Original article

Longitudinal changes of biochemical markers and bone mineral density in hyperthyroid patients during antithyroid drug therapy

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Abstract

Objective : The aim of the present study was to clarify whether patients with Graves' disease who have lost bone mass can restore bone mass to age-matched control levels by antithyroid drug therapy.

Patient/Materials and Methods : One male and 16 female patients (aged 21-71 years, mean±SE 39.9±16.5) with untreated Graves' disease were included in the study. Methimazole or propylthiouracil was given to all of the patients. Biochemical markers (serum N-mid osteocalcin (OC_{N-mid}), alkaline phosphatase (ALP), type I collagen C-terminal telopeptide (sCTx), urinary pyridinoline (Pyr), deoxypyridinoline (Dpyr) and type I collagen C-terminal telopeptide (uCTx) and bone mineral density at the distal one third of the radius were assessed prior to treatment, and in the first, third, sixth and twelfth months of treatment.

Results : All biochemical markers had increased significantly 12 months after treatment compared with the baseline values (OC_{N-mid}, $p<0.05$; ALP, $p<0.01$; sCTx, $p<0.05$; Pyr, Dpyr, uCTx, $p<0.01$). Among the biochemical markers, urinary Pyr and

Dpyr had decreased the most prominently 12 months after treatment. However, BMD at the distal one third of the radius did not improve after 12 months of treatment.

Conclusion : Based on assessments of BMD at the distal one third of the radius, one year is not enough to restore bone mass using antithyroid drug therapy in patients with Graves' disease.

Key words : bone formation markers, bone resorption markers, bone mineral density, hyperthyroidism

Introduction

Since Fraser *et al.* reported in 1971 on severe osteoporosis and fractures following hyperthyroidism, attention has been paid to thyroid hormone-induced osteoporosis¹⁾. Histomorphometric study and metabolic markers of bone turnover confirmed that excess thyroid hormone resulted in high turnover^{2,3,4)}. Both bone resorption and bone formation markers have been shown to significantly increase in patients with untreated hyperthyroidism and in patients undergoing thyroid hormone replacement^{5,6)}. Solomon *et al.* reported that in those with a history of thyroid disorders there might be a propensity for fractures to occur earlier in life⁷⁾.

In patients with hyperthyroidism, it is important

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to assess metabolic markers of bone turnover and bone mineral density (BMD) during the course of therapy in order to prevent further bone loss and fractures. In one study of patients with hyperthyroidism, BMD in the appendicular bone decreased more prominently than that in the axial bone since porosity in the cortical bone was more severe than that in the cancellous bone⁵. The distal one third of the radius is one of the sites often used to monitor BMD in patients with hyperthyroidism, because one third of the distal radius is relatively rich in the cortical bone⁸. Whether patients who have lost bone mass can be restored to age-matched control levels is still debatable. More data using longitudinal monitoring of biochemical markers and BMD will be necessary to assess bone metabolism during antithyroid drug therapy. In the last decade, various bone specific biochemical markers have been developed. Urinary type I collagen C-terminal telopeptide is one of the specific bone resorption markers as well as urinary pyridinium crosslinks⁹. Recently, type I collagen C-terminal telopeptide in sera has also been used, and it accurately reflects bone resorption in patients with metabolic bone disorders¹⁰.

In this study, we evaluated bone turnover in patients with hyperthyroidism who are undergoing antithyroid drug therapy for 12 months. This was done by using the novel biochemical markers and BMD in the distal one third of the radius.

Materials and Methods

Subjects

The study included one male and 16 female patients (aged 21-71 years, mean $\pm$ SE ; 39.9 $\pm$ 16.5) with untreated Graves' disease who had been admitted to an out-patient clinic (Ishigaki Clinic). The diagnosis of Graves' disease was made if one or more clinical symptoms, including thyrotoxic symptoms (tachycardia, weight loss, finger tremor), a diffuse goiter and exophthalmos, and abnormal levels of thyroid-related hormones, were

found. The latter is defined as low serum levels of thyroid stimulating hormone (TSH) in combination with high serum free thyroxine (FT4), free triiodothyronine (FT3) and TSH binding inhibiting antibody (TBII) levels. All patients started to take antithyroid drugs (5-30 mg daily doses of methimazole) at the beginning of the study. Propylthiouracil (50-300 mg daily) was substituted when the patient had side effects to methimazole. Blood and urine samples were taken between 0900 h and 1100 h and BMD at one third of the distal radius was measured prior to therapy and 1, 3, 6 and 12 months after the beginning of treatment. The patients had not been taking a medicine such as estrogen, glucocorticoids, anticonvulsants, vitamin D or calcium supplements that might have affected calcium and mineral metabolism. No patients were treated with radioiodine or surgical therapy prior to the study. No patients had diseases that were known to affect bone metabolism. Informed consent was obtained from all patients.

Biochemical markers

Serum FT3 and FT4 were measured by a radioimmunoassay. Serum TSH was measured by a highly sensitive immunoradiometric assay (IRMA). Serum TBII was measured by a radioreceptor assay. Reference ranges were 3.8-6.6 pmol/l for FT3, 12.8-23.1 pmol/l for FT4, 0.4-4.0 mU/l for TSH and less than 15% for TBII. Serum total alkaline phosphatase (ALP) was determined by routine laboratory methods.

Urinary pyridinoline (Pyr) and deoxypyridinoline (Dpyr) were measured using high performance liquid chromatography after acid hydrolysis using a Glison ASPEC (automated sample preparation with extraction column). This procedure follows Pratt *et al*¹¹. In our protocol, the intra-and interassay coefficients of variation were 6.4% and 5.9% for Pyr and 6.0% and 6.0% for Dpyr, respectively.

Urinary type I collagen C-terminal telopeptide (uCTX) was measured by using a CrossLaps ELISA kit, the same procedure as in Bonde *et al*⁹.

The intra- and interassay coefficients of variation were less than 6% and 8%, respectively. Values of urinary Pyr, Dpyr and CTx were corrected for urinary creatinine concentration, which was measured with an autoanalyser.

The serum concentration of osteocalcin (OC_{N-mid}) was measured in an enzyme-linked immunosorbent assay (ELISA) (two-site N-MID Osteocalcin, Osteometer BioTech A/S, Denmark)¹²⁾. The intra- and inter assay coefficients of variation were less than 4.2% and 4.0%, respectively, and the detection limit was 2.0 µg/l.

The serum levels of type I collagen C-terminal telopeptide (sCTx) were measured by using the recently developed specific ELISA that Bonde et al. discussed¹⁰⁾. The intra- and interassay coefficients of variation were 6.7% and 9.2%, respectively.

Bone mineral density measurements

Bone mineral density (BMD) at the distal one third of the radius in the left arm was measured using dual energy X-ray absorptiometry (DEXA ; DCS-600EX, Aloka, Tokyo). Using this method, the coefficient of variation for repeated short-term phantom measurements was less than 0.5% and in vivo reproducibility was less than 1 %. Values were expressed as BMD Z-scores which were calculated as SDs from the mean BMD values in a control group of 555 healthy females aged 20-80 years.

Statistical analysis

Data was analyzed using a Statview 4.02 program on a Macintosh computer. Differences in two related samples were analyzed with two-way analysis of variance (ANOVA). To eliminate the effect of the baseline values on following longitudinal values, percent changes from the initial values were calculated at each time point. Percent changes in metabolic bone markers were analyzed using one-way analysis of variance (ANOVA). Correlations between changes in thyroid-related hormones and metabolic bone markers and BMD were calculated by simple

regression analysis. All values were expressed as mean±SE. Statistical significance was defined as $p^{**}<0.01$ or $p^{*}<0.05$.

Results

Table 1 shows the anthropometric data, basal levels of thyroid hormones and biochemical markers, and Z-scores of BMD at the distal one third of the radius. Prior to the antithyroid drug therapy, mean values of FT3, FT4 and TBII were above the reference range and TSH was below the reference range. Mean values of bone resorption and formation markers were above the reference range. Mean Z-scores of BMD at the distal one third of the radius had negative values. Based on these results, the patients were characterized as having higher bone turnover and lower bone mass than people in age-matched control groups. Serial changes in thyroid-related hormones during antithyroid drug therapy are shown in Figure 1. The levels of FT3, FT4 and TBII tended to decrease and the levels of TSH to increase after starting antithyroid drug therapy. Specifically, the levels of TBII decreased significantly compared to the baseline levels at 3, 6 and 12 months after the start of treatment. Serum levels of OC_{N-mid} decreased significantly at all points (1, 3, 6 and 12 months) after the start of treatment and serum ALP had decreased significantly 6 and 12 months after treatment was started (Figure 2). Urinary CTx decreased significantly after 3, 6 and 12 months, and sCTx decreased significantly after 6 and 12 months of treatment (Figure 3). Both urinary Pyr and Dpyr decreased significantly after 3, 6 and 12 months of treatment (Figure 4). Percent changes in the mean values from the baseline of each biochemical parameter are shown in Figure 5. Percent decreases from the initial values of the bone resorption markers (uCTx, sCTx, Pyr and Dpyr) were more prominent than those of the bone formation markers (OC_{N-mid} and ALP). In particular, urinary Pyr and Dpyr tended to decrease more than other markers after 12 months of treatment. However, p values did not reach

Table 1 Baseline data of 17 patients.

Male : Female	1 : 16	
Age (years)	39.9±16.5	
Height (cm)	155.1±6.9	
Weight (kg)	50.1±7.1	
BMI (kg/m ²)	20.8±2.2	Reference range
TSH (mU/l)	0.08±0.24	0.30-3.90
FT3 (pmol/l)	11.3±7.9	4.0-6.5
FT4 (pmol/l)	33.0±26.9	10.9-22.1
TBII (%)	39.6±27.1	< 15
OC _{N-mid} (μg/l)	51.1±5.5	5.8-39.4 ^a
ALP (IU/l)	358.2±42.6	80-260
uCTx (μg/mmol creat.)	889±169	not available
sCTx (pM)	4916±742	not available
Pyr (nmol/mmol creat.)	777.2±97.7	10.6-46.4 ^b
Dpyr (nmol/mmol creat.)	176.8±24.3	1.1-13.1 ^b
Z-scores for BMD	-0.92±0.32	

Data are shown as the mean±SE.

BMI, body mass index ; TSH, thyroid stimulating hormone ; FT3, triiodothyronine ; FT4, thyroxine ; TBII, TSH binding inhibiting antibody ; OC_{N-mid}, N-mid osteocalcin ; ALP, alkaline phosphatase ; uCTx, urinary type I collagen C-terminal telopeptide ; sCTx, serum type I collagen C-terminal telopeptide ; Pyr, pyridinoline ; Dpyr, deoxypyridinoline ; BMD, bone mineral density

^aMean±2SD in 93 postmenopausal women (Ref. 28)

^bMean±2SD in 45 postmenopausal women (Ref. 29)

Figure 1 Longitudinal changes of thyroid functions during the follow-up (upper panel : FT3, FT4 and TSH, lower panel : TBII). Data are shown as the mean±SE. **P<0.01 vs. month 0.

FT3, triiodothyronine ; FT4, thyroxine ; TSH, thyroid stimulating hormone ; TBII, TSH binding inhibiting antibody

Figure 2 Longitudinal changes of bone formation markers (upper panel : OC_{N-mid}, lower panel : ALP). Data are shown as the mean±SE. *P<0.05, **P<0.01 vs. month 0. The shaded area indicates the reference range.

OC_{N-mid}, N-mid osteocalcin ; ALP, alkaline phosphatase

Figure 3 Longitudinal changes of bone resorption markers (upper panel : uCTX, lower panel : sCTX). Data are shown as the mean±SE. *P < 0.05, **P < 0.01 vs. month 0.

uCTX, urinary type I collagen C-terminal telopeptide
sCTX, serum type I collagen C-terminal telopeptide

Figure 4 Longitudinal changes of bone resorption markers (upper panel : urinary Pyr, lower panel : urinary Dpyr). Data are shown as the mean±SE. *P < 0.05, **P < 0.01 vs. month 0. The shaded area indicates the reference range.

Pyr, pyridinoline ; Dpyr, deoxypyridinoline

significance because of the large standard deviation of each bone marker. Percent changes of urinary Pyr and Dpyr were significantly larger than percent changes of serum ALP at 6 months of treatment (Pyr vs. ALP : $p=0.003$, Dpyr vs. ALP : $p=0.008$). However, at the final measurement

Figure 5 Percent changes of the mean values from the baseline of each biochemical parameter. Data are shown as the mean values. Percent changes of Pyr and Dpyr were significantly greater than serum ALP at 6 months of treatment (Pyr and Dpyr vs. ALP : ** $p < 0.01$).

OC, N-mid osteocalcin ; ALP, alkaline phosphatase ; uCTX, urinary type I collagen C-terminal telopeptide ; sCTX, serum type I collagen C-terminal telopeptide ; Pyr, pyridinoline ; Dpyr, deoxypyridinoline

Figure 6 Longitudinal changes in Z-scores for bone mineral density (BMD) at the distal one third of the radius.

BMD, bone mineral density

neither bone formation nor bone resorption markers had decreased to the levels in the reference ranges. The longitudinal changes in the Z-scores of BMD at the distal one third of the radius are shown in Figure 6. Bone mineral density did not change significantly throughout the study. Correlations between thyroid-related hormones and metabolic bone markers and BMD were evaluated. Percent changes of serum FT3 and FT4 from the baseline values at 12 months after treatment were significantly correlated with percent changes of uCTX and sCTX (percent (%))

changes of FT3 vs. uCTx : $r=0.452$, $p=0.033$, % changes of FT3 vs. sCTx : $r=0.489$, $p=0.025$, % changes of FT4 vs. uCTx : $r=0.594$, $p=0.009$. % changes of FT4 vs. sCTx : $r=0.587$, $p=0.009$). Percent changes of TSH or TBII did not correlate with bone resorption or formation markers. Changes in the thyroid-related hormones did not correlate with changes in radius BMD.

Only one female patient among all of the subjects did not have the thyroid function go into remission at the end of study. All metabolic bone markers of this patient decreased by 22-62% (uCTx : -22.7% , sCTx : -28.5% , Pyr : -38.5% , Dpyr : -53.6% , OC_{N-mid} : -62.0% and ALP : -39.6%), and Z-scores of radius BMD increased by $+0.85$ SD compared to the baseline values at the final measurement. In the 16 patients with remission, percent changes were -42.7 ± 19.6 (mean $\pm$ SE) % for uCTx, $-47.1 \pm 14.2\%$ for sCTx, $-77.3 \pm 5.6\%$ for Pyr, $-77.8 \pm 4.2\%$ for Dpyr, $-36.9 \pm 15.1\%$ for OC_{N-mid} , and $-39.2 \pm 6.9\%$ for ALP, while Z-scores of radius BMD decreased by -0.21 ± 0.15 SD compared to the baseline values.

Discussion

We investigated longitudinal changes of biochemical markers and BMD at the distal one third of the radius after administration for 12 months of antithyroid drugs in patients with Graves' disease. In this study, both bone resorption and formation markers decreased concurrently with a decrease in thyroid functions. We found a significant correlation between percent changes of FT3 and FT4 and percent changes in urinary or serum type I collagen breakdown products (uCTx and sCTx). This result indicates that for patients with Graves' disease serum levels of FT3 and FT4 could be an indicator of bone resorption during antithyroid drug therapy.

Bone resorption markers decreased in the early phase of antithyroid drug therapy. This finding is consistent with previous studies^{13,14,15,16}. However, in these studies the time required to see changes

in bone formation markers during antithyroid therapy varied^{14,15,16,17}. Garnero *et al.* found a rapid and acute fall in osteocalcin levels in patients with hyperthyroidism after one month of treatment¹⁷. In our study, one bone formation marker (OC_{N-mid}) decreased significantly from baseline at one month, but bone resorption markers (Pyr, Dpyr, uCTx) did not decrease significantly until after 3 months. Thyroid hormone exerts an effect on osteoblastic bone formation directly through thyroid hormone receptor on the osteoblast ; however, it exerts an indirect effect on osteoclastic bone resorption through interleukin^{6,18,19,20}. Our results suggest that a rapid decrease in OC_{N-mid} might be the direct effect of antithyroid drugs through thyroid hormone receptor on the osteoblast. N-mid osteocalcin (OC_{N-mid}) could be a more sensitive marker for monitoring bone turnover in the early phase of treatment of Graves' disease than bone resorption markers.

In 1996, Miyakawa *et al.* measured serum de novo synthetic products of type I collagen (PICP) and degradation products of type I collagen (ICTP) in patients with Graves' disease undergoing antithyroid drug therapy²¹. They found a significant decrease in both markers and a significant increase in the PICP/ICTP ratio at 6 months of treatment. Siddiqi *et al.* used the coupling index (Z-scores of formation marker minus Z-scores of resorption marker) for evaluating the imbalance of bone formation and bone resorption in patients with hyperthyroidism who were undergoing therapy¹⁵. According to their results, the coupling index changed from a negative value to a positive value during the antithyroid drug therapy. At the end of our study, the decrease of bone resorption markers was more prominent than that of bone formation markers. This result suggests that high bone turnover with predominant bone resorption tended to change to a balanced turnover state after antithyroid drug therapy, although the coupling index was not calculated in our study.

For two years beginning in 1985, Toh *et al.* monitored the radius bone mineral contents of 23

male patients with Graves' disease undergoing radioiodine or antithyroid drug therapy²². In their study, bone mineral loss in the patients improved, but a return to the level of age-matched controls was not achieved after 2 years of therapy. Furthermore, in spite of successful antithyroid therapy bone mineral contents significantly decreased after 1 year of treatment when compared to the initial values. Siddiqi *et al.* examined BMD in 17 patients with Graves' disease who were undergoing therapy¹⁵. They found a significant increase in lumbar spine and femoral neck BMD after 1 year of treatment. However, in their study initial mean values of lumbar spine and femoral neck BMD Z-scores were both positive. Subjects in their study might have been patients whose osteoporosis was less severe than those in our study. Nagasaka *et al.* reported a significant increase in both the cortical bone striation index in the metacarpals and lumbar spine BMD after 1 and 2 years of antithyroid drug therapy in patients with Graves' disease, although the study had relatively few subjects¹⁶. Several recent studies have reported significant increases in lumbar and femoral neck BMD during antithyroid therapy in patients with hyperthyroidism^{23,24}.

A significant change in BMD in the distal one third of the radius was not observed in our study. This lack of a significant change can be accounted for as follows. First of all, biochemical parameters of bone turnover did not decline to the reference range by the final measurement although they did decrease significantly after the therapy when compared to the baseline values. There might be a time lag between changes in biochemical parameters and the change in BMD. The former would precede the latter. Therefore, in future studies it will be necessary to assess the biochemical parameters and BMD for a longer period of time. Second, a change in BMD could be dependent upon the site of the BMD measurement. In both Toh's study and ours, BMD or BMC at the distal radius was measured, and it did not improve during the 1 year of treatment²².

However, lumbar spine or femoral neck BMD was measured in other studies, and after 1 year of treatment there was significant improvement in these BMD. In our previous study, the radius BMD accurately reflected bone loss in patients with untreated Graves' disease⁹. The lumbar spine and femoral neck are both weight-bearing bones, while the distal radius is not a weight bearing-bone. It is possible that not only antithyroid drugs but also mechanical stress could affect BMD. Third, Suwanwalaikom *et al.* investigated osteoblast gene expression response of L-thyroxine administration in rats²⁵. They found a differential gene expression response of alkaline phosphatase and osteocalcin in the vertebra and femur. Their results indicated that there could be site selectivity in osteoblast gene expression response of drug therapy in patients with hyperthyroidism. The radius may not respond to antithyroid drugs as well as vertebra or femur in regards to osteoblast gene expression. Finally, the sample size in our study was relatively small. More subjects will be necessary to discuss whether forearm BMD in patients with hyperthyroidism increases after antithyroid drug intervention.

Only one patient did not go into remission after 12 months of treatment. In this patient, radius BMD increased and metabolic bone markers decreased at the final measurement when compared to the initial values although thyroid function was not under-controlled. However, because only one patient did not go into remission, data could not be compared between the patients with remission and those without remission.

Since postmenopausal women lack estrogen protection against bone loss, it is probable that restoration of bone mass in premenopausal women would be greater than that in postmenopausal women. Unfortunately, since the number of subjects was too small to divide into pre- and postmenopausal groups, we could not compare BMD in the premenopausal patients in our study with BMD in the postmenopausal patients.

There are two conservative therapy options for patients with Graves' disease. One is antithyroid drug administration like that undertaken in this study. The other option is radioiodine therapy. In postmenopausal hyperthyroid patients with initially low lumbar BMD, BMD increased significantly by 6.5% after a 2-year treatment of radioiodine, while in the study by Obermayer-Pietsch *et al.* it decreased by 4.3% in the initially normal BMD group²⁶⁾. The effectiveness of radioiodine therapy for patients with Graves' disease might vary depending upon the bone quality and quantity before the treatment. Azizi *et al.* reported on a ten-year follow-up to methimazole or radioiodine treatment for hyperthyroid patients²⁷⁾. According to their results, a decrease in bone turnover and conversion of the mineral balance to positive occurred earlier in the methimazole-treated group than in the radioiodine-treated group. However, there is still controversy about which intervention 'antithyroid drug or radioiodine, is superior when considering recovery of bone loss in patients with hyperthyroidism.

Conclusion

Bone turnover decreased through the use of antithyroid drugs, but when assessing BMD at the distal one third of the radius, one year of treatment was not enough to restore bone mass in patients with Graves' disease.

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