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New insights into the metabolic-bone crosstalk in active acromegaly

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Abstract

Introduction: Body composition (BC) and adipokines share bone active properties and display an altered profile in acromegaly. The fibroblast growth factor 23 (FGF23)/α-Klotho system, also involved in bone metabolism, is upregulated in growth hormone (GH) excess states. Hence, we aimed to investigate their impact on bone in active acromegaly, compared to controls.

Material and methods: BC, bone mineral density (BMD) (via dual X-ray absorptiometry), serum adipokines (leptin, adiponectin, resistin), parathyroid hormone (PTH), FGF23, α-Klotho, and osteocalcin were assessed in a cross-sectional study enrolling 35 patients with active acromegaly (Acro), compared to 35 sex, age, and body mass index (BMI) one-to-one matched healthy controls (CTL).

Results: The Acro group had higher bone density scores ($p < 0.05$), lower visceral fat depots ($p = 0.011$), and lower serum leptin ($p < 0.001$) but elevated adiponectin ($p < 0.001$) and resistin ($p = 0.001$) concentrations when compared to the CTL group. α-Klotho was not related to the GH/IGF1 axis in the Acro group. Resistin was higher in both diabetic and non-diabetic Acro compared to CTL ($p < 0.05$). Age and BC were the main independent BMD predictors in regression analysis in both groups, while IGF1 was a positive predictor of osteocalcin levels in the Acro ($\beta = 0.48$, $p = 0.006$). The correlations between adipokines, the FGF23/α-Klotho system, and bone parameters, respectively, were lost after adjusting for age and BC.

Conclusions: Age and BC were the main independent BMD predictors in the acromegalic patients with active disease, while IGF1 was independently associated with serum osteocalcin concentrations. The role of α-Klotho in evaluating acromegaly and the associated osteopathy in the long-term appears to be limited. Our study is among the first to report significant serum resistin changes in patients with active acromegaly, opening new insights in the GH-mediated insulin resistance. The GH-resistin relationship merits further investigations.
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Key words: *active acromegaly; body composition; adipokines; bone*

Introduction

Skeletal fragility is increased in acromegaly, regardless of the bone density [1, 2]. Growth hormone (GH) excess is associated with increased bone turnover, deterioration of bone microarchitecture, and increased risk of vertebral fractures [3]. Besides the classic acromegaly-related risk factors [gonadal status, the presence of diabetes mellitus (DM), vitamin D deficiency, or overtreatment [1]], additional alterations provoked by GH excess, such as body composition (BC) [4], adipokines [5], and fibroblast growth factor 23 (FGF23)/α-Klotho axis modifications [6], may also impact bone. The altered BC in acromegaly — increased lean mass (LM) and reduced fat mass (FM), but with ectopic intermuscular or trunk deposition — is GH mediated [4], directly or via chronic inflammation [7]. Leptin usually reflects the amount of adipose tissue, and blunted leptin levels are restored after successful surgery of acromegaly [8]. Both DM and cardiovascular disease (CVD) (frequent complications of acromegaly) are associated with low levels of adiponectin and higher resistinaemia — negatively correlated with BMD in the general population [7, 9]. Data approaching the BC-adipokines-BMD relationship in acromegaly are, however, scarce [10, 11].

The FGF23/α-Klotho system negatively regulates phosphate and vitamin D metabolism, having an overall detrimental impact upon bone metabolism [12]. α-Klotho was proposed as a marker for acromegaly activity because the higher levels encountered in active states dropped after treatment, but the determinant mechanism remains to be elucidated [13]. To our knowledge, the impact of FGF23/α-Klotho on bone has not yet been evaluated in the acromegalic population.

We aimed to evaluate the particularities of the FGF23/α-Klotho axis and adipokine spectrum in relation to BC, and their influence upon bone density and turnover, in active acromegaly.

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Material and methods

Study design

We performed a cross sectional, case-control study, enrolling 35 patients with active acromegaly (Acro; after selecting the 127 acromegalic patients who attended our Endocrinology Department for diagnostic or follow-up between April 2019 and February 2020, according to the criteria described below) and 35 age-, sex-, and body mass index (BMI)-matched, apparently healthy controls (CTL; general population volunteers referred by the general practitioner to our outpatient department for a health check-up in the same period of time, who met the criteria below and agreed to take part in the study). One-to-one individual matching was used to avoid gender, age, and BMI as confounders.

Patients with active acromegaly (regardless of the disease-related comorbidities or therapy type, gender, gonadal status, being at first evaluation or at follow-up), between 20 and 80 years old were included in the Acro group. According to the criteria of the current guidelines of the Endocrine Society [14] and according to our national protocol, respectively, active disease was defined as [1] insulin-like growth factor 1 (IGF1) levels above normal range for age and [2] failure of GH to suppress to less than 1 ng/mL during a 75 mg oral glucose tolerance test or 24 h GH mean value ≥ 2.5 ng/mL in Acro with DM.

Exclusion criteria in the Acro group were represented by pregnancy, bone active therapy (other than vitamin D supplements), confirmed or suspected secondary causes of osteoporosis other than acromegaly related — hypogonadism and DM (e.g. primary hyperparathyroidism, thyrotoxicosis, hypercortisolism, major bone trauma, chronic kidney disease, inflammatory bowel disease, congenital bone disease, anorexia nervosa, malignancy, sarcoidosis, antioestrogen/antiandrogen therapy), and suspected or confirmed multiple endocrine neoplasia. In order to minimize the additional factors affecting bone metabolism, DM patients treated with thiazolidinediones or insulin were also excluded.

All-cause secondary osteoporosis, the use of bone active drugs other than vitamin D supplements, the presence of DM, CVD, and pregnancy were also exclusion criteria in the CTL group.

Medical history was recorded, and physical examination (including anthropometric measurements) was performed for both groups. A fasting morning blood draw of 20 mL for serum determinations (serum aliquots were stored at –80°C until analysis) and dual X-ray absorptiometry (DXA) for BMD and BC assessment were performed, respectively.

Hypogonadism was defined as [1] menopause — more than 12 months since natural cessation of menstrual cycles or [2] gonadotropin deficiency due to the evolution of the disease.

The study adhered to the Declaration of Helsinki. The institutional ethics committee approved the protocol (20.03.2019), and all patients gave written informed consent before entering the study.

Measurements

Body mass index was calculated as weight (kg)/[height (m)]^2. Serum levels of leptin, adiponectin, resistin, FGF23, α-Klotho, IGF1, GH, and osteocalcin were quantified using commercially available ELISA Research kits (Elabscience Biotechnology, USA). Serum parathyroid hormone (PTH) was quantified by electrochemiluminescence (ECLIA), using commercial kits (Advia Centaur Intact PTH Assay, Siemens Healthcare Diagnostics Inc., USA). Free T4, cortisol, oestradiol (in women), testosterone (in men), follicle-stimulating hormone (FSH), and luteinizing hormone (LH) were routinely measured in the Acro group via ECLIA (Immulite 2000 Immunoassay System, Siemens). Serum concentrations of calcium, phosphate, and glucose were determined by colorimetry (Cobas 6000 analyser, Roche). HbA1c was assessed via the ion-exchange high-performance liquid chromatography (HPLC) method.

BMD at the lumbar spine (the mean BMD value for L1-L4 lumbar vertebrae), femoral neck, 1/3 radius, and whole-body levels were measured via DXA (Hologic Delphi A; Hologic Inc., USA) by 2 homologated technicians, according to standard protocol. Low bone mass was defined as T-score < –1 SD at the level of the lumbar spine or hip for postmenopausal women and men above 50 years of age, and Z-score < –2 SD for premenopausal women and men under 50 years of age [15].

BC parameters (total FM, trunk FM, lower limbs FM, total LM) were also determined by whole-body DXA scan. Trunk-to-leg fat ratio was calculated as trunk FM (g)/lower limbs FM (g).

Statistical analysis

Statistical analysis was performed using SPSS software (SPSS version 20.0 for Windows, IBM SPSS Inc.). Data are expressed as mean ± standard error of the mean (SEM). Normal distribution of data was verified using the Shapiro-Wilk test. Between-group and subgroup differences were assessed by [1] Student's paired t-test (for normally distributed data) or the non-parametric Mann-Whitney U test (for skewed data) for 2 variables and [2] ANOVA for 3 or more variables. Pearson correlation (for normally distributed data) or Spearman rank correlation (for skewed data) was used to evaluate any given relationships between the assessed parameters. Significant correlations were further introduced in multiple regression analysis to determine independent predictors of bone parameters in the Acro and CTL groups, respectively. The level of significance was established according to a p-value < 0.05.

Results

The general characteristics of the 35 patients with active acromegaly (10 newly diagnosed and 25 long-standing Acro with a mean time elapsed from initial diagnosis of 132.77 ± 25.1 months) are presented in Table 1. At the time of observation, 25 were hypogonadal, 18 were diabetic, and 22 had evidence of CVD (arterial hypertension, cardiac hypertrophy, or heart failure). All had normal functioning adrenal axis (mean serum cortisol = 10.26 ± 0.74 μg/dL; 2 patients were under glucocorticoid substitutive treatment), normal free T4 levels (mean serum free T4 = 1.11 ± 0.03 ng/dL; 13 patients were under levothyroxine replacement therapy), normal serum calcium (9.7 ± 0.08 mg/dL), and phosphate (4.05 ± 0.1) and were rather obese (Tab. 1). Seventeen Acro group members had low bone mass (all of them also associated hypogonadism; 2 had prior history of fragility fractures), while 18 exhibited DXA values in the normal range. Five patients were under vitamin D3 supplementation. Osteocalcin levels were increased (Tab. 1; reference range: 9–42 ng/mL).

Acro had higher lumbar spine and femoral neck Z-scores, but a lower trunk-to-leg fat ratio compared to CTL, respectively (Tab. 1). Serum adipokines, but not FGF23 and PTH, also varied significantly between the 2 groups (Tab. 1).

Correlations

Acro

Significant correlations in the Acro group between age, BC, serum parameters and bone density and osteocalcin, respectively, are depicted in Table 2. IGF1, BMI, LM, and trunk-to-leg fat ratio were all positively correlated

Table 1. *General characteristics of the study participants*

Parameter	Acro (n = 35)	CTL (n = 35)	p-value
Age [y]	55.2 ± 2.33	54.14 ± 2.52	0.76
Men:Women	12:23	12:23	–
Menopause (n/total women)	18/23	18/23	–
Newly diagnosed/long-standing disease	10/25	–	
Active disease time [mo]*	90.32 ± 20.44	–	–
BMI [kg/m^2]	31.63 ± 0.98	31.44 ± 0.89	0.88
Total FM [kg]	31.17 ± 2.01	32.05 ± 1.76	0.74
FM (%)	34.94 ± 1.4	36.04 ± 1.26	0.56
Total LM [kg]	54.61 ± 2.26	54.12 ± 2.19	0.88
LM (%)	62.46 ± 1.33	61.43 ± 1.2	0.57
Trunk-to-leg fat ratio	1.22 ± 0.04	1.42 ± 0.06	0.011
Lumbar BMD [g/cm^2]	1.022 ± 0.033	0.923 ± 0.03	0.022
Lumbar Z-score	0.66 ± 0.22	-0.19 ± 0.15	0.002
Femoral neck BMD [g/cm^2]	0.836 ± 0.042	0.814 ± 0.026	0.66
Femoral neck Z-score	0.98 ± 0.16	0.47 ± 0.16	0.03
Total hip BMD [g/cm^2]	0.991 ± 0.029	0.952 ± 0.028	0.33
Total hip Z-score	0.85 ± 0.18	0.55 ± 0.15	0.2
1/3 radius BMD [g/cm^2]	0.669 ± 0.021	0.688 ± 0.019	0.51
1/3 radius Z-score	0.31 ± 0.17	0.41 ± 0.15	0.66
Whole-body BMD [g/cm^2]	1.089 ± 0.021	1.061 ± 0.025	0.39
IGF-1 [ng/mL] (mean for age)	437.54 ± 30.19 (234.46 ± 7.27)	–	–
Basal GH [ng/mL]	7.45 ± 1.67	–	–
Mean GH/24 h [ng/mL] (n = 18)	5.06 ± 1.59	–	–
GH at glucose suppression test [ng/mL] (n = 17)	10.18 ± 2.89	–	–
Glycaemia [mg/dL]	113.88 ± 6.28	84.15 ± 2.2	< 0.001
Osteocalcin [ng/mL]	58.46 ± 9.73	–	–
Leptin [ng/mL]	14.03 ± 1.99	51.23 ± 0.89	< 0.001
Adiponectin [ug/mL]	35.98 ± 1.51	14.18 ± 4.03	< 0.001
Resistin [ng/mL]	18.91 ± 3.18	6.59 ± 0.66	0.001
PTH [pg/mL]	42.46 ± 4.57	48.26 ± 3.6	0.32
FGF23 [pg/mL]	81.75 ± 10.66	75.51 ± 3.12	0.58
Klotho [ng/mL]	9.15 ± 1.74	–	–
Hypogonadism (n/total)	25/35	18/35	–
Low/normal bone mass	17/18	20/15	–
Fractures (n/total)	2/35	0/35	–
Diabetes mellitus (n/total)	18/35	0/35	–
HbA_{1c} in DM patients (%)	7.04 ± 0.26	–	–
CVD (n/total)	22/35	–	–
Surgery (n)	14	–	–
SST analogues (n)	18	–	–
Pegvisomant (n)	7	–	–
Radiotherapy (n)	10	–	–
Dopamine agonists (n)	14	–	–

*in long-standing patients; Data are expressed as mean ± standard error of the mean; ADPN — adiponectin; BMD — bone mineral density; BMI — body mass index; CTL — control group; CVD — cardiovascular disease; DM — diabetes mellitus; FGF23 — fibroblast growth factor 23; FM — fat mass; FN — femoral neck; GH — growth hormone; HbA_{1c} — glycated haemoglobin; IGF-1 — insulin-like growth factor-1; LM — lean mass; mo — months; PTH — parathormone; SST — somatostatin; WB — whole-body; y — years

Table 2. *Correlations between age, body composition, serum parameters, bone density, and osteocalcin, respectively, in the Acro group (only significant correlations are shown)*

	Lumbar BMD	FN BMD	Total Hip BMD	1/3 radius BMD	WB BMD	OCN	Leptin	ADPN	Resistin
Age	–0.45 p = 0.08	–	–0.63 p < 0.001	–0.61 p < 0.001	–0.72 p < 0.001	–	–	0.46 p = 0.011	0.42 p = 0.022
BMI	–	–	0.36 p = 0.041	–	–	–	0.76 p < 0.001	–	–
Total FM	–	–	–	–	–	–	0.79 p < 0.001	–	–
Total LM	–	0.521 p = 0.003	0.55 p = 0.001	0.65 p < 0.001	0.68 p < 0.001	–	–	–0.55 p = 0.003	–
Trunk–to–leg fat ratio	–	–	0.4 p = 0.027	0.48 p = 0.006	0.51 p = 0.004	–	–	–	–
Leptin	–	–	–	–	–	–	1	–	–
Resistin	–0.46 p = 0.015	–	–	–	–	–	–	–	1
ADPN	–	–	–0.54 p = 0.003	–	–	–	–	1	–
FGF23	–	–	–	–	–	–	–	–	–
Klotho	–	–	–	–	–	0.52 p = 0.003	–	–	–
PTH	–	–0.54 p = 0.002	–0.55 p = 0.03	–	–	–	–	0.42 p = 0.031	–
IGF1	0.35 p = 0.048	–	0.39 p = 0.024	–	–	0.51 0.004	–	–	–
Basal GH	–	–	–	–	–	–	–0.4 p = 0.031	–	–
Glycaemia	–	–	–	–	–	–	–	–	0.5 p = 0.007

ADPN — adiponectin; BMD — bone mineral density; BMI — body mass index; FGF23 — fibroblast growth factor 23; FM — fat mass; FN — femoral neck; GH — growth hormone; IGF-1 — insulin like growth factor-1; LM — lean mass; OCN — osteocalcin; PTH — parathormone, WB — whole-body

with BMD, while age, PTH, adiponectin, and resistin were negatively correlated with bone density at various sites. IGF1 and α-Klotho were positively correlated with osteocalcin (Tab. 2).

Basal GH was inversely correlated with leptin (r = –0.4, p = 0.031) and FM (r = –0.58, p = 0.001). IGF1 was not correlated with BC parameters or with BMI (data not shown). Likewise, we did not find any significant correlations between basal GH, IGF1, and the FGF23/α-Klotho axis in the whole group, nor when analysed separately (newly diagnosed and long-standing Acro)(data not shown).

CTL

Similarly to the Acro group, BMI, LM, and trunk-to-leg fat ratio were positively correlated with BMD, while age was negatively related to bone density in the CTL group. FGF23 was also negatively correlated with BMD in the CTL group. Contrary to the Acro patients, no significant correlations were found between adipokines and bone parameters or between PTH and BMD, respectively (Table 3).

Multiple regression analysis

Significant correlations were further introduced in unadjusted (Model 1) and gender-adjusted (Model 2) multiple regression analysis, with BMD at various sites and osteocalcin as the dependent variables, respectively (Table 4 — only significant predictors are shown).

BC (LM and trunk-to-leg fat ratio — positive) and age (negative) were the main independent BMD predictors in both groups in unadjusted models (Model 1, Tab. 4); PTH was also negatively associated with femoral neck BMD in the Acro group. The influence of LM was lost in CTL, while the independent BMD predictors remained essentially unchanged in the Acro group after adjusting for gender (Model 2, Tab. 4).

Multiple regression analysis was performed to assess any independent effects of basal GH and FM upon serum leptin concentrations, respectively. FM, but not

Table 3. *Correlations between age, body composition, serum parameters, and bone density, respectively, in the CTL group (only significant correlations are shown)*

	Lumbar BMD	FN BMD	Total Hip BMD	1/3 radius BMD	WB BMD	Leptin	ADPN	Resistin
Age	–0.7 $p < 0.001$	–0.57 $p < 0.001$	–0.51 $p < 0.001$	–0.71 $p < 0.001$	–0.7 $p < 0.001$	–	–	–
BMI	–	–	0.37 $p = 0.03$	–	–	0.48 $p = 0.018$	–	–
Total FM	–	–	–	–	–	0.58 $p = 0.003$	–	–
Total LM	0.58 $p < 0.001$	0.7 $p < 0.001$	0.76 $p < 0.001$	0.8 $p < 0.001$	–	–	–0.41 $p = 0.048$	–
Trunk-to-leg fat ratio	–	–	0.46 $p = 0.006$	0.42 $p = 0.012$	0.42 $p = 0.012$	–	–	–
Leptin	–	–	–	–	–	1	–	–
Resistin	–	–	–	–	–	–	0.48 $p = 0.018$	1
ADPN						–	1	
FGF23	–0.44 $p = 0.008$	–0.41 $p = 0.014$	–0.41 $p = 0.014$	–0.38 $p = 0.025$	–	–	–	–
PTH	–	–	–	–	–	–	–	–

ADPN — adiponectin; BMD — bone mineral density; BMI — body mass index; CTL — control group; FGF23 — fibroblast growth factor 23; FM — fat mass; FN — femoral neck; LM — lean mass; PTH — parathormone; WB — whole-body

Table 4. *Multiple regression analysis in the Acro and CTL groups, respectively*

		Dependent variable	Acro				CTL			
			Significance	Predictor	Beta	p-value	Significance	Predictor	Beta	p-value
Model 1	1	Lumbar BMD	–	–	–	–	$R^2 = 0.56$ $p < 0.001$	Age	–0.48	0.004
	2	FN BMD	$R^2 = 0.514$ $p < 0.001$	Total LM PTH	0.46 –0.56	0.005 0.001	$R^2 = 0.59$ $p < 0.001$	LM	0.53	0.001
	3	Total hip BMD	–	–	–	–	$R = 0.63$ $p < 0.001$	LM	0.53	0.008
	4	1/3 radius BMD	$R^2 = 0.545$ $p < 0.001$	Age	–0.34	0.042	$R^2 = 0.76$ $p < 0.001$	Age LM	–0.38 0.48	0.003 0.002
	5	WB BMD	$R^2 = 0.668$ $p < 0.001$	Age Trunk-to-leg fat ratio	–0.5 0.28	0.002 0.036	$R^2 = 0.65$ $p < 0.001$	Age Trunk-to-leg fat ratio	–0.69 0.38	< 0.001 0.001
	6	Osteocalcin	$R^2 = 0.37$ $p = 0.002$	IGF1	0.48	0.005	NA	NA	NA	NA
Model 2	1	Lumbar BMD	–	–	–	–	$R^2 = 0.58$ $p < 0.001$	Age	–0.5	0.003
	2	FN BMD	$R^2 = 0.52$ $p = 0.001$	Total LM PTH	0.52 –0.55	0.012 0.001	–	–	–	–
	3	Total hip BMD	$R^2 = 0.82$ $p = 0.001$	PTH	–0.36	0.023	–	–	–	–
	4	1/3 radius BMD	$R^2 = 0.65$ $p < 0.001$	Age	–0.36	0.019	$R^2 = 0.82$ $p < 0.001$	Age	–0.4	0.001
	5	WB BMD	$R^2 = 0.67$ $p < 0.001$	Age Trunk-to-leg fat ratio	–0.51 0.28	0.002 0.04	$R^2 = 0.7$ $p < 0.001$	Age Trunk-to-leg fat ratio	–0.54 0.25	< 0.001 0.035
	6	Osteocalcin	$R^2 = 0.37$ $p = 0.007$	IGF1	0.48	0.006	NA	NA	NA	NA

Model 2 = Model 1 adjusted for gender; Acro — acromegaly group; BMD — bone mineral density; CTL — control group; FN — femoral neck; LM — lean mass; NA — not assessed; PTH — parathormone; WB — whole-body

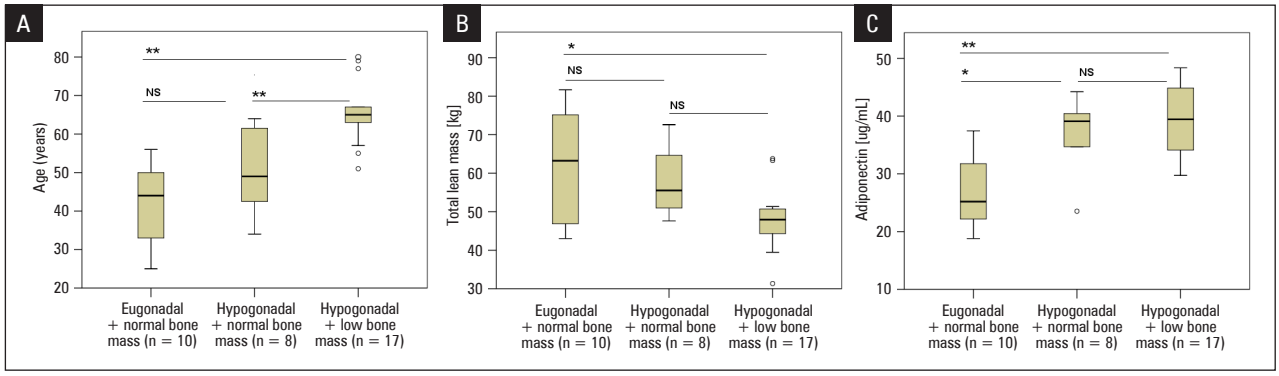

Figure 1. *Comparison of age (A), total lean mass (B), and serum adiponectin levels (C) between eugonadal Acro patients with normal bone mass (n = 10) versus hypogonadal Acro patients with normal bone mass (n = 8) versus hypogonadal Acro patients with low bone mass (n = 17); *p < 0.05; **p < 0.01, NS — non-significant*

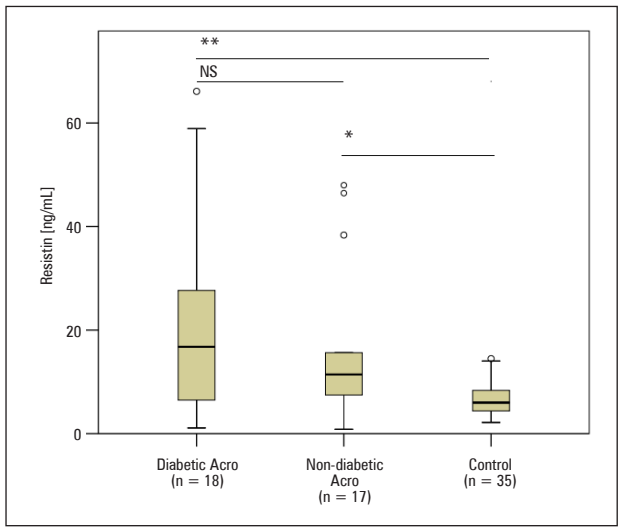

Figure 2. *Serum resistin levels in diabetic patients with acromegaly (Acro) vs. non-diabetic Acro patients versus the control group (CTL). *p < 0.05; **p < 0.01; NS — non-significant*

basal GH, remained an independent predictor of serum leptin levels after adjusting for BMI ($\beta = 0.61$, p = 0.016).

Subgroup analysis in Acro

Initial diagnosis (n = 10) *vs*. long-standing Acro (n = 25)

While age, BMI, BMD, BC, serum adipokines, osteocalcin, glycaemia, and FGF23 were similar between the 2 subgroups (data not shown), the newly diagnosed Acro patients had significantly higher serum IGF1 concentrations (587.18 ± 75.47 *vs*. 377.68 ± 20.76 ng/mL, p = 0.023) and higher, although non-significantly, α-Klotho levels (11.57 ± 3.59 *vs*. 8.27 ± 2.01 ng/mL, p = 0.41) compared to their long-standing counterparts.

Hypogonadism and low bone mass

Hypogonadal Acro patients had significantly lower total hip (p = 0.002), 1/3 radius (p = 0.004), and whole-body (p = 0.003) BMD compared to eugonadal Acro patients, respectively. IGF1, leptin, resistin, PTH, FGF23, and α-Klotho did not differ significantly between eugonadal Acro patients with normal bone mass (n = 10), hypogonadal Acro patients with normal bone mass (n = 8), and hypogonadal Acro patients with low bone mass (n = 17) (data not shown). However, age, adiponectin, and LM varied significantly between the 3 subgroups (Fig. 1).

Diabetes mellitus

Bone density, BMI, BC, age, IGF1, basal GH, leptin, adiponectin, FGF23, and α-Klotho did not differ significantly according to the presence (n = 18) or absence (n = 17) of DM (data not shown). Non-diabetic Acro patients tended to have lower resistin concentrations compared to diabetic Acro patients (15.9 ± 4.26 ng/mL *vs*. 21.48 ± 4.69 ng/mL, p = 0.4) but still exhibited significantly higher serum resistin values when compared to the CTL group (15. 9 ± 4.26 *vs*. 6.59 ± 0.66, p = 0.048, Fig. 2).

Cardiovascular disease

Insulin-like growth factor 1, basal GH, adipokines, α-Klotho, and FGF23 serum concentrations did not differ significantly according to the presence (n = 22) or absence (n = 13) of CVD (data not shown).

Discussion

Body composition (LM and trunk-to-leg fat ratio), PTH, and age were the main BMD predictors in obese acromegalic patients with active disease in the current study, while IGF1 was a positive predictor of serum osteocalcin levels. Acromegalic patients exhibited a different distribution of fat mass and a corresponding dysregulation of the adipokine profile when compared to the general population, but no independent impact of the adipokines upon bone density and metabolism

was observed. Our study is among the first to report significantly higher resistin concentrations in both diabetic and non-diabetic acromegalic patients compared to CTL. α-Klotho was not related to the GH/IGF1 axis in our study, suggesting a limited role (if any) of α-Klotho in the follow-up of acromegalic patients with active disease. The current study is the first to investigate the bone impact of the FGF23/ α-Klotho axis in patients with active acromegaly.

Acromegalic osteopathy is characterized by increased bone turnover, increased cortical porosity — despite the specific increased cortical thickness — due to high remodelling, and also reduced trabecular bone volume — closely related to gonadal function [3, 11].

Lumbar BMD was significantly higher in Acro compared to age-, sex-, and BMI-matched CTL in our study. Although acromegalic patients often share degenerative changes and vertebral deformities due to chronic GH exposure leading to an overestimation of lumbar spine BMD [16]; we also found higher femoral neck Z-scores. The anabolic effects of GH prevail upon the cortical bone, while DXA cannot assess the variable distribution of cortical and trabecular bone, thus possibly explaining increased BMD in some skeletal sites [17]. Acro patients also had elevated serum osteocalcin concentrations — closely related to the IGF1 level — revealing an accelerated bone turnover, and thus confirming previously reported data [17]. Gonadal function is a main BMD determinant in acromegaly as well [17]. Age, bone mass and gonadal function are strongly inter-related in all populations [18]. Indeed, we reported lower BMD in the hypogonadal Acro patients, and age proved to be one of the main BMD predictors in both the Acro and CTL groups in our study. In agreement with other studies reporting a critical role for PTH in bone metabolism and health of the hip [19, 20], we also found a negative association between PTH and hip BMD in the Acro group.

Body composition was the other main BMD predictor in both Acro and CTL subjects, besides age and PTH. The GH-mediated lipolysis, whole-body protein synthesis and water retention cause lean tissue increase and fat loss in acromegaly [7, 21]. LM is considered to increase bone mass through mechanical stress [22]. Direct data assessing the bone impact of BC changes in acromegalic patients are, however, scarce and lacking a control group [11]. We found that [1] obese Acro patients with active disease had similar LM to their matched reference population, but higher bone mass, [2] the relationship between IGF1 and BMD is lost after adjusting for BC, but IGF1 is independently associated with bone turnover, and [3] that LM positively predicted BMD in the Acro group, after adjusting for gender. Thus, LM seems to play a greater role in BMD regulation in acromegaly, while IGF1 specifically impacts bone turnover.

A reduction in visceral fat in active acromegaly was also reported by others [11, 23–25]. Although significantly lower in the obese Acro group with active disease compared to the CTL group, the trunk-to-leg fat ratio independently predicted BMD in both groups. We also previously reported positive independent effects of trunk-to-leg fat ratio upon BMD in overweight postmenopausal women [26]. Kim et al. [27] found android fat to be positively related to BMD, but negatively related to trabecular bone score in postmenopausal women. Although adipokines were proposed to mediate the relationship between fat and bone [27], no significant associations between adipokines and BMD were found in multiple regression analysis in any of the study groups, after adjusting for age and BC.

Nonetheless, the release of adipokines may account for the insulin resistance seen in acromegaly, despite a rather favourable BC [7]. Similarly to other studies, we report lower leptin concentrations that mirror the GH-regulated fat tissue depots [28, 29].

Insulin-sensitizing, anti-inflammatory, and anti-atherogenic adiponectin is negatively correlated with visceral adiposity [30]. Similarly to previous data, higher adiponectin concentrations were encountered in Acro patients exhibiting lower visceral fat in our study [31, 32]. On the other hand, patients suffering from acromegaly often associate CVD and insulin resistance, which are usually accompanied by hypoadiponectinaemia (32), which has also been described in this particular population [10]. Gurbulak et al. [31] hypothesized that adiponectin concentrations are elevated as a consequence of GH-lowering therapies. Serum adiponectin did not vary significantly according to the presence of CVD, DM, or disease duration (newly diagnosed versus long-standing active acromegaly) in the current research. Also, the negative relationship between adiponectin and total hip BMD was lost after adjusting for age and BC (the differences in serum adiponectin between eugonadal and hypogonadal were probably mediated by age and LM). The negative correlation between LM and adiponectin, also reported by others (10), may reflect the link between muscle mass and the corresponding degree of insulin sensitivity. Recently, adiponectin was demonstrated to be merely a marker of insulin sensitivity, to have neutral effects upon non-fatal CV adverse events, but to paradoxically increase all-cause and CV mortality [33]. Hence, adiponectin merits further consideration regarding its potential as a CV risk marker in acromegaly.

To our knowledge, only one study investigated the role of resistin in acromegaly-related metabolic disruption and reported similar serum resistin levels between

18 patients and corresponding controls [34]. Resistin is an adipocyte-derived hormone that "resists" insulin actions, therefore reflecting decreased insulin sensitivity [34]. We report significantly higher resistinaemia in Acro compared to CTL, despite lower visceral adipose depots. Resistin itself stimulates GH secretion in the pituitary [35], while GH increases resistin expression in animal studies [36]; this may explain the high resistin concentrations even in non-diabetic Acro patients compared to the general population in our study, especially because serum resistin levels were not significantly influenced by the presence of CVD. Further research is needed to verify if GH increases insulin resistance via the direct induction of resistin expression in human subjects.

Acromegaly is the sole acquired disease accompanied by an excess of serum α-Klotho [37], probably as a counterregulatory mechanism to the hyperactivity of the GH/IGF1 axis: while inhibiting the activation of the IGF1 receptor, it also opposes the GH effects upon calcium and phosphate metabolism [38, 39]. Although newly diagnosed Acro had a tendency to be associated with higher α-Klotho concentrations compared to the long-standing active Acro, we did not find any significant correlations between the GH/IGF1 axis and α-Klotho in our acromegaly patients. Moreover, α-Klotho was recently demonstrated in animal models to be a negative regulator of bone formation, independently of FGF23 [12]. To our knowledge, our study is the first to investigate any relationship between the FGF23/α-Klotho axis and bone parameters in acromegaly. Although serum α-Klotho initially correlated with a high bone turnover, the relationship was lost in regression analysis. While it is true that our relatively small group of Acro patients was rather heterogenous with regards to disease duration and therapy, α-Klotho might be increased predominantly in particular subgroups of acromegaly — like the ones carrying the d3-GH receptor phenotype, and might be useful in determining patients at higher risk for recurrence despite low post-surgical GH levels [40]. Nonetheless, the assays used to measure serum α-Klotho render poorly standardized results, and this may impede the general interpretation of literature data [37].

Our study is limited by the small number of Acro patients enrolled — also impeding gender separate analysis, the cross-sectional design hindering the monitoring of patients and the heterogenous characteristics of the study patients, especially regarding disease duration (newly diagnosed or long-standing). However, our study is among the first to investigate any given relationship between the FGF23/α-Klotho system and bone parameters, and also one of the first evaluating the impact of metabolic changes related to BC and adipokines on bone mass in acromegaly. The presence of a reference group is nonetheless one of the strengths of the study.

Conclusions

Age and BC are the main BMD predictors in obese acromegalic patients with active disease, while bone turnover is independently related to IGF levels. Serum adipokines mirror the acromegaly-related metabolic changes, but do not appear to exert independent bone actions. Our study is among the first to report increased serum resistin in both diabetic and non-diabetic active Acro patients compared to the reference population, and the first to report the absence of a significant bone impact of the FGF23/ α-Klotho axis in patients with active acromegaly. The usefulness of α-Klotho in assessing the activity of acromegaly on the long-term seems therefore rather limited.

Conflict of interest

The authors declare no conflict of interest.

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