GROWTH HORMONE (GH) and insulin-like growth factor-I (IGF-I) are anabolic hormones for bone with important regulating effects on bone metabolism not only in childhood, but also in adulthood [1]. In active acromegaly, GH excess increases bone turnover, which is characterized predominantly by bone formation, resulting in an overall increase in BMD [2-4], although aging, sex, and hypogonadism in such patients is usually correlated with decreased BMD [3-5]. Although BMD in acromegalic patients should decrease after the treatment of transsphenoidal surgery (TSS), our previous study showed a rapid decline in bone turnover markers but not BMD in acromegalic patients at first 1 year after TSS [6]. While this observation is meaningful, the follow-up period of that study might not have been sufficient to evaluate BMD in acromegalic patients after TSS. In this study, we measured levels of bone turnover markers and BMD for 3 years after TSS in patients with active acromegaly.

Subjects and Methods

This prospective study recruited 22 Japanese patients with untreated acromegaly who had been hospitalized at our department between April 2009 and May 2012. We excluded 4 patients for either receiving bisphosphonate treatment (n=3) or reaching menopause during the study (n=1). A total of 18 Japanese patients (10 males and 8 females) with untreated acromegaly (median age: 52 years, range: 34-68 years; median body mass index [BMI]: 24.1 kg/m², range: 20.8-30.9
kg/m²) were included in the final analysis (Table 1). The diagnosis of acromegaly and hypogonadism was described previously [6]. Hypoadrenalism was defined as a failed cortisol response (absolute value < 18 μg/dL) to an insulin tolerance test (ITT). Hypothyroidism was defined as a free T4 level below the normal laboratory reference range (absolute value < 0.8 ng/dL). GH deficiency (GHD) was not routinely assessed.

None of the patients had a history of bone fracture based on interviews, and none were taking any drugs to treat osteoporosis, including anti-osteoporosis drugs, vitamin D, or calcium supplementation at the start of or throughout the study.

The study protocol was reviewed and approved by the ethics committee of Osaka University (reference no: 08116-5, UMIN no: 000004665) and performed in accordance with the Helsinki declaration. All patients provided informed consent before participation in the study.

**Methods**

All acromegaly patients underwent TSS. After TSS, serum GH levels were measured following a 75 g oral glucose tolerance test (OGTT). Patients in whom serum GH levels did not fall below 1 ng/mL after OGTT received pharmacological therapy with either or both long-acting agonists of somatostatin or dopamine agonists to control GH hypersecretion.

In all patients, GH, IGF-I, bone formation marker (serum bone alkaline phosphatase: BAP), bone resorption marker (urinary type I collagen cross-linked N-telopeptide: urinary NTx), and BMD were measured before surgery and at 3 months, 1 year, and 3 years after surgery as in our previous study [6].

**Statistical analysis**

The measured variables are expressed as median values (1st and 3rd quartiles). The time courses of all data were analyzed using generalized estimating equations (GEEs). Two-sided \( P<0.05 \) denoted a statistically significant difference. All statistical analyses were performed with R version 3.1.0 (R Core Team, Vienna, Austria).

**Results**

Eleven patients were considered to have reached remission, based on suppression of GH levels below 1 ng/mL after OGTT following TSS. GH hypersecretion in the remaining seven patients was not completely controlled by TSS. Of these seven patients, three were treated with long-acting somatostatin analog, one received long-acting somatostatin analog and dopamine agonists, one received long-acting somatostatin analog and GH receptor antagonist, and two received dopamine agonists after TSS.

The characteristics of all acromegalic patients are summarized in Table 1. Eight patients were diagnosed with hypogonadism, two with hypoadrenalism, and three with diagnosed hypothyroidism at the beginning of this study. Hormone replacement therapy was performed in patients with hypoadrenalism and hypothyroidism; however, no therapy was conducted for those with hypogonadism. The hormone replacement therapy regimens described above were not altered during the study period.

GH and IGF-I levels were significantly decreased at 3 months, 1 year, and 3 years after surgery (Table 2). Serum IGF-I levels were controlled in all patients to within age- and sex-adjusted reference ranges for a Japanese population [7]. While GHD was not routinely assessed, no patients had an IGF-I SD score less than −1 SD at 1 year, and only one patient was less than −1 SD at 3 years.

**Bone Metabolism**

**Baseline bone markers measurements**

At baseline, levels of bone formation marker (BAP, median: 24.5 μg/L, range: 13.4-56.5 μg/L; reference range: male 3.7-20.9 μg/L, female 2.9-14.5 μg/L) and bone resorption marker (urinary NTx, median: 102 nmol·BCE/mmol·CRE, range: 34-354 nmol·BCE/mmol·CRE; ref-
Postoperative BMD in acromegaly

Baseline BMD measurements

At baseline, median BMD at the lumbar spine was 1.01 g/cm² (range: 0.92-1.14 g/cm²), median T-score was −0.2 (range: −0.9-1.1), and median Z-score was 0.2 (range: −0.1-1.4) (Table 2). Median BMD at the femoral neck was 0.77 g/cm² (range: 0.72-0.85 g/cm²), median T-score was −0.5 (range: −0.8-0.3), and median Z-score was 0.7 (range: −0.4-1.3) (Table 2). No patients had osteoporosis at the lumbar spine or femoral neck at baseline, but 4 (22%) had osteopenia. Six patients (33%) had osteoporosis at the 33% radius at baseline, and 3 (17%) had osteopenia.

Longitudinal BMD measurements after 3 years of follow-up

GEE showed no decrease in BMD at 3 months, 1 year, or 3 years after TSS at any measured bone sites (Fig. 1 A-C). No significant changes were noted in male or female patients in BMD throughout the study (Fig. 1 D-I), nor were any differences in percent-change in BMD found after TSS, relative to pre-surgery values. Of note, neither the presence of hypogonadism nor BMD level at baseline had any influence on changes in BMD during the study (data not shown). In order to investigate the effect of decline in GH levels

Table 2 Postoperative changes in bone metabolism and BMD.

<table>
<thead>
<tr>
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<th>Baseline</th>
<th>3 months</th>
<th>1 year</th>
<th>3 year</th>
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<tbody>
<tr>
<td>GH (μg/L)</td>
<td>6.5 (2.9-13.1)</td>
<td>0.9 (0.6-1.8)**</td>
<td>0.7 (0.4-1.2)**</td>
<td>0.7 (0.5-1.3)**</td>
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<tr>
<td>IGF-I (μg/L)</td>
<td>593 (394-758)</td>
<td>199 (161-320)**</td>
<td>186 (162-243)**</td>
<td>158 (131-221)**</td>
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<td>IGF-I S.D. score</td>
<td>7.0 (4.8-8.4)</td>
<td>1.4 (0.8-3.1)**</td>
<td>1.6 (0.3-2.0)**</td>
<td>0.6 (0.1-1.5)**</td>
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<tr>
<td>BAP (μg/L)</td>
<td>24.5 (20.2-35.2)</td>
<td>22.7 (17.4-29.4)</td>
<td>13.4 (10.2-17.0)***</td>
<td>10.2 (8.7-13.7)***</td>
</tr>
<tr>
<td>Urinary NTx (nmol·BCE/mmol·CRE)</td>
<td>102 (80-140)</td>
<td>57 (45-68)**</td>
<td>41 (33-58)**</td>
<td>31 (18-58)**</td>
</tr>
<tr>
<td>BAP/urinary NTx ratio</td>
<td>0.25 (0.13-0.62)</td>
<td>0.44 (0.16-1.17)**</td>
<td>0.33 (0.14-1.69)</td>
<td>0.35 (0.15-1.03)*</td>
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Lumbar spine

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<tbody>
<tr>
<td>BMD (g/cm²)</td>
<td>1.01 (0.92-1.14)</td>
</tr>
<tr>
<td>T-score</td>
<td>-0.2 (-0.9-1.1)</td>
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<tr>
<td>Z-score</td>
<td>0.2 (-0.1-1.4)</td>
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Femoral neck

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<tr>
<td>BMD (g/cm²)</td>
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</tr>
<tr>
<td>T-score</td>
<td>-0.5 (-0.8-0.3)</td>
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<tr>
<td>Z-score</td>
<td>0.7 (-0.4-1.3)</td>
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33% Radius

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<tr>
<td>BMD (g/cm²)</td>
<td>0.65 (0.56-0.74)</td>
</tr>
<tr>
<td>T-score</td>
<td>-1.0 (-2.6-1.0)</td>
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<tr>
<td>Z-score</td>
<td>0.0 (-1.7-1.6)</td>
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GH, growth hormone; IGF-I, insulin-like growth factor-I; BAP, serum bone alkaline phosphatase; urinary NTx, urinary type I collagen cross-linked N-telopeptide; BMD, bone mineral density.

Comparison vs baseline. * P< 0.05, ** P< 0.01, *** P< 0.001

The measured variables are expressed as median values (1st and 3rd quartiles).

dence range: 9.3-54.3 nmol·BCE/mmol·CRE) in acromegalic patients were high before TSS compared with respective reference ranges (Table 2). The frequency of abnormal BAP elevation was 83% (men: 70%, women: 100%), and the frequency of abnormal urinary NTx elevation was 83% (men: 90%, women: 75%).

Longitudinal bone marker measurements after 3 years of follow-up

BAP was significantly decreased at 1 and 3 years after TSS, but not at 3 months, compared with the baseline (Table 2). In contrast, urinary NTx was significantly decreased at all follow-up points after TSS compared with the baseline (Table 2). These results demonstrated high levels of bone formation and resorption in acromegalic patients before TSS, with both decreasing after TSS. Further, the decrease in bone resorption occurred earlier than that of bone formation after TSS. BAP/urinary NTx ratio was significantly increased at 3 months and 3 years after TSS compared with the baseline value (Table 2). The decline of bone turnover following TSS was bone resorption dominantly.

BMD measurements

Baseline BMD measurements

At baseline, median BMD at the lumbar spine was 1.01 g/cm² (range: 0.92-1.14 g/cm²), median T-score was −0.2 (range: −0.9-1.1), and median Z-score was 0.2 (range: −0.1-1.4) (Table 2). Median BMD at the femoral neck was 0.77 g/cm² (range: 0.72-0.85 g/cm²), median T-score was −0.5 (range: −0.8-0.3), and median Z-score was 0.7 (range: −0.4-1.3) (Table 2). Median BMD at the 33% radius was 0.65 g/cm² (range: 0.56-0.74 g/cm²), median T-score was −1.0 (range: −2.6-1.0), and median Z-score was 0.0 (range: −1.7-1.6) (Table 2). No patients had osteoporosis at the lumbar spine or femoral neck at baseline, but 4 (22%) had osteopenia. Six patients (33%) had osteoporosis at the 33% radius at baseline, and 3 (17%) had osteopenia.

Longitudinal BMD measurements after 3 years of follow-up

GEE showed no decrease in BMD at 3 months, 1 year, or 3 years after TSS at any measured bone sites (Fig. 1 A-C). No significant changes were noted in male or female patients in BMD throughout the study (Fig. 1 D-I), nor were any differences in percent-change in BMD found after TSS, relative to pre-surgery values. Of note, neither the presence of hypogonadism nor BMD level at baseline had any influence on changes in BMD during the study (data not shown). In order to investigate the effect of decline in GH levels
Fig. 1 Change in bone mineral density (BMD) after transsphenoidal surgery (TSS) (Z-score).
BMD of all patients (n=18, A, B, C), male (n=10, D, E, F) and female (n=8, G, H, I) patients was not decreased at 3 months or 1 or 3 years after TSS. BMD of three patients (2 males; ■ and 1 female; ●) who satisfies the following criteria: 1) under 45 years old, 2) reached remission by TSS only (serum GH levels below 0.4 ng/mL after a 75 g oral glucose tolerance test (OGTT) with normal serum IGF-I levels (IGF-I S.D. score < 1.5)), and 3) not required hormone replacement therapy after TSS (J, K, L).
after TSS excluding subject heterogeneity, we selected the BMD of three patients (2 males and 1 female) who satisfies the following criteria: 1) under 45 years old, 2) remission by TSS by itself (serum GH levels below 0.4 ng/mL after an OGTT with normal serum IGF-I levels (IGF-I S.D. score < 1.5)), and 3) not required hormone replacement therapy after TSS (Fig. 1 J-L). Their BMD at the lumbar spine and the femoral neck increased at the 3 years after TSS. On the other hand, the BMD at the 33% radius was not changed.

**Discussion**

In the present study, treatment of acromegalic patients with TSS significantly reduced bone turnover over the long term. However, BMD was not decreased in the 3 years after TSS.

We previously reported changes in levels of bone turnover markers and BMD in acromegalic patients in the first year following TSS [6]. In the present study, BMD remained stable up to three years after TSS, despite significant changes in levels of bone turnover makers. The results of BMD are explained by changes in bone metabolism, with reduction of bone resorption occurring much earlier than bone formation due to a post-surgery decline in GH levels. Further, in the current study, BAP/urinary NTx ratio was significantly increased at 3 months and 3 years after TSS. Previous studies have reported that the ratio of procollagen type I N-propeptide (PINP) and C-terminal cross-linking telopeptide (CTX-I), other bone turnover markers, reflects the bone remodeling balance [8]. The increase of BAP/urinary NTx ratio after TSS in acromegalic patients suggests that bone formation is superior to bone resorption. The discrepancy in this bone remodeling balance may explain the maintenance of BMD after TSS in acromegalic patients. We also found that BMD at the lumbar spine and the femoral neck increased at the 3 years after TSS in only three acromegalic patients whose condition were suitable for the change of GH levels after TSS.

Recently, Claessen et al. reported that BMD at the lumbar spine and total hip did not change markedly in 15 acromegalic patients in remission for 31 months after TSS [9]. Further, they noted no marked differences in BMD between men and women. These authors’ results seem consistent with our present findings; however, of note, their acromegalic patients had been in remission for an average of 18.1 years (range: 11-28 years) at the beginning of their study. Their study point might therefore have been too late to evaluate the effect of GH decline due to treatment for acromegaly on BMD. Our study has the distinct advantage of having started evaluation of BMD before TSS.

Mazziotti et al. reported that BMD decreased significantly at the femoral neck but did not change at the lumbar spine in acromegalic patients after 3 years of follow-up [10]. Their results regarding BMD at the femoral neck differ from ours, likely because the frequency of hormone replacement for hypopituitarism in their patients was much higher than in our patients (replacement for hypoadrenalism, 56% vs. 11%; replacement for hypothyroidism, 52% vs. 17%). Glucocorticoid replacement was independently associated with reduced BMD at the femoral neck and lumber spine and an increased frequency of osteopenia in women with ACTH insufficiency [11]. In primary hypothyroidism patients, supraphysiological doses of L-thyroxine decreased BMD at the femoral neck and lumber spine [12]. These previous findings suggest that hormone replacement for hypoadrenalism and hypothyroidism may reduce BMD. Alternatively, difference in number or quality of cured acromegaly between previous reports and current study may explain.

The risk of bone fracture is still unclear in active acromegalic patients [13-15] as well as after treatment [9, 10]. However, whether or not maintenance of BMD after TSS reduced risk of fracture is unclear, as we were unable to evaluate the risk of bone fracture in the current study. Further, we did not evaluate bone quality. Clearly, a study about bone quality and a prospective study to evaluate the incidence of bone fractures in a large number of acromegalic patients will be needed to clarify these points.

In conclusion, the present study demonstrated that a rapid decline in GH levels after TSS does not affect BMD up to 3 years after surgery in a limited number of heterogenous patients with treated acromegaly, despite significant changes in levels of bone turnover markers.

**Disclosures**

The authors declare no personal financial or institutional interest in this article.
References