# In acromegaly, increased bone mineral density (BMD) is determined by GH-excess, gonadal function and gender

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*Submitted: August 29, 2007* *Accepted: September 5, 2007*

*Key words:* **acromegaly; growth hormone; hypogonadism; bone mineral density; bone metabolism; osteocalcin; type I collagen carboxyterminal telopeptide**

Neuroendocrinol Lett 2007; **28**(5):621–628 **PMID:** 17984937 NEL280507A31 

## Abstract

**OBJECTIVES**: The aim of our study was to evaluate bone metabolism and bone mineral density (BMD), and to indicate the main determinants of these parameters in a large group of patients with active acromegaly.

**METHODS**: A group of 121 active acromegalics, aged 23–80 years, from a single endocrinological center was studied. Serum GH, IGF-I, LH, FSH, PRL, estradiol/testosterone, osteocalcin (OC), type I collagen carboxyterminal telopeptide (ICTP) as well as BMD by DXA at spine $L_2$–$L_4$, femoral neck, Ward's triangle and trochanter were measured.

**RESULTS**: Serum OC and ICTP concentrations were elevated (mean±SEM: 31.7±2.2 μg/L, $p<0.001$; 7.3±0.5 μg/L, $p<0.001$, respectively), and positively correlated with each other, as well as with IGF-I. BMD (Z-scores) was increased at $L_2$–$L_4$, femoral neck and trochanter (0.35±0.15, $p=0.016$; 0.60±0.11, $p<0.001$ and 0.59±0.13, $p<0.001$; respectively). The main determinants of Z-scores and ICTP were gonadal status and gender, while of OC was IGF-I. Eugonadal acromegalics had higher than normal serum OC and ICTP, as well as Z-scores at all measured sites. Hypogonadal patients (2/3 of the population) had significantly higher serum ICTP concentrations and lower BMD at all sites, when compared to eugonadal acromegalics. Thirty five percent of hypogonadal subjects had T-score<–1. Men had significantly higher serum ICTP and lower Z-scores than women.

**CONCLUSIONS**:

1. In active acromegaly, enhanced IGF-I-dependent bone turnover and increased BMD is observed.
2. In hypogonadal acromegalics, high bone resorption decreases BMD and may lead to osteoporosis.
3. There is a smaller increase in bone resorption and greater increase in BMD in women with acromegaly than in men.

ORIGINAL ARTICLE

**Abbreviations**

| | |
|---|---|
| BMD | - bone mineral density |
| DXA | - dual-energy X-ray absorptiometry |
| OC | - osteocalcin |
| ICTP | - type I collagen carboxyterminal telopeptide |
| GH | - growth hormone |
| IGF-I | - insulin-like growth factor 1 |
| PRL | - prolactin |
| FSH | - follicle stimulating hormone |
| LH | - luteinizing hormone |
| T | - testosterone |
| E2 | - estradiol |

## INTRODUCTION

Growth hormone (GH) is known to increase both bone turnover and bone mineral density (Inzucchi and Robbins, 1994). Acromegaly has been utilized as a model for studies on the effects of prolonged GH-excess on bone. However, the disease is relatively rare and often coexists with other conditions influencing bone metabolism and structure, of which hypogonadism seems to be the most relevant (Thorner *et al*., 1992).

Previous studies on bone mineral density (BMD) in acromegaly gave conflicting results. Lumbar spine BMD was reported by several authors as increased (Kaji *et al*., 2001; Scillitani *et al*., 1997; Seeman *et al*., 1982), whereas by others – as normal (Ho PJ *et al*., 1992; Kotzman *et al*., 1993; Lesse *et al*., 1998; Scillitani *et al*., 2003) or even decreased (Diamond *et al*., 1989; Ezzat *et al*., 1993; Longobardi *et al*., 1998). In most studies, BMD of the proximal femur was described high (Kaji *et al*., 2001; Kotzman *et al*., 1993; Lesse *et al*., 1998; Scillitani *et al*., 2003) or normal (Ho PJ *et al*., 1992; Longobardi *et al*., 1998; Scillitani *et al*., 1997). These discrepancies usually resulted from a small sample size, including patients with an inactive disease and lack of stratification according to gonadal status.

Most biochemical studies showed increased concentrations of both bone formation and resorption markers in active acromegaly (Bolanowski *et al*., 2002; De la Piedra *et al*., 1988; Ezzat *et al*., 1993; Halse and Gordeladze, 1981; Ho PJ *et al*., 1992; Kaji *et al*., 2001; Marazuela *et al*., 1993; Piovesan *et al*., 1994; Scillitani *et al*., 1997; Terzolo *et al*., 1993). However, all of the previously investigated groups of patients with acromegaly were small and so the authors did not observe any significant influence of superimposed hypogonadism (Bolanowski *et al*., 2002; Ezzat *et al*., 1993; Ho PJ *et al*., 1992; Scillitani *et al*., 1997), gender nor other factors on bone turnover markers.

The aim of this study was to evaluate factors determining bone mineral density (BMD) and bone metabolism in acromegaly.

## MATERIAL AND METHODS

*Patients*

A group of 142 patients with active acromegaly, referred to our department, was studied. The diagnosis of active acromegaly was based on clinical features and contemporarly accepted biochemical criteria i.e. serum GH levels not suppressible below 1 μg/L after an oral glucose tolerance test (OGTT) and IGF-I above normal range for sex and age (Giustina *et al*., 2000). We excluded ten patients with coexisting hyperthyroidism, one with hyperparathyroidism, six with gigantism, one with rheumatoid arthritis and three with malignant diseases (2 – colon cancer, 1 – renal cancer). Finally, 121 patients with active acromegaly, 70 women and 51 men, aged 46.8±1.0 years (range, 23–80 years) were enrolled. None of the included subjects was vegetarian nor on a diet modifying vitamin D or calcium intake, had osteoporotic fractures, suffered from renal or hepatic impairment nor consumed substances affecting bone metabolism. All patients had pituitary tumors visualized by computed tomography or/and magnetic resonance. A macroadenoma was present in 103 subjects (58 women, 45 men) and a microadenoma in 18 subjects (12 women, 6 men). The duration of acromegaly, estimated from clinical information and photographs, was 9.0±0.5 years (range, 1–26 years). The protocol was approved by the faculty ethical committee and informed consent was obtained from every patient.

*Patients' classification*

Subjects were analyzed as a whole group and in subgroups, according to gonadal status (eugonadal, hypogonadal) and gender.

To assess pituitary and gonadal function, serum estradiol in women and testosterone in men, LH, FSH and PRL were measured. Patients were categorized as hypogonadal if women were amenorrheic for at least one year and had serum estradiol concentration below 30 pg/ml (110 pmol/L) and men had decreased libido and/or erectile disfunction for at least one year and serum testosterone concentration below 3.5 ng/mL (12 nmol/L).

Thirty-eight subjects (24 women, 14 men) were diagnosed eugonadal, and 83 (46 women, 37 men) hypogonadal. The duration of hypogonadism, estimated by interviewing patients, was 8.6±0.7 years (range, 1–30 years). Hypogonadothropic hypogonadism was found in 43 patients (13 women, 30 men), and hyperprolactinaemia – in 16 patients (10 women, 6 men). Four women underwent hystero-oophorectomy (uterus myomatosus), one – ovariectomy (bilateral cysts), and eighteen were postmenopausal. One man had hypergonadothropic hypogonadism.

The influence of gender on bone was analyzed in a group of patients under 50 (45 women and 36 men), to exclude the effect of age-dependent gonadal hormone deficiency.

Additional analysis was performed for the group of eighteen postmenopausal acromegalic women with reference to menstruating acromegalics and to data of postmenopausal healthy population. Patients were diagnosed postmenopausal if they were over 50 years old,

had amenorrhea for at least one year, low serum estradiol and high LH/FSH concentrations, and had no evidence of other causes of primary hypogonadism.

*Bone density measurements*

Bone mineral density was measured by dual-energy X-ray absorptiometry (DXA) in the lumbar vertebrae ($L_2$–$L_4$) and nondominant proximal femur using Lunar Expert densitometer (Lunar Radiation Corp., Madison WI, USA). To exclude clinically silent vertebral fractures we performed lumbar spine morphometry based on lateral scans together with standard lumbar P-A scans. The coefficients of variation for measurements made at regions: $L_2$–$L_4$, femoral neck, Ward's triangle and greater trochanter were 1.8, 2.7, 5.2 and 2.9%, respectively. All scans were performed and analyzed by the same operator without knowledge of other data available for the patients. BMD was assessed in 118 patients. We excluded from the analysis three lumbar spine scans (2 postmenopausal women, 1 hypogonadal man) and three proximal femur scans (1 eugonadal and 1 postmenopausal woman, 1 eugonadal man) because of severe arthritis. To elaborate the BMD results we utilized the USA reference population provided by the manufacturer, which was found to be compatible with the Polish population in large cohort studies and was accepted for use in Poland by the Polish Society for Endocrinology (Jaworski *et al*., 2002; Kochański and Badurski, 2002; Nowak *et al*., 2003). Each result was referred to age-, sex-, weight- and ethnicity-matched healthy control group, and was expressed as a standard deviation Z-score. To diagnose osteoporosis in accordance with WHO recommendations, T-scores, which compare BMD to the sex-matched young adult (aged 20–40) reference population, were also determined. For each subject the lowest measured T-score value at $L_2$–$L_4$, femoral neck and trochanter was taken into consideration (Lewiecki *et al*., 2004). According to the position statement of International Society for Clinical Densitometry, T-score≤–2.5 was used for diagnosising osteoporosis in postmenopausal women, men 65 yrs and older and hypogonadal men. As all the subjects were white, T-score≤–2.5 was also utilized for diagnosis of osteoporosis in the group of hypogonadal women (Lewiecki *et al*., 2004).

*Biochemical markers of bone metabolism*

Patients' serum samples were collected between 08.00 and 10.00 h, after an overnight fast, and stored at –20 °C until assayed. Serum osteocalcin (OC) was measured in 54 women (15 eugonadal, 39 hypogonadal) and 32 men (8 eugonadal, 24 hypogonadal) by immunoradiometric assay (IRMA) using ELSA-OSTEO kit (CIS Bio International, France). Sensitivity of the method was 0.4 µg/L (0.07 nmol/L), intra- and interassay CVs were 3.8–3.9% and 4.5–5.2% respectively. To compare serum OC concentrations obtained from subjects of different gender and age, each result was additionally expressed as standardized OC value, in accordance to the formula:

$$\mathbf{OC_{std} = OC - OC_{mean} / OC_{max} - OC_{mean},}$$

where:
$OC_{std}$ –standarized OC value,
OC –patient's serum OC concentration (µg/L),
$OC_{mean}$ –mean serum OC concentraton in the reference group (µg/L),
$OC_{max}$ –maximal serum OC concentraton in the reference group (µg/L).

Serum type I collagen carboxyterminal telopeptide (ICTP) was measured in 55 women (17 eugonadal, 38 hypogonadal) and 34 men (9 eugonadal, 25 hypogonadal) by RIA (Orion Diagnostica, Finland). Sensitivity of the method was 0.5 µg/L, intra- and interassay CVs were 3.5–9.4% and 5.6–9.0% respectively. The reference values were: 2.1–5.6 µg/L for female and 2.1–5.0 µg/L for male. To compare serum ICTP concentrations obtained from men and women, standardized ICTP were calculated, in accordance to the formula:

$$\mathbf{ICTP_{std} = ICTP - ICTP_{mean} / ICTP_{max} - ICTP_{mean},}$$

where:
$ICTP_{std}$ –standarized ICTP value,
ICTP –patient's serum ICTP concentration (µg/L),
$ICTP_{mean}$ –mean serum ICTP concentraton in the reference group (µg/L),
$ICTP_{max}$ –maximal value of reference interval for ICTP (µg/L).

*Statistics*

Results were expressed as mean ± SEM. Significance was assumed at $p<0.05$. Multiple regression analysis was performed for Z-scores, OC, $OC_{std}$, ICTP and $ICTP_{std}$ as dependent variables, and thirteen independent variables: age, gender, weight, height, serum GH concentration, serum IGF-I concentration, duration of acromegaly, presence of macroadenoma, adrenocortical insufficiency, hypothyroidism, hypogonadism, duration of hypogonadism and diabetes. The comparison between the patients and the reference population as well as between all subgroups was performed by Student's unpaired *t* test and by the Mann-Whitney U test as appropriate.

## RESULTS

BMD in the whole group of 121 patients with active acromegaly was 1.267±0.020 g/cm$^2$ at $L_2$–$L_4$, 1.065±0.015 g/cm$^2$ at femoral neck, 0.876±0.017 g/cm$^2$ at Ward's triangle and 0.926±0.014 g/cm$^2$ at trochanter. Z-scores of acromegalic patients were increased in all of the measured sites, except Ward's triangle (Table 2, Figure 1). In 5% (6) of the active acromegalics the lowest value of T-scores measured at $L_2$–$L_4$, femoral neck and trochanter was <–2.5, in 20% (24) <–1, in 31% (37) >0, in 13% (15) >1 and in 6% (7) >2.5.

**Table 1.** Demographical and clinical characteristics of the whole group of patients studied according to gonadal status, patients ≤ 50 y studied according to gender and postmenopausal patients compared to eugonadal acromegalic women.

| | All patients (n=121) | | | Patients ≤ 50 (n=81) | | | Women with gonadal function adequate to age (n=42) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Eugonadal | Hypogonadal | p-value *E/H* | Women | Men | p-value *F/M* | Eugonadal | Postmenopausal | p-value *EF/PMF* |
| **Sex** (F/M) | 24 / 14 | 46 / 37 | ns | 45 / – | – / 36 | – | 24 / – | 18 / – | – |
| **Age** (years) | 43.8±1.4 | 48.1±1.4 | ns | 41.2±1.0 | 39.5±1.2 | ns | 43.5±1.2 | 60.9±2.1 | <0.001 |
| **Height** (cm) | 169.8±1.6 | 168.4±1.0 | ns | 164.8±0.8 | 177.0±1.3 | <0.001 | 163.7±1.0 | 159.5±1.8 | 0.007 |
| **Weight** (kg) | 84.5±2.4 | 84.8±1.6 | ns | 78.7±2.2 | 94.6±2.2 | <0.001 | 78.0±2.2 | 79.5±2.2 | ns |
| **BMI** (kg/m$^2$) | 29.2±0.6 | 29.9±0.5 | ns | 28.9±0.7 | 30.2±0.6 | ns | 29.1±0.8 | 31.3±0.8 | ns |
| **Duration of acromegaly** (years) | 7.8±0.8 | 9.6±0.6 | ns | 7.9±0.7 | 9.2±1.0 | ns | 7.0±1.0 | 9.8±1.3 | ns |
| **Micro-/macroadenoma** | 10 / 28 | 8 / 75 | 0.017 | 7 / 38 | 4 / 32 | ns | 7 / 17 | 4 / 14 | ns |
| **GH** (μg/L) | 43.3±18.6 | 27.9±5.7 | ns | 26.5±6.5 | 60.8±21.3 | ns | 24.6±8.3 | 8.1±2.2 | ns |
| **IGF-I** (μg/L) | 974.2±68.3 | 883.0±40.1 | ns | 849.6±58.1 | 1038.5±68.6 | 0.038 | 891.2±89.5 | 779.5±69.3 | ns |
| **Hypogonadism** | – | 83 | ns | 20 / 25 | 11 / 25 | ns | – | 18 | – |
| **Duration of hypogonadism** (years) | – | 8.6±0.7 | – | 6.1±0.7 | 6.3±0.9 | ns | – | 12.8±1.9 | – |
| **Adrenal insufficiency** | 1 | 27 | <0.001 | 14 | 7 | ns | 1 | 1 | ns |
| **Hypothyroidism** | 3 | 26 | 0.008 | 13 | 10 | ns | 3 | 1 | ns |
| **Diabetes** | 5 | 27 | ns | 6 | 3 | ns | 3 | 7 | 0.021 |

Mean serum OC concentration and $OC_{std}$ increased, when compared to healthy population values ($p<0.001$, Table 2, Figure 1). The ICTP concentration and $ICTP_{std}$ were also significantly elevated ($p<0.001$, Table 2, Figure 1).

There was a weak negative correlation between duration of hypogonadism and Z-scores in the lumbar vertebrae ($R=-0.268$, $p=0.004$), but not in other locations. We did not find any correlation between duration of acromegaly, size of the adenoma, GH or IGF-I concentration, coexisting diabetes, adrenal insufficiency or hypothyroidism and Z-scores.

Serum OC and ICTP concentrations significantly positively correlated with each other ($R=0.606$, $p<0.001$) and with serum IGF-I ($R=0.399$, $p<0.001$ and $R=0.279$, $p=0.008$, respectively).

Serum ICTP and $ICTP_{std}$ negatively correlated with BMD expressed in Z-scores in the lumbar vertebrae ($n=83$; $R=-0.288$, $p=0.008$ and $R=-0.285$, $p=0.009$, respectively), femoral neck ($n=84$; $R=-0.227$, $p=0.037$ and $R=-0.217$, $p=0.048$, respectively), Ward's triangle ($n=84$; $R=-0.240$, $p=0.028$ and $R=-0.237$, $p=0.03$, respectively) and trochanter ($n=84$; $R=-0.392$, $p<0.001$ and $R=-0.372$, $p=0.001$, respectively).

To identify factors determining Z-scores, OC and ICTP in acromegaly, a multiple regression analysis was performed. Z-scores of all measured sites correlated with hypogonadism and female gender (at $L_2$–$L_4$: $R=0.577$, $p<0.001$, $\beta=-0.487$ and $\beta=0.337$, respectively; at femoral neck: $R=0.546$, $p<0.001$, $\beta=-0.363$ and $\beta=0.470$, respectively; at Ward's triangle: $R=0.568$, $p<0.001$, $\beta=-0.481$ and $\beta=0.407$, respectively; at trochanter: $R=0.571$, $p<0.001$, $\beta=-0.329$ and $\beta=0.495$, respectively). The only independent variable correlating with serum OC and $OC_{std}$ was IGF-I level ($R=0.516$, $p<0.031$, $\beta=0.399$ and $R=0.534$, $p<0.018$, $\beta=0.354$, respectively). ICTP and $ICTP_{std}$ correlated mainly with female gender ($R=0.618$, $p<0.001$, $\beta=-0.467$ and $R=0.656$, $p<0.001$, $\beta=-0.535$, respectively), and less strongly with hypogonadism ($R=0.618$, $p<0.001$, $\beta=0.226$ and $R=0.656$, $p<0.001$, $\beta=0{,}214$, respectively).

Eugonadal and hypogonadal patients did not significantly differ in sex, age, body mass index, mean serum GH and IGF-I concentrations or duration of acromegaly (Table 1).

In the eugonadal group, BMD significantly increased in all measured sites (Table 2, Figure 1). Of all the locations, BMD expressed in Z-scores was significantly lower in hypogonadal than in eugonadal acromegalics (Table 2, Figure 1).

In the group of eugonadal acromegalics, none of the patients had T-score≤–2.5, 2 men (5%) had T-score<–1, 38% (14) had T-score>0, 16% (6) >1 and 19% (7) >2.5.

**Table 2.** Bone mineral density (BMD) expressed in Z-scores and bone metabolism markers.

| | All patients | | Eugonadal | | | Hypogonadal | | | p-value E/H |
|---|---|---|---|---|---|---|---|---|---|
| | Mean ± SEM | p-value | n | Mean ± SEM | p-value | n | Mean ± SEM | p-value | |
| **Z-score $L_2$–$L_4$** | 0.35 ± 0.15 | 0.016 | 37 | 1.48 ± 0.28 | <0.001 | 78 | -0.18 ± 0.14 | ns | <0.001 |
| **Z-score Neck** | 0.60 ± 0.11 | <0.001 | 35 | 1.18 ± 0.21 | <0.001 | 80 | 0.35 ± 0.13 | 0.006 | 0.001 |
| **Z-score Ward's triangle** | 0.05 ± 0.12 | ns | 35 | 0.82 ± 0.23 | <0.001 | 80 | -0.28 ± 0.12 | 0.017 | <0.001 |
| **Z-score Trochanter** | 0.59 ± 0.13 | <0.001 | 35 | 1.39 ± 0.25 | <0.001 | 80 | 0.24 ± 0.13 | ns | <0.001 |
| **OC** | 31.7 ± 2.2 | <0.001 | 23 | 25.2 ± 2.5 | 0.028 | 63 | 34.0 ± 2.8 | <0.001 | ns |
| **$OC_{std}$** | 0.78 ± 0.16 | <0.001 | 23 | 0.40 ± 0.16 | 0.017 | 63 | 0.91 ± 0.21 | <0.001 | ns |
| **ICTP** | 7.32 ± 0.49 | <0.001 | 26 | 5.4 ± 0.6 | <0.001 | 63 | 8.1 ± 0.6 | <0.001 | 0.009 |
| **$ICTP_{std}$** | 2.07 ± 0.27 | <0.001 | 26 | 1.01 ± 0.29 | <0.001 | 63 | 2.51 ± 0.35 | <0.001 | 0.011 |

osteocalcin (OC; in µg/L, 1 µg/L=0.171 nmol/L) and type I collagen carboxyterminal telopeptide (ICTP; in µg/L) concentrations, as well as $OC_{std}$ and $ICTP_{std}$ values in the whole group of acromegalic patients, eugonadal and hypogonadal subjects. n = sample size. p-value refers to comparison with reference population. PE/H refers to comparison between eugonadal and hypogonadal group. ns – not significant

On the contrary, 7% (6) of hypogonadal subjects had osteoporosis and 27% (22) – T-score<–1, whereas 26% (21) had T-score>0, 11% (9) >1, and none had T-score>2.5.

Serum OC and ICTP concentrations, as well as $OC_{std}$ and $ICTP_{std}$ were elevated in both eugonadal and hypogonadal groups (Table 2, Figure 1). However, ICTP and $ICTP_{std}$ were significantly higher in hypogonadal acromegalic serum than in eugonadal subjects (Table 2, Figure 1).

Demographic and clinical features of women and men did not differ significantly, except for weight, height and IGF-I concentrations, which were greater in men (Table 1).

As shown in Figure 2, women, compared to men, had significantly higher Z-scores of lumbar vertebrae (0.74±0.26 vs. –0.06±0.25, p=0.032), femoral neck (0.81±0.18 vs. 0.14±0.19, p=0.012), Ward's triangle (0.35±0.16 vs. –0.30±0.25, p=0.026) and trochanter (1.08±0.19 vs. 0.13±0.21, p=0.001). Women also had lower $ICTP_{std}$ values than men (1.10±0.27 vs. 3.65±0.62, p<0.001).

In the postmenopausal group, BMD expressed in Z-scores increased in the femoral neck and trochanter, but not in the lumbar vertebrae nor Ward's triangle (Figure 3). Postmenopausal patients had lower Z-scores in the lumbar vertebrae (0.62±0.36 vs. 1.86±0.34, p=0.020), Ward's triangle (0.23±0.27 vs. 1.06±0.25, p=0.027) and trochanter (0.84±0.31 vs. 1.83±0.26, p=0.019) than eugonadal women, whereas the difference in Z-scores in the femoral neck (1.06±0.30 vs. 1.48±0.27) was not significant (Figure 3).

In a multiple regression analysis, performed for women with gonadal function adequate to age (eugonadal and postmenopausal), hypogonadism was the only important independent variable negatively correlating with Z-scores in all measured sites (at $L_2$–$L_4$: R=0.661, p<0.016, β=–0.465, femoral neck: R=0.609, p<0.047, β=–0.315, Ward's triangle: R=0.719, p<0.002, β=–0.439 and trochanter: R=0.704, p<0.003, β=–0.439).

Of the postmenopausal group 11% (2) had osteoporosis, 17% (3) – osteopenia, whereas in 39% (7) T-score was >0, in 22% (4) >1, and none had T-score>2.5.

## DISCUSSION

In our group of 121 patients with active acromegaly, 70% was hypogonadal, which was high, but comparable with the prevalence of hypogonadism in other large cohort studies, where hypogonadism occurred in 50–80% of acromegalic patients (Ezzat *et al*., 1993; Kaltsas *et al*., 1999; Katznelson *et al*., 2001; Kayath and Vieira, 1997; Scillitani *et al*., 2003). The most common causes of hypogonadism are destruction of the pituitary and hypeprolactinaemia. Additionally, as the disease occurs mainly in the fourth decade of life and is often diagnosed late (Thorner *et al*., 1992; Bolanowski *et al*., 2006b), many acromegalics are postmenopausal women and elderly men, in whom age-dependent sex steroid deficiency is observed.

Despite a high percentage of hypogonadal patients, BMD in the whole acromegalic group increased in three of the four measured sites (Table 2, Figure 1). However, 25% of the studied subjects had T-score<–1, which was comparable to the observations of Kayath and Vieira (1997). It confirms large discrepancies in BMD results of acromegalic patients and indicates, that besides GH-excess, there are other factors strongly influencing BMD in this population.

Despite low BMD in a quarter of the enrolled patients, none of them had osteoporotic fracture in anamnesis nor visualized vertebral fracture in the lumbar mor-

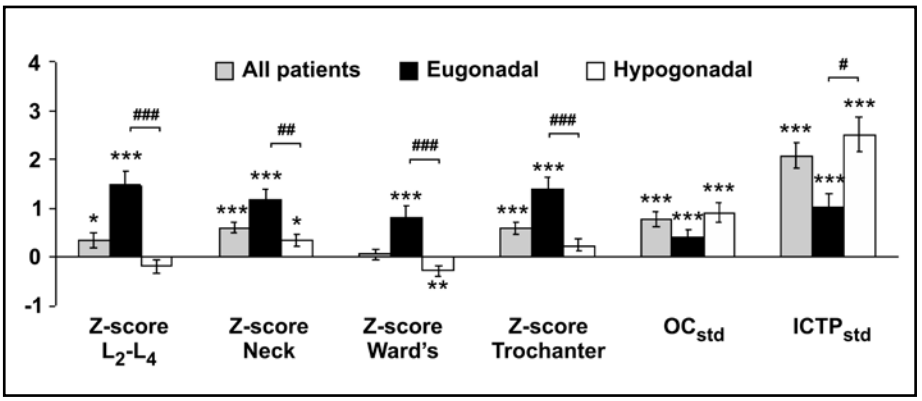


**Figure 1.** Bone mineral density (BMD) expressed in Z-scores measured at $L_2$–$L_4$, femoral neck, Ward's triangle and trochanter, $OC_{std}$ and $ICTP_{std}$ values of the whole group of acromegalic patients, eugonadal and hypogonadal subjects. Results are expressed as mean ± SEM. *p<0.02, **p<0.01, ***p<0.001 vs. reference population, #p<0.02, ##p=0.01, ###p<0.001 between eugonadal and hypogonadal acromegalics. 0 = mean value of the reference population.

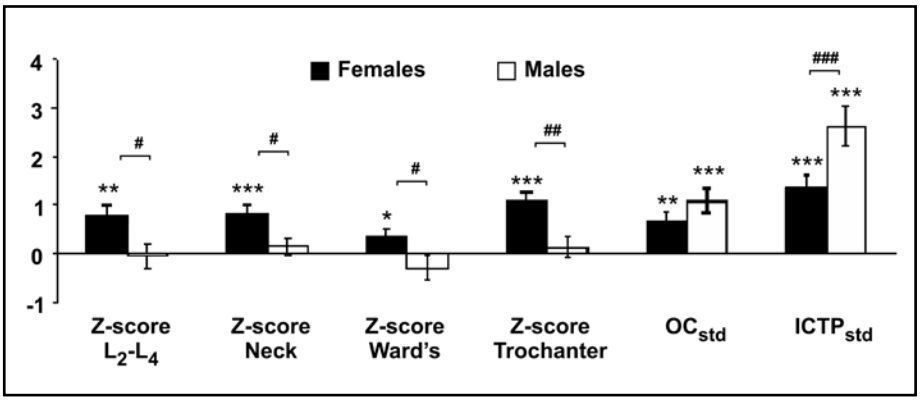


**Figure 2.** Bone mineral density (BMD) expressed in Z-scores measured at $L_2$–$L_4$, femoral neck, Ward's triangle and trochanter, $OC_{std}$ and $ICTP_{std}$ values of the acromegalic women and men ≤ 50 y. Results are expressed as mean ± SEM. *p<0.05, **p<0.01, ***p<0.001 vs. reference population, #p<0.05, ##p<0.01 between women and men. 0 = mean value of the reference population.

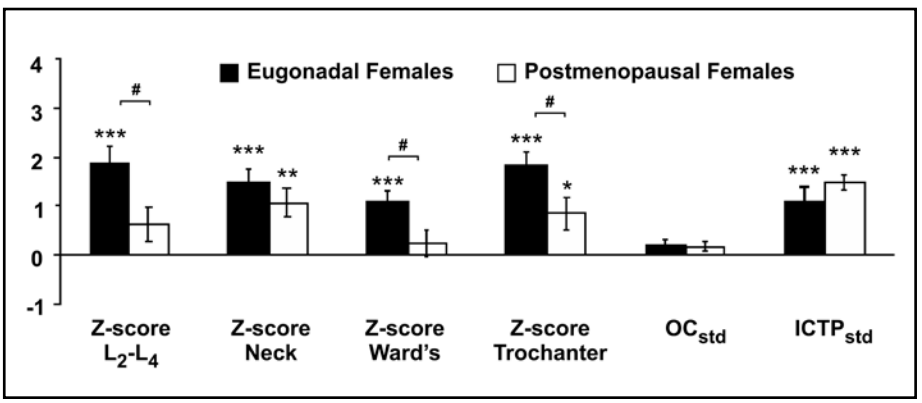


**Figure 3.** Bone mineral density (BMD) expressed in Z-scores measured at $L_2$–$L_4$, femoral neck, Ward's triangle and trochanter, $OC_{std}$ and $ICTP_{std}$ values of the acromegalic eugonadal and postmenopausal women. Results are expressed as mean ± SEM. *p<0.05, **p<0.01, ***p<0.001 vs. reference population, #p<0.05 between eugonadal and postmenopausal acromegalic women. 0 = mean value of the reference population.

phometry. These observations are consistent with the study of Vestergaard and Mosekilde (2004), who related a decreased fracture risk in active acromegaly.

In our study the multiple regression analysis of thirteen various independent variables showed, that the main determinants of BMD (Z-scores) were gonadal function and gender.

In accordance with previous data (Lesse *et al.*, 1998; Scillitani *et al.*, 2003; Seeman *et al.*, 1982), in eugonadal subjects with acromegaly we observed significantly increased Z-scores in all sites (Table 2, Figure 1). All but two of them had normal or higher than normal BMD expressed in T-scores.

In the whole acromegalic group we found increased OC, $OC_{std}$, ICTP and $ICTP_{std}$ values, as well as a positive correlation between OC and ICTP, which indicates high bone turnover in acromegaly. It confirms the results of some of the studies on small groups of acromegalic patients, which described high levels of bone formation and resorption markers, as well as positive correlations between them (Ezzat *et al.*, 1993; Halse and Gordeladze, 1981; Kotzmann *et al.*, 1993; Ueland *et al.*, 2001).

De la Piedra *et al.* (1988), Marazuela *et al.* (1993), Piovesan *et al.* (1994) and Terzolo *et al.* (1993) had shown earlier that OC concentrations positively correlated with IGF-I. Unlike these authors, we did not find a correlation between GH and OC. Similarly, ICTP positively correlated with IGF-I, but not with GH, as shown by Scillitani *et al.* (1997) . Therefore, our results indicate, that GH stimulates bone turnover through IGF-I rather than directly.

In a multiple regression analysis, IGF-I concentration independently determined OC and $OC_{std}$, but not ICTP or $ICTP_{std}$, which suggests increased BMD in

acromegaly due to a stronger influence of IGF-I on bone formation than resorption. The negative influence of hypogonadism on bone density of acromegalic patients has been shown by several authors. Lesse *et al.* (1998) observed low lumbar spine and femoral neck BMD in hypogonadal acromegalic patients**;** however they studied only 18 patients (6 eugonadal,12 hypogonadal), of whom eight had the active disease. Scillitani *et al.* (2003) and Kayath and Vieira (1997) reported significantly lower BMD of the spine in hypogonadal compared to eugonadal acromegalics, but the difference in BMD was not significant in the proximal femur. In our study, hypogonadal acromegalics had significantly lower BMD than eugonadal patients in all sites (Table 2, Figure 1).

Like most authors, we used criteria according to which postmenopausal women were included in the hypogonadal group (Diamond *et al.*, 1989; Ezzat *et al.*, 1993; Ho PJ *et al.*, 1992; Lesse *et al.*, 1998). Therefore, utilizing Z-scores could cause the overestimation of BMD of the hypogonadal group. On the other hand, if estrogens and GH have a synergic effect on bone, classifying postmenopausal women into the eugonadal group (Scillitani *et al.*, 2003) might lead to the underestimation of BMD. Mean BMD value (Z-scores) of the hypogonadal acromegalic subjects was approximately normal (Table 2, Figure 1**);** however 35% of them had T-score<–1 and 7% were diagnosed osteoporotic (T-score≤–2.5).

To the best of our knowledge, we are the first to show higher bone resorption marker concentrations in hypogonadal acromegalics compared to eugonadal subjects (Table 2, Figure 1). It is probably the result of a relatively large group of patients studied, when compared with previous reports (Scillitani *et al.*, 1997). Additionally, in our study ICTP concentrations negatively correlated with BMD in all locations. These results confirm, that hypogonadism in acromegaly leads to bone loss by stimulation of bone resorption.

We utilized Z-scores, $OC_{std}$ and $ICTP_{std}$ to cancel the difference in BMD of the subjects caused by sex. However, gender was one of two independent variables correlating with Z-scores, and the most important variable determining $ICTP_{std}$ in the whole acromegalic group. We showed that, in acromegalic women compared to men, both lumbar spine and proximal femur BMD was relatively higher (Figure 2). Our results are in accordance with Tütüncü *et al.* (2004), who observed increased BMD in women, but not in men with acromegaly. Bolanowski *et al.* (2002; 2006a) also showed the influence of sex on BMD of acromegalic subjects. Others, however, did not find a significant difference in BMD between acromegalic men and women (Scillitani *et al.*, 2003; Kayath and Vieira, 1997). Contrary to our results, a study regarding therapy with recombinant human GH (rhGH) showed a higher increase in BMD in men (Johansson *et al.*, 1999).

In our study $ICTP_{std}$ was significantly higher in men than in women (Figure 2). There was the same tendency for $OC_{std}$, but the difference was insignificant. This observation is in accordance with the study of Burman *et al.* (1997), in which men treated with rhGH had higher bone metabolism markers concentrations than women. The difference between men and women in Z-scores, as well as in $ICTP_{std}$, was similar in eugonadal and hypogonadal subjects (data not shown).

In our study, postmenopausal women had increased BMD in the femoral neck and trochanter, but not in the lumbar vertebrae or Ward's triangle (Figure 3). These results are in accordance with Scillitani *et al.* (1997), who revealed that, in amenorrheal acromegalics (the majority of whom was postmenopausal), lumbar spine BMD was not higher than in gonadal status matched controls.

Despite using Z-scores, which cancel the difference in BMD caused by age and physiological menopause in healthy subjects, BMD significantly increased more in eugonadal, compared to postmenopausal acromegalics (Figure 3). Our results indicate, that GH-excess influences BMD less strongly after menopause, which suggests the synergic effect of GH and estrogens on human bones**.**

Several studies in vitro and in vivo proved, that estrogens influence not only GH secretion (Friend *et al.*, 1996; Ho KK and Weissberger, 1992; Ho KK *et al.*, 1996), but also its function (Slootweg *et al.*, 1997). Gonadal hormones were also shown to facilitate the influence of GH on bone (Sandstedt *et al.*, 1996a; Slootweg *et al.*, 1997). Our observations are in accordance with the studies, in which intact ovaries and testes were a prerequisite for an optimal stimulatory effect of elevated GH levels on bone in mice (Ohlsson *et al.*, 1998; Sandstedt *et al.*, 1996a; Sandstedt *et al.*, 1996b). As the data on the synergic effect of GH and gonadal hormones are limited, it seems to be a very interesting problem for further investigations.

Osteopenia was present only in 17% and osteoporosis in 11% of postmenopausal acromegalics, whereas in NHANES III studies on the normal population of postmenopausal women, osteopenia was found in 34–50% and osteoporosis in 17–20% of subjects (Looker *et al.*, 1998). It seems, therefore, that GH-excess in acromegaly partially prevents postmenopausal bone loss.

To the best of our knowledge, our study was the first in which not only BMD, but also bone metabolism were determined in a large group of acromegalics in a single endocrinological center.

## CONCLUSIONS

1. In active acromegaly, enhanced IGF-I-dependent bone turnover and increased bone mineral density (BMD) is observed.
2. In hypogonadal acromegalics (2/3 of the population), high bone resorption decreases BMD and may lead to osteoporosis.
3. There is a smaller increase in bone resorption and greater increase in BMD in women with acromegaly than in men.

## ACKNOWLEDGEMENTS

This study was supported by 501-2-1-08-81/04 CMKP grant.

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