distal radius. Data are presented as mean $\pm$ S.E.M.

Variable	Acromegaly* (n = 16)	GH deficiency (n = 16)	Control* (n = 16)	Pairwise P value		
				(Acromegaly vs control)†	(GH deficiency vs control)	(Acromegaly vs GH deficiency)
Total area (mm ²)	383.5 $\pm$ 19.3	351.3 $\pm$ 13.6	350.3 $\pm$ 14.4	NS	NA	NA
Total density (mg HA/cm ³)	343.9 $\pm$ 17.2	337.4 $\pm$ 9.1	354.3 $\pm$ 14.9	NS	NA	NA
Cortical area (mm ²)	96.7 $\pm$ 7.5 (100.9 $\pm$ 8.4)	65.5 $\pm$ 2.4	71.2 $\pm$ 2.3 (71.2 $\pm$ 2.3)	<0.0001 (0.0009); 0.0006	0.0013 (0.0009)	NS <0.0001
Cortical density (mg HA/cm ³)	814.1 $\pm$ 11.1	842.2 $\pm$ 11.1	848.7 $\pm$ 9.1	0.0596	NA	NA
Cortical perimeter (mm)	87.7 $\pm$ 2.3 (87.4 $\pm$ 2.6)	81.2 $\pm$ 1.3	80.8 $\pm$ 1.8 (80.8 $\pm$ 1.8)	0.0194 (0.0410); 0.0141	0.0320 (0.0410)	0.0438 NS
Cortical thickness (mm)	1.101 $\pm$ 0.078 (1.144 $\pm$ 0.083)	0.832 $\pm$ 0.039	0.891 $\pm$ 0.042 (0.891 $\pm$ 0.042)	0.0038 (0.0078); 0.0231	0.0308 (0.0078)	NS 0.0039
Cortical pore volume (mm ³)	38.49 $\pm$ 5.99 (40.41 $\pm$ 7.32)	15.54 $\pm$ 1.84	15.27 $\pm$ 1.81 (15.27 $\pm$ 1.81)	<0.0001 (0.0008); <0.0001	0.0001 (0.0008)	NS 0.0001
Cortical porosity (%)	4.1 $\pm$ 0.4 (4.2 $\pm$ 0.4)	2.6 $\pm$ 0.3	2.4 $\pm$ 0.3 (2.4 $\pm$ 0.3)	0.0008 (0.0016); 0.0004	0.0015 (0.0016)	NS 0.0035
Trabecular area (mm ²)	279.8 $\pm$ 18.7	278.4 $\pm$ 12.7	272.8 $\pm$ 14.7	NS	NA	NA
Trabecular bone density (mg HA/cm ³)	163.9 $\pm$ 9.2 (176.2 $\pm$ 7.8)	200.8 $\pm$ 6.8	208.6 $\pm$ 8.9 (208.6 $\pm$ 8.9)	0.0010 (0.0138); 0.0034	0.0015 (0.0138)	NS 0.0083
Meta trabecular density (mg HA/cm ³)	232.1 $\pm$ 8.8 (243.8 $\pm$ 7.4)	255.3 $\pm$ 6.8	267.6 $\pm$ 8.3 (267.6 $\pm$ 8.3)	0.0107 (0.0472); 0.0226	0.0087 (0.0472)	NS
Inner trabecular density (mg HA/cm ³)	116.8 $\pm$ 9.8 (129.5 $\pm$ 8.7)	163.2 $\pm$ 7.2	167.9 $\pm$ 9.6 (167.9 $\pm$ 9.6)	0.0003 (0.0079); 0.0012	0.0007 (0.0079)	NS 0.0017
Trabecular thickness (mm)	0.071 $\pm$ 0.002 (0.072 $\pm$ 0.002)	0.079 $\pm$ 0.003	0.085 $\pm$ 0.003 (0.085 $\pm$ 0.003)	0.0049 (0.0067); 0.0029	0.0036 (0.0067)	NS 0.0931
Trabecular number (/mm)	1.93 $\pm$ 0.09	2.11 $\pm$ 0.05	2.04 $\pm$ 0.06	NS	NA	NA
Trabecular separation (mm)	0.46 $\pm$ 0.03	0.39 $\pm$ 0.01	0.41 $\pm$ 0.01	0.0311 (NS); NS	NS	NS 0.0338
Inhomogeneity of trabecular network (mm)	0.23 $\pm$ 0.02 (0.19 $\pm$ 0.02)	0.16 $\pm$ 0.01	0.17 $\pm$ 0.01 (0.17 $\pm$ 0.01)	0.0042 (NS); 0.0283	0.0199 (NS)	NS 0.0058
Compressive stiffness (N/mm)	123674 $\pm$ 8979	104628 $\pm$ 4219	109056 $\pm$ 3374	0.0684	NA	NA
Failure load (N)	6193 $\pm$ 440	5284 $\pm$ 208	5487 $\pm$ 158	0.0755	NA	NA

*Data and P values shown within parentheses represent estimates after excluding subjects with hypogonadism. When several P values are listed in the same cell, these represent data from unadjusted analyses, followed by analyses excluding patients with hypogonadism (within parentheses), followed by analyses adjusting for serum testosterone levels. Additional analyses adjusting for BMI did not change the statistical significance of these findings (not shown).

HA, hydroxyapatite; NA, not applicable; NS, not significant.

Figure 2

Representative images depicting bone microarchitecture of the left (non-dominant) radius of a patient with acromegaly (A, A'), growth hormone deficiency (B, B') and a healthy subject (C, C'). Top panels (A, B, C) show cortical and trabecular microarchitecture, including thickened cortex and deficient trabecular bone in the patient with acromegaly. Bottom panels (A', B', C') highlight cortical porosity, which is increased in the patient with acromegaly. Scale bars (5 mm) are shown.

trabecular volumetric BMD were reported to be decreased in the proximal femur of patients with acromegaly (19). Our findings are broadly consistent with these observations on trabecular microarchitecture, but also provide important novel insights into cortical microarchitecture and the mechanical properties of bone in patients with active acromegaly. Maffezzoni *et al.* recently reported on bone microarchitecture in patients with acromegaly. In their study, cortical abnormalities were not found in the entire study population in comparison with controls, but were reported in patients with acromegaly and vertebral fractures studied in comparison with those who did not have fractures (20). Malgo *et al.* identified abnormalities in impact microindentation in patients with acromegaly, suggesting deficient cortical bone properties in this population (21).

Of note, serum IGF-I levels have been associated with cortical area in obese men without GH excess (22). In our study, the association between serum IGF-I level and cortical area persisted after adjusting for BMI, suggesting an independent role for GH influencing cortical bone microarchitecture in patients with acromegaly.

In addition to the presence of hypogonadism in a subgroup of patients with acromegaly, serum calcium levels were higher (albeit within the normal range) and serum SHBG levels were lower in acromegaly compared to controls in agreement with previously published observations (23, 24). The alterations of bone microarchitecture and estimated strength in patients with acromegaly reported in the present study remained robust after excluding patients with hypogonadism or after adjusting for serum testosterone levels, indicating that these deficiencies are not a consequence of testosterone

deficiency. Hypogonadism has been associated with decreased aBMD and increased fracture risk in patients with acromegaly (2, 25).

Another strength of the present study involves the assessment of individuals with active GH excess (rather than controlled acromegaly) at the time of evaluation. Furthermore, gender-specific comparisons were made between patients with abnormal GH secretion and healthy controls in the present investigation. This aspect of study design helped us avoid potential bias, which could have been introduced if patients of both genders were analyzed together. This is of importance, since there are well-documented differences in bone microarchitecture between women and men (26, 27, 28).

Of note, GHD was not associated with significant abnormalities of bone microarchitecture or strength in the present study, which cannot be explained on the basis of testosterone levels, since these were not different between patients with GHD and controls. It is possible that differences in bone microarchitecture or strength may still exist between men with GHD and healthy individuals, but are of smaller magnitude than those between patients with acromegaly and healthy subjects, and thus could not be detected in a study of our sample size. It is also possible that any abnormalities in bone microarchitecture or strength in men with GHD were blunted by the effects of previous GH replacement, which had been administered in a subgroup of our patient population (even though there were no statistically significant differences between patients with GHD who had previously received GH replacement and those who had not). Published data suggest that aBMD may continue to increase for 18 months after the cessation of GH

Table 4 Bone microarchitecture and estimated bone strength of the tibia. Data are presented as mean $\pm$ S.E.M.

Variable	Acromegaly* (n=16)	GH deficiency (n=16)	Control* (n=16)	Overall P value†	Pairwise P value		
					(acromegaly vs control)†	(GH deficiency vs control)	(acromegaly vs GH deficiency)
Total area (mm ²)	880.8 $\pm$ 39.2	920.9 $\pm$ 31.8	836.6 $\pm$ 29.8	NS	NA	NA	NA
Total density (mg HA/cm ³)	313.9 $\pm$ 11.1	313.9 $\pm$ 8.8	348.7 $\pm$ 17.2	0.0995	NA	NA	NA
Cortical area (mm ²)	176.9 $\pm$ 8.3	148.2 $\pm$ 7.9	167.5 $\pm$ 9.1	0.0598	NA	NA	NA
Cortical density (mg HA/cm ³)	832.3 $\pm$ 7.7	824.6 $\pm$ 13.2	852.0 $\pm$ 12.0	NS	NA	NA	NA
Cortical perimeter (mm)	117.4 $\pm$ 2.5	120.1 $\pm$ 2.2	114.1 $\pm$ 2.0	NS	NA	NA	NA
Cortical thickness (mm)	1.511 $\pm$ 0.069 (1.534 $\pm$ 0.075)	1.244 $\pm$ 0.072	1.492 $\pm$ 0.103 (1.492 $\pm$ 0.103)	0.0498 (NS); 0.0651	NS	0.0991	0.0706
Cortical pore volume (mm ³)	95.56 $\pm$ 9.61	91.62 $\pm$ 10.70	76.24 $\pm$ 10.22	NS	NA	NA	NA
Cortical porosity (%)	6.4 $\pm$ 0.5	7.1 $\pm$ 0.8	5.8 $\pm$ 0.7	NS	NA	NA	NA
Trabecular area (mm ²)	699.8 $\pm$ 37.9	767.1 $\pm$ 36.9	665.5 $\pm$ 35.8	NS	NA	NA	NA
Trabecular bone density (mg HA/cm ³)	175.5 $\pm$ 7.8 (184.3 $\pm$ 6.8)	204.6 $\pm$ 7.8	211.6 $\pm$ 9.1 (211.6 $\pm$ 9.1)	0.0082 (0.0289); 0.0304	0.0096 (0.0289)	NS	0.0427
Meta trabecular density (mg HA/cm ³)	245.4 $\pm$ 7.4 (254.3 $\pm$ 5.7)	265.7 $\pm$ 8.7	279.1 $\pm$ 9.1 (279.1 $\pm$ 9.1)	0.0239 (0.0371); 0.0928	0.0186 (0.0371)	NS	NS
Inner trabecular density (mg HA/cm ³)	128.0 $\pm$ 8.4 (136.7 $\pm$ 7.9)	163.3 $\pm$ 7.8	165.8 $\pm$ 9.4 (165.8 $\pm$ 9.4)	0.0047 (0.0300); 0.0121	0.0088 (0.0300)	NS	0.0152
Trabecular thickness (mm)	0.074 $\pm$ 0.002	0.077 $\pm$ 0.002	0.082 $\pm$ 0.003	NS	NA	NA	NA
Trabecular number (/mm)	1.99 $\pm$ 0.08	2.23 $\pm$ 0.08	2.17 $\pm$ 0.07	0.0994	NA	NA	NA
Trabecular separation (mm)	0.44 $\pm$ 0.02	0.38 $\pm$ 0.02	0.39 $\pm$ 0.01	0.0363 (NS); NS	0.0790	NS	0.0526
Inhomogeneity of trabecular network (mm)	0.20 $\pm$ 0.01	0.16 $\pm$ 0.01	0.16 $\pm$ 0.01	0.0235 (NS); 0.0759	0.0546	NS	0.0369
Compressive stiffness (N/mm)	280353 $\pm$ 13267	276296 $\pm$ 10421	282446 $\pm$ 10248	NS	NA	NA	NA
Failure load (N)	14091 $\pm$ 665	13937 $\pm$ 538	14083 $\pm$ 458	NS	NA	NA	NA

*Data and P values shown within parentheses represent estimates after excluding subjects with hypogonadism. When several P values are listed in the same cell, these represent data from unadjusted analyses, followed by analyses excluding patients with hypogonadism (within parentheses), followed by analyses adjusting for serum testosterone levels. Additional analyses adjusting for BMI did not change the statistical significance of these findings (not shown).

HA, hydroxyapatite; NA, not applicable; NS, not significant.

replacement in adults with GHD (29). However, there are no published data on the effects of GH replacement on bone microarchitecture in adults with hypopituitarism.

Limitations of the present study include its cross-sectional nature, which does not allow us to infer causality. In addition, our study did not assess bone microarchitecture in the central skeleton, including the spine. It should be noted that the μ FEA data are limited to bone compression. In addition, these analyses assume no abnormality in the degree of bone mineralization. Data on morphometric vertebral fractures are not available in the present study, as vertebral imaging was not obtained. By design, our study enrolled only men. Further studies are needed to examine the effects of GH excess or deficiency on bone microarchitecture and estimated strength in women. Studies of larger size will be needed in order to further investigate possible abnormalities of bone microarchitecture in GHD. Our study was not designed to distinguish between effects on bone microarchitecture mediated by GH itself vs those mediated by IGF-I in patients with acromegaly.

Additional studies are also needed in order to characterize bone microarchitecture in patients with monogenic GHD due to genetic etiologies or compare bone microarchitecture in patients with GHD and anorexia, which is characterized by GH resistance.

In conclusion, our findings indicate that acromegaly is associated with alterations in both cortical and trabecular bone microarchitecture without changes in estimated bone strength in the radius or tibia of men. In this population, the microarchitecture of trabecular bone is impaired, which may influence these patients' fracture risk. Studies in women as well as longitudinal studies in patients of both genders are needed in order to further elucidate the relationship between bone microarchitecture or estimated strength and fracture risk in patients with GH excess.

Declaration of interest

A K has consulted for Chiasma and is a member of the scientific advisory board of Crinetics; B M K B is the PI of institution-directed research grants from Novartis, Novo Nordisk, OPKO and Versartis and has received occasional consulting honoraria from Ipsen, Novartis, Novo Nordisk, OPKO, Pfizer and Versartis; E L is a member of the scientific advisory board and has a financial interest in OXT Therapeutics, Inc; N A T is the PI of institution-directed research support from Novartis, Novo Nordisk and Pfizer. The rest of the authors have no disclosures.

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References

- Giustina A, Mazziotti G & Canalis E. Growth hormone, insulin-like growth factors, and the skeleton. *Endocrine Reviews* 2008 **29** 535–559. (doi:10.1210/er.2007-0036)
- Mazziotti G, Biagioli E, Maffezzoni F, Spinello M, Serra V, Maroldi R, Floriani I & Giustina A. Bone turnover, bone mineral density, and fracture risk in acromegaly: a meta-analysis. *Journal of Clinical Endocrinology and Metabolism* 2015 **100** 384–394. (doi:10.1210/jc.2014-2937)
- Mazziotti G, Bianchi A, Bonadonna S, Cimino V, Patelli I, Fusco A, Pontecorvi A, De Marinis L & Giustina A. Prevalence of vertebral fractures in men with acromegaly. *Journal of Clinical Endocrinology and Metabolism* 2008 **93** 4649–4655. (doi:10.1210/jc.2008-0791)
- Mazziotti G, Bianchi A, Porcelli T, Mormando M, Maffezzoni F, Cristiano A, Giampietro A, De Marinis L & Giustina A. Vertebral fractures in patients with acromegaly: a 3-year prospective study. *Journal of Clinical Endocrinology and Metabolism* 2013 **98** 3402–3410. (doi:10.1210/jc.2013-1460)
- Madeira M, Neto LV, de Paula Paranhos Neto F, Barbosa Lima IC, Carvalho de Mendonca LM, Gadelha MR & Fleiuss de Farias ML. Acromegaly has a negative influence on trabecular bone, but not on cortical bone, as assessed by high-resolution peripheral quantitative computed tomography. *Journal of Clinical Endocrinology and Metabolism* 2013 **98** 1734–1741. (doi:10.1210/jc.2012-4073)
- Mazziotti G, Bianchi A, Bonadonna S, Nuzzo M, Cimino V, Fusco A, De Marinis L & Giustina A. Increased prevalence of radiological spinal deformities in adult patients with GH deficiency: influence of GH replacement therapy. *Journal of Bone and Mineral Research* 2006 **21** 520–528. (doi:10.1359/jbmr.060112)
- Mazziotti G, Doga M, Frara S, Maffezzoni F, Porcelli T, Cerri L, Maroldi R & Giustina A. Incidence of morphometric vertebral fractures in adult patients with growth hormone deficiency. *Endocrine* 2016 **52** 103–110. (doi:10.1007/s12020-015-0738-z)
- Holmes SJ, Economou G, Whitehouse RW, Adams JE & Shalet SM. Reduced bone mineral density in patients with adult onset growth hormone deficiency. *Journal of Clinical Endocrinology and Metabolism* 1994 **78** 669–674. (doi:10.1210/jcem.78.3.8126)
- Hitz MF, Jensen JE & Eskildsen PC. Bone mineral density in patients with growth hormone deficiency: does a gender difference exist? *Clinical Endocrinology* 2006 **65** 783–791. (doi:10.1111/j.1365-2265.2006.02667.x)
- Barake M, Klibanski A & Tritos NA. Effects of recombinant human growth hormone therapy on bone mineral density in adults with growth hormone deficiency: a meta-analysis. *Journal of Clinical Endocrinology and Metabolism* 2014 **99** 852–860. (doi:10.1210/jc.2013-3921)
- Ho KK. Consensus guidelines for the diagnosis and treatment of adults with GH deficiency II: a statement of the GH Research Society in association with the European Society for Pediatric Endocrinology, Lawson Wilkins Society, European Society of Endocrinology, Japan Endocrine Society, and Endocrine Society of Australia. *European Journal of Endocrinology* 2007 **157** 695–700. (doi:10.1530/EJE-07-0631)
- Paffenbarger RS Jr, Wing AL & Hyde RT. Physical activity as an index of heart attack risk in college alumni. *American Journal of Epidemiology* 1978 **108** 161–175. (doi:10.1093/oxfordjournals.aje.a112608)

Clinical Study	P P B Silva, F G Amlashi and others	Bone microarchitecture in acromegaly	177:5	420
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- 13 Bystrom C, Sheng S, Zhang K, Caulfield M, Clarke NJ & Reitz R. Clinical utility of insulin-like growth factor 1 and 2; determination by high resolution mass spectrometry. *PLoS ONE* 2012 **7** e43457. (doi:10.1371/journal.pone.0043457)
- 14 Boutroy S, Buxsein ML, Munoz F & Delmas PD. In vivo assessment of trabecular bone microarchitecture by high-resolution peripheral quantitative computed tomography. *Journal of Clinical Endocrinology and Metabolism* 2005 **90** 6508–6515. (doi:10.1210/jc.2005-1258)
- 15 Boutroy S, Van Rietbergen B, Sornay-Rendu E, Munoz F, Buxsein ML & Delmas PD. Finite element analysis based on in vivo HR-pQCT images of the distal radius is associated with wrist fracture in postmenopausal women. *Journal of Bone and Mineral Research* 2008 **23** 392–399. (doi:10.1359/jbmr.071108)
- 16 Pistoia W, van Rietbergen B, Lochmuller EM, Lill CA, Eckstein F & Rueggsegger P. Estimation of distal radius failure load with micro-finite element analysis models based on three-dimensional peripheral quantitative computed tomography images. *Bone* 2002 **30** 842–848. (doi:10.1016/S8756-3282(02)00736-6)
- 17 Faje AT, Karim L, Taylor A, Lee H, Miller KK, Mendes N, Meenaghan E, Goldstein MA, Buxsein ML, Misra M *et al.* Adolescent girls with anorexia nervosa have impaired cortical and trabecular microarchitecture and lower estimated bone strength at the distal radius. *Journal of Clinical Endocrinology and Metabolism* 2013 **98** 1923–1929. (doi:10.1210/jc.2012-4153)
- 18 Marcus R, Peritz E & Gabriel KR. On closed testing procedures with special reference to ordered analysis of variance. *Biometrika* 1976 **63** 655–660. (doi:10.1093/biomet/63.3.655)
- 19 Valassi E, Crespo I, Malouf J, Llauger J, Aulinas A, Marin AM, Biagetti B & Webb SM. Reduction of trabecular and cortical volumetric bone mineral density at the proximal femur in patients with acromegaly. *European Journal of Endocrinology* 2016 **174** 107–114. (doi:10.1530/EJE-15-0931)
- 20 Maffezzoni F, Maddalo M, Frara S, Mezzone M, Zorza I, Baruffaldi F, Doglietto F, Mazziotti G, Maroldi R & Giustina A. High-resolution-cone beam tomography analysis of bone microarchitecture in patients with acromegaly and radiological vertebral fractures. *Endocrine* 2016 **54** 532–542. (doi:10.1007/s12020-016-1078-3)
- 21 Malgo F, Hamdy NA, Rabelink TJ, Kroon HM, Claessen KM, Pereira AM, Biermasz NR & Appelman-Dijkstra NM. Bone material strength index as measured by impact microindentation is altered in patients with acromegaly. *European Journal of Endocrinology* 2017 **176** 339–347. (doi:10.1530/EJE-16-0808)
- 22 Bredella MA, Lin E, Gerweck AV, Landa MG, Thomas BJ, Torriani M, Buxsein ML & Miller KK. Determinants of bone microarchitecture and mechanical properties in obese men. *Journal of Clinical Endocrinology and Metabolism* 2012 **97** 4115–4122. (doi:10.1210/jc.2012-2246)
- 23 Ajmal A, Haghshenas A, Attarian S, Barake M, Tritos NA, Klibanski A, Miller KK & Nachtigall LB. The effect of somatostatin analogs on vitamin D and calcium concentrations in patients with acromegaly. *Pituitary* 2014 **17** 366–373. (doi:10.1007/s11102-013-0514-0)
- 24 Kaltsas GA, Mukherjee JJ, Jenkins PJ, Satta MA, Islam N, Monson JP, Besser GM & Grossman AB. Menstrual irregularity in women with acromegaly. *Journal of Clinical Endocrinology and Metabolism* 1999 **84** 2731–2735. (doi:10.1210/jcem.84.8.5858)
- 25 Mazziotti G, Bianchi A, Cimino V, Bonadonna S, Martini P, Fusco A, De Marinis L & Giustina A. Effect of gonadal status on bone mineral density and radiological spinal deformities in adult patients with growth hormone deficiency. *Pituitary* 2008 **11** 55–61. (doi:10.1007/s11102-007-0069-z)
- 26 Khosla S, Riggs BL, Atkinson EJ, Oberg AL, McDaniel LJ, Holets M, Peterson JM & Melton LJ 3rd. Effects of sex and age on bone microstructure at the ultradistal radius: a population-based noninvasive in vivo assessment. *Journal of Bone and Mineral Research* 2006 **21** 124–131. (doi:10.1359/JBMR.050916)
- 27 Riggs BL, Melton LJ 3rd, Robb RA, Camp JJ, Atkinson EJ, Oberg AL, Rouleau PA, McCollough CH, Khosla S & Buxsein ML. Population-based analysis of the relationship of whole bone strength indices and fall-related loads to age- and sex-specific patterns of hip and wrist fractures. *Journal of Bone and Mineral Research* 2006 **21** 315–323. (doi:10.1359/JBMR.051022)
- 28 Sode M, Burghardt AJ, Kazakia GJ, Link TM & Majumdar S. Regional variations of gender-specific and age-related differences in trabecular bone structure of the distal radius and tibia. *Bone* 2010 **46** 1652–1660. (doi:10.1016/j.bone.2010.02.021)
- 29 Biller BM, Sesmilo G, Baum HB, Hayden D, Schoenfeld D & Klibanski A. Withdrawal of long-term physiological growth hormone (GH) administration: differential effects on bone density and body composition in men with adult-onset GH deficiency. *Journal of Clinical Endocrinology and Metabolism* 2000 **85** 970–976. (doi:10.1210/jcem.85.3.6474)

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