Original

Evaluation of Bone Strength in Women with Graves' Disease by Ultrasound Bone Densitometry

Yoshiyuki BAN, Yoshio BAN, Matsuo TANIYAMA
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Abstract: Bone mineral density (BMD) decreases in Graves' disease due to the high rate of bone turnover with a predominance of resorption. Studies have evaluated the use of dual energy X-ray absorptiometry (DEXA) for investigating bone metabolism. Bone strength is a function of BMD and bone quality. Quantitative ultrasound (QUS), which involves no radiation exposure and uses portable equipment, assesses both BMD and bone quality. This study investigated the relationship between broadband ultrasound attenuation (BUA), speed of sound (SOS), and stiffness to BMD measured at the calcaneus in a closely matched region of interest (ROI); and the relationship between BMD and ultrasound parameters to markers of bone metabolism in female patients with Graves' disease. We investigated 9 patients with untreated Graves' disease (group G: mean age, 41.0 ± 7.6 years) and 19 patients in remission from Graves' disease (group RG: mean age, 55.7 ± 5.0 years). Ultrasound parameters by QUS correlated significantly with calcaneus BMD in the ROI only for group RG, suggesting that bone quality played an additional role. In 8 patients who were in remission for more than 5 years, the Z-scores for ultrasound parameters of the calcaneus assessed by QUS were in the negative range. No significant correlation was found between blood or urine markers and ultrasound parameters or calcaneus BMD. These results show that the lack of a significant correlation between ultrasound parameters and BMD of the calcaneus in our present group G indicates that BUA and SOS are affected by mineral content and by other material and structural properties. In addition, even Graves' disease patients who are in long-term remission may not have had recovery of bone density to normal levels following hyperthyroidism.

Key words: Graves' disease, ultrasound parameters, bone mineral density

Introduction

Hyperthyroidism is associated with increased bone turnover\textsuperscript{1). Bone mineral density (BMD) has been shown to decrease in hyperthyroidism due to the high rate of bone turnover with a predominance of resorption\textsuperscript{2). Thus, hyperthyroidism has been considered to be a risk factor for osteoporosis and fractures\textsuperscript{3-4). Cross-sectional studies of subjects with hyperthyroidism have confirmed reduced BMD at various skeletal sites\textsuperscript{5-12). It is unclear

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whether the bone loss induced by hyperthyroidism predominantly affects the axial or the peripheral skeleton. Furthermore, one study suggested that the increased tendency of bone fractures in hyperthyroidism was mediated by low BMD and bone quality\textsuperscript{[13]}.

Bone strength is a function of BMD and bone quality. Quantitative ultrasound (QUS), which does not involve radiation exposure and uses portable equipment, assesses BMD and bone quality. \textit{In vivo} studies have shown the value of ultrasound to predict osteoporotic fractures\textsuperscript{[14-16]}, even when corrected for BMD\textsuperscript{[17]}. Speed of sound (SOS) is theoretically related to density; in contrast, broadband ultrasound attenuation (BUA) has no direct theoretical relation to density. Lin \textit{et al.}\textsuperscript{[18]} found no significant relation between SOS in cores taken from human vertebral bodies and apparent density (dry weight of specimen/total volume), while BUA correlated significantly with apparent density \textit{in vitro} (r=0.63, p<0.00001). Correlations between BUA and density are often higher in \textit{in vitro}\textsuperscript{[19-21]} studies than in \textit{in vivo} ones\textsuperscript{[22,23]}. A close relationship between ultrasound parameters and bone mineral density would limit the extent of structural information attributable to SOS or BUA.

The relationship between ultrasound and BMD \textit{in vivo} has been studied in patients with Graves' disease\textsuperscript{[22,23]}. Gomez Acotto \textit{et al.}\textsuperscript{[23]} measured BMD of the lumbar spine, femoral neck, total skeleton, and body composition using dual energy X-ray absorptiometry (DEXA) and QUS parameters of the calcaneus, and suggested that hyperthyroidism equally affects cortical and trabecular bones and bone quality. Ultrasound analysis, which generally measures in the calcaneus due to its accessibility, suitable shape, and high trabecular content, is often compared with BMD measurements at the lumbar spine or femoral neck. Recent studies have investigated the relationship between ultrasound and BMD at the calcaneus\textsuperscript{[24,25]}. The present study investigated the relationship between BUA, SOS, and stiffness to BMD measured at the calcaneus in a closely matched region of interest (ROI); and the relationship between BMD and ultrasound parameters to markers of bone metabolism in female patients with Graves' disease. Our study represents the first comparison of ultrasound and DEXA measurements of the calcaneus in patients with hyperthyroidism.

Subjects and Methods

Subjects

A total of 9 patients with untreated Graves' disease (group G: mean age, 41.0±7.6 years) and 19 patients in remission from Graves' disease (group RG: mean age, 55.7±5.0 years) were studied. Details regarding the patient groups are shown in Table 1. Graves' disease was diagnosed on the basis of clinical symptoms and biochemical confirmation of hyperthyroidism, such as diffuse goitre, ophthalmopathy, elevated radioactive iodine uptake, and elevated thyroid hormone levels. Remission of Graves' disease was defined as follows: thyroid-stimulating hormone (TSH) in the negative range for 6 months and subsequently in the euthyroid state for a period of at least six months after cessation of antithyroid drug. Remission ranged from 0.5 to 22.0 years. A total of 5 patients from group G and 18 patients from group RG were postmenopausal. The postmenopausal state was defined as amenorrhea for at least 12 months. A total of 4 patients from group G and 1 patient from group RG were premenopausal, and all of them reported generally regular menses. No patients reported a history of rheumatoid arthritis, diabetes mellitus, or alcohol abuse, or
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any other serious medical disorder. No patients had been treated with thiazide diuretics, calcium, vitamin D, estrogen, bisphosphonate, or bone-active medications for 12 months prior to this study. All patients gave informed consent for study procedures. The research protocol was approved by the ethics committee of our hospital.

Ultrasound measurements

Ultrasound measurements of the calcaneus were made for BUA in dB Mhz⁻¹ in the frequency range of 200–600 kHz and for SOS in m⁻¹ using a Lunar Achilles system (Lunar Corp., Madison, WI, USA), which consists of two 2.54-cm diameter unfocused transducers mounted coaxially approximately 9.5 cm apart. Acoustic coupling is accomplished by submerging the transducers and the heel in room temperature water with surfactant to wet the foot. The right foot was measured in all subjects. A third computer-calculated parameter from the BUA and SOS values is the stiffness index (expressed as a percentage of the young adult value), which is a combination of normalized BUA and SOS. To calculate stiffness, BUA and SOS are normalized and expressed as a percentage of the mean value for young normal women as calculated from the following equations:

\[
\text{Normalized BUA} = \frac{(\text{BUA} - 50)}{75} \times 100% \\
\text{Normalized SOS} = \frac{(\text{SOS} - 1380)}{180} \times 100% \\
\]

The short-term precision errors (coefficients of variation) were 0.1% for SOS, 0.9% for BUA, and 0.8% for stiffness index, while the precision errors in vivo ranged from 0.2% to 0.7% for SOS, from 0.4% to 1.4% for BUA, and from 0.2% to 0.9% for stiffness index. The standard values for ultrasound measurements in each decade of life have been calculated and previously reported for 842 females ranging in age from 20 to 79 years. This previously studied group, which was representative of the general population, served as a control reference to calculate age-adjusted Z-scores for our patients using the following formula:

\[
\text{Patient X} - \left( \frac{\text{mean normal}}{\text{SD of mean normal}} \right) \times X \\
\]

where X = BUA, SOS, or stiffness index

DEXA measurements

BMD was measured using a Hologic QDR 2000 DXA machine (Hologic, Waltham, MA). Calcaneus BMD measurements were made using an ROI based on anatomical markers and located in the posterior part of the calcaneus. The ROI is a circle (area 2 cm²) positioned in the posterior part of the calcaneus approximately equidistant from its sides. Data were acquired from the whole calcaneus profile view using a lumbar spine scanning protocol. Results were expressed as BMD (g cm⁻²). Coefficients of variation in our laboratory were less than 2%.

Biochemical markers

Following an overnight fast, early morning blood and urine samples were collected from the subjects. Serum and urine were stored at −70°C until assays. Serum free triiodothyronine (FT3) and free thyroxine (FT4) concentrations were measured by radioimmunoassay. Serum thyroid-stimulating hormone (TSH) concentration was measured with a highly sensitive immunoradiometric assay (IRMA). Reference ranges were 2.2 to 4.1 pg/ml for
FT3, 0.88 to 1.81 ng/dl for FT4, and 0.35 to 3.73 μU/ml for TSH. The serum concentration of bone-type alkaline phosphatase (B-ALP) was measured with densitometric scan following polyacrylamide disc gel electrophoresis (disc-PAGE). Osteocalcin (OC) was measured in serum with IRMA. Materials for both assays were obtained from Mitsubishi Kagaku BCL (Tokyo, Japan). Intra- and interassay coefficients of variation were less than 10%.

Urinary hydroxyproline (Hp) and deoxypyridinoline (Dpyr) were measured with high-performance liquid chromatography (HPLC; Japan Spectroscopic, Tokyo, Japan). Intra-assay coefficients of variation were less than 2.8% for Hp and 5.2% for Dpyr; interassay coefficients of variation were less than 3.1% for Hp and 3.2% for Dpyr. Values of urinary Hp and Dpyr were corrected for urinary creatinine concentration, which was measured with an autoanalyzer.

**Statistical analysis**

All measurement results are presented as mean ± standard deviation (X ± SD). The relationship between ultrasound parameters and calcaneus BMD in the anatomical ROI were expressed as Pearson product moment correlation coefficient (r), which represents the magnitude of the relation and p-value (p), which represents the reliability of the relationship. Statistical correlations were also determined with the Spearman rank correlation method. The significance of differences between study groups was obtained with paired and unpaired Student's t-tests. Throughout the study p-values less than or equal to 0.05 were considered significant.

**Results**

Serum TSH concentrations were higher in group RG than in group G, and serum FT3 and FT4 concentrations were higher in group G than in group RG (Table 1). Serum B-ALP and OC concentrations and urinary Hp and Dpyr are shown in Table 2. Serum OC concentration and urinary Hp and Dpyr were higher in group G than in group RG (Table 2).

Table 3 shows the average values of ultrasound parameters and their Z-score values in both groups. Although there was no significant difference between the Z-score values for

<table>
<thead>
<tr>
<th>Table 1. Clinical characteristics and biochemistry of the two groups according to thyroid function</th>
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<tr>
<td></td>
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<tr>
<td>Age (years)</td>
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<tr>
<td>Age range (years)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
</tr>
<tr>
<td>TSH (0.35–3.73 μU/ml)</td>
</tr>
<tr>
<td>FT3 (2.2–4.1pg/ml)*</td>
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<tr>
<td>FT4 (0.88–1.81ng/dl)*</td>
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</table>

Data are mean±SD. *p<0.05 vs. group RG, **p<0.0001 vs. group RG. ‘Normal ranges are shown in parentheses. BMI, body mass index ; TSH, thyroid-stimulating hormone ; FT3, free triiodothyronine ; FT4, free thyroxine.
Table 2. Mean values of bone mineral density of the calcaneus and bone turnover markers in female patients in the G and RG groups.

<table>
<thead>
<tr>
<th></th>
<th>group G</th>
<th>group RG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>less than 60 years old</td>
<td>60 or more years old</td>
</tr>
<tr>
<td>Calcaneus BMD in the anatomical ROI (g/cm²)</td>
<td>0.55±0.13</td>
<td>0.55±0.06</td>
</tr>
<tr>
<td>S-bone type ALP (IU/L)</td>
<td>136.2±47.7</td>
<td>100.6±25.0</td>
</tr>
<tr>
<td>S-osteocalcin (ng/mL)</td>
<td>11.7±4.2</td>
<td>7.7±2.2</td>
</tr>
<tr>
<td>U-total hydroxyproline (nmol/mm mol creat)</td>
<td>62.8±24.6</td>
<td>20.9±3.6**</td>
</tr>
<tr>
<td>U-total deoxypyridinoline (nmol/mm mol creat)</td>
<td>23.9±13.2</td>
<td>6.0±1.6**</td>
</tr>
</tbody>
</table>

S, serum; U, urine; BMD, bone mineral density; ROI, region of interest; ALP, alkaline phosphatase. Data are means±SD except where noted. *p<0.05 vs. group G, **p<0.01 vs. group G.

Table 3. Mean values of ultrasound parameters and Z-score values in G and RG group.

<table>
<thead>
<tr>
<th></th>
<th>group G</th>
<th>group RG</th>
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<tbody>
<tr>
<td></td>
<td>Mean±SD</td>
<td>Z-score</td>
</tr>
<tr>
<td>SOS (m/s)</td>
<td>1511±22</td>
<td>-1.53±1.45</td>
</tr>
<tr>
<td>BUA (dB/MHz)</td>
<td>107.1±6.7</td>
<td>-0.60±1.17</td>
</tr>
<tr>
<td>Stiffness (%)</td>
<td>74.4±9.9</td>
<td>-1.28±0.87</td>
</tr>
</tbody>
</table>

SOS, speed sound; BUA, broadband ultrasound attenuation. Data are means±SD except where noted. *p<0.05, **p<0.001 compared with the normal values determined by Yamazaki et al(27).

Table 4. Relationship between ultrasound parameters and calcaneus BMD in the anatomical ROI.

<table>
<thead>
<tr>
<th></th>
<th>group G</th>
<th>group RG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcaneus BMD in the anatomical ROI (g/cm²)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>r</td>
<td>p</td>
</tr>
<tr>
<td>SOS (m/s)</td>
<td>0.292</td>
<td>0.5018</td>
</tr>
<tr>
<td>BUA (dB/MHz)</td>
<td>0.253</td>
<td>0.5630</td>
</tr>
<tr>
<td>Stiffness (%)</td>
<td>0.298</td>
<td>0.4920</td>
</tr>
</tbody>
</table>

BMD, bone mineral density; ROI, region of interest; SOS, speed of sound; BUA, broadband ultrasound attenuation.

BUA, SOS, and stiffness in the G and RG groups, the Z-score values for all ultrasound parameters were in the negative ranges in both groups (Table 3). The Z-score values for SOS in both groups and the stiffness in group RG were significantly below the normal values determined by Yamazaki et al(27).

Table 4 shows the relationship between ultrasound parameters with calcaneus BMD in the anatomical ROI for groups G and RG. All ultrasound parameters correlated significantly with calcaneus BMD in the anatomical ROI in group RG but not in group G (Table 4). Comparison of the Z scores for ultrasound parameters between short-term (less than 5 years) and long-term (5 or more years) remission in group RG are shown in Table 5. In
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Table 5. Comparison of the Z scores for ultrasound parameters between short-term (less than 5 years) and long-term (5 or more years) remission.

<table>
<thead>
<tr>
<th></th>
<th>Short-term (n=11)</th>
<th>Long-term (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOS (Z-score)</td>
<td>$-1.72 \pm 0.97^{**}$</td>
<td>$-2.62 \pm 1.49^{**}$</td>
</tr>
<tr>
<td>BUA (Z-score)</td>
<td>$-0.09 \pm 0.14$</td>
<td>$-1.06 \pm 2.04$</td>
</tr>
<tr>
<td>Stiffness (Z-score)</td>
<td>$-1.06 \pm 0.59^{**}$</td>
<td>$-1.79 \pm 1.11^{*}$</td>
</tr>
</tbody>
</table>

SOS, speed of sound; BUA broadband ultrasound attenuation. *$p<0.01$, $p<0.005$ compared with the normal values determined by Yamazaki et al (27)

Table 6. Correlations between biochemical markers of turnover and thyroid hormones in group G.

<table>
<thead>
<tr>
<th></th>
<th>B-ALP</th>
<th>OC</th>
<th>Hp</th>
<th>Dpyr</th>
</tr>
</thead>
<tbody>
<tr>
<td>FT3</td>
<td>0.234</td>
<td>0.025</td>
<td>0.176</td>
<td>0.847*</td>
</tr>
<tr>
<td>FT4</td>
<td>0.183</td>
<td>0.217</td>
<td>0.267</td>
<td>0.857*</td>
</tr>
</tbody>
</table>

FT3, free triiodothyronine; FT4 free thyroxine; B-ALP, bone type alkaline phosphatase; OC, osteocalcin; Hp, hydroxyproline; Dpyr, deoxypyridinoline. *$p<0.05$, Spearman correlation.

8 long-term remission patients, the Z scores for ultrasound parameters of the calcaneus assessed by QUS were still in the negative ranges (Table 5). Ultrasound parameters were lower, though not significant, for patients in long-term remission than for those in short-term remission. The Z-score values for SOS and stiffness in both short-term and long-term remission were significantly lower than normal.

Table 6 shows correlations between biochemical markers of turnover and thyroid hormones in group G. Significant positive correlations were observed between serum FT3, FT4, and urinary Dpyr (Table 6). No biochemical markers of turnover correlated significantly with TSH, FT3, or FT4 (data not shown). No significant correlation was found between either blood or urine markers and either ultrasound parameters or calcaneus BMD (data not shown).

Discussion

We investigated the relationship between BUA, SOS, and stiffness to BMD measured in an anatomical ROI of the calcaneus and the relationship between BMD and ultrasound parameters to markers of bone metabolism in female patients with Graves' disease. Thyroid hormones stimulate both osteoclast and osteoblast activity in vitro [38]. We found statistically significant positive correlations between FT3, FT4 and Dpyr in group G (Table 6). The biochemical marker Dpyr clearly reflected elevated levels of bone turnover in patients with Graves' disease.

In our study, BMD in an anatomical ROI of the calcaneus but not in the fixed ROI, which was positioned at a set distance from the reference axes to imitate the position of fixed ultrasound transducers, was employed as the index of BMD measurement. The position
of a fixed ROI on a DEXA scan indicates the path of the ultrasound beam. For some subjects this ROI lay partially outside the bone or included the edge of the calcaneus. Kang and Speller et al.\textsuperscript{25)} found that the correlation coefficient of ultrasound parameters and BMD of the calcaneus was not significant at a fixed ROI compared with an anatomical ROI. Our study shows that all ultrasound parameters correlated significantly with calcaneus BMD in the anatomical ROI in group RG but not in group G (Table 4). This result is similar to the value observed in an \textit{in vivo} study by Kang \textit{et al.}\textsuperscript{25)} who measured ultrasound parameters and calcaneus BMD in an anatomical ROI in normal subjects and found a correlation between BUA and calcaneus BMD in the anatomical ROI of $r=0.78$ ($p<0.0001$). The lack of significant correlation between ultrasound parameters and BMD of the calcaneus in our present group G indicates that ultrasound propagation in the bone is extremely complex and that BUA and SOS are affected by mineral content and by other material and structural properties\textsuperscript{29)}.

Although there was no significant difference between the Z-score values for BUA, SOS, and stiffness in both groups, the Z-score values for all ultrasound parameters were in the negative ranges (Table 3). These findings are similar to those observed in an \textit{in vivo} study by Gomez Acotto \textit{et al.}\textsuperscript{23)} who measured 24 women with untreated Graves’ disease and found that all ultrasound parameters were lower than those in the control groups. Because the ultrasound parameters were within the negative ranges in group RG, we compared the Z scores for ultrasound parameters between short-term and long-term remission (Table 5). Our finding that long-term remission was not associated with significantly lower ultrasound parameters compared to those for short-term remission suggests that even if patients are in remission, their bone loss following hyperthyroidism is not restored to normal levels.

Although restoration of bone loss following hyperthyroidism has rarely been reported, a few studies using DEXA measurement indicated that it takes about 4 years to recover from reversible bone loss following hyperthyroidism\textsuperscript{10, 30–32)}. These results contradict our findings for long-term remission. Our data suggest that, in addition to antithyroid drug therapy, active calcium or vitamin D therapy are necessary to prevent bone loss in patients with Graves’ disease, especially postmenopausal women.

In conclusion, the present results show that the lack of significant correlation between ultrasound parameters and BMD of the calcaneus in group G indicates that BUA and SOS are affected by mineral content and by other material and structural properties. In addition, even Graves’ disease patients who are in long-term remission may not have had recovery of bone density to anywhere near normal levels.

References


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