BONE CHANGES IN HYPERTHYROIDISM:
INTERRELATIONSHIPS BETWEEN BONE MORPHOMETRY,
THYROID FUNCTION AND
CALCIUM-PHOSPHORUS METABOLISM

By
Leif Mosekilde, Flemming Melsen, Jens Peder Bagger,
Olaf Myhre-Jensen and Niels Schwartz Sørensen

ABSTRACT

Iliac-crest biopsies were obtained from 40 untreated hyperthyroid patients after double-labelling with tetracycline. Histomorphometric analyses were performed on undecalcified and decalcified bone sections. The morphometric and chemical data were compared with those in normal control groups and the results of the morphometric analyses were related to thyroid function and to chemical quantities of calcium-phosphorus metabolism.

The bone turn-over was increased in the hyperthyroid patients with an increase both in bone resorption and bone formation. Serum alkaline phosphatase was increased and positively correlated to the amount of osteoid and to the extent of tetracycline-labelled surfaces. The osteoclastic resorption was positively and the bone formation inversely correlated to thyroid activity. This might explain the reduced amount of trabecular bone found in hyperthyroidism. The most striking feature was, however, a pronounced increase in osteoclastic activity in cortical bone followed by increased porosity. The osteoclastic resorption in cortical bone was positively correlated to serum concentrations and urinary excretions of calcium and phosphorus. This indicates that increased cortical osteoclastic resorption is mainly responsible for bone mineral mobilisation in hyperthyroidism.
We have previously reported on increased serum concentrations and urinary excretions of calcium and phosphorus in patients with hyperthyroidism (Mosekilde & Christensen 1977). The demonstrated changes in calcium-phosphorus metabolism were positively correlated to the degree of hyperthyroidism. The serum concentration of parathyroid hormone was found decreased and inversely correlated to the serum calcium concentration. It was suggested that increased bone mineral mobilisation was mainly responsible for the chemical changes.

The histological bone changes in hyperthyroidism have previously been described as similar to osteitis fibrosa (Hunter 1930; Askanazy & Rutishauser 1933; Follis 1953), osteoporosis (Follis 1953; Cook et al. 1959) or osteomalacia (Nielsen 1952; Follis 1953). In recent investigations using histomorphometric analysis the hyperthyroid bone changes have, however, been described as a specific disease characterized by increased turn-over in trabecular bone and increased re-modeling and porosity in cortical bone (Meunier et al. 1972; Melsen & Mosekilde 1977).

We have investigated the interrelationships between morphometric bone changes, thyroid function tests and chemical quantities of calcium-phosphorus metabolism in 40 hyperthyroid patients in order to clarify the influence of increased serum levels of thyroid hormone(s) on bone.

MATERIALS AND METHODS

The investigation comprised 40 patients with untreated hyperthyroidism: 33 women aged 16-79 years (mean 44) and 7 men aged 29-57 years (mean 48). Diffuse toxic goitre was found in 19 patients, nodular toxic goitre in 18 patients and 3 patients had non-goitrous hyperthyroidism. The diagnosis was based on clinical symptoms and signs, measurements of total serum thyroxine concentration (S-T₄), serum triiodothyronine uptake test (S-T₃-test) and absolute [¹³²I]iodine uptake. All patients had a normal serum creatinine value and none of the patients were immobilized.

S-T₄ was measured by a competitive protein binding assay (Tetralute ¹²₅I Reagent kit, Ames). S-T₃-test was carried out using ¹²³I as a tracer (Thyopac-3, the Radiochemical Centre, Amersham) and expressed in reciprocal values and in per cent of a normal standard. The free thyroxine index (FTI) was calculated as the product of S-T₄ and S-T₃-test and expressed in arbitrary units (Irvine & Standeven 1968). Fasting serum concentrations of calcium, phosphorus and alkaline phosphatase were measured by standard laboratory methods and the mean of measurements of serum samples from three different days was calculated. Serum calcium was corrected for individual variations in serum albumin concentration (serum calcium (corr.)) (Pedersen 1972) and calculated as the calcium concentration corresponding to an albumin concentration of 700 μmol/l. Serum albumin was analysed according to Doumas et al. (1971). The urinary excretion of calcium, phosphorus and creatinine was determined on a non-restricted diet as the mean of three 24-h collections.

Transcortical biopsies were obtained from the right iliac crest after double-labelling with tetracycline (600 mg of demethylchlortetracycline given orally 14, 13, 4 and 3 days before biopsy). The following measurements were performed on sections of
decalcified and undecalcified bone in order to quantitate alterations in bone mass, bone resorption and bone mineralization (Melsen & Mosekilde 1977).

(I) Trabecular bone

Absolute volume of trabecular bone (AVTB) in per cent of total bone volume; osteoid surfaces (OS) in per cent of total trabecular bone surfaces; relative osteoid volume (OV) in per cent of total trabecular bone volume; mean width of osteoid seams (WOS) in μm, as the mean of four extreme measurements in all surfaces covered with osteoid; trabecular osteoclastic resorption surfaces (RS) in per cent of total trabecular bone surfaces; calcification rate (CR) in μm per day, as the mean distance between the fluorescent tetracycline lines in all double-labelled zones divided by interval in days between the two labelings; active trabecular calcification surfaces (ATCS) as the tetracycline-labelled surfaces in per cent of total trabecular bone surfaces, and active osteoid calcification surfaces (AOCS) as tetracycline-labelled surfaces in per cent of osteoid covered surfaces. The measurement of tetracycline-labelled surfaces was performed in ultraviolet light on 20 μm thick undecalcified, unstained sections.

(II) Cortical bone

Mean thickness of external and internal cortex in μm, as the mean of ten measurements of both; porosity of cortical bone in per cent of total cortical bone area; Haversian canals with active osteoclastic resorption (CAR) in per cent of total number of canals, and the mean size of peri-osteocytic lacunes (POL) in μm², as the mean of 50 randomly selected lacunes.

The control group for bone morphometry comprised 30 individuals of the same age and sex distribution as the hyperthyroid patients.

Statistical significance of differences in group means was determined by Wilcoxon test for two samples and correlation coefficients by Spearman’s rank correlation (R). Linear regression analysis (r) was used in figures showing the correlations.

Table 1.

Chemical quantities of calcium-phosphorus metabolism in hyperthyroid patients and controls.

<table>
<thead>
<tr>
<th></th>
<th>S-calcium (corrected) mmol/l</th>
<th>S-phosphorous mmol/l</th>
<th>S-alkaline phosphatase U/l</th>
<th>U-calcium¹</th>
<th>U-phosphorus²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>x</td>
<td>2.508</td>
<td>1.18</td>
<td>147</td>
<td>430</td>
<td>1965</td>
</tr>
<tr>
<td>se (N)</td>
<td>0.006 (160)</td>
<td>0.02 (60)</td>
<td>4 (60)</td>
<td>13 (60)</td>
<td>45 (60)</td>
</tr>
<tr>
<td>Hyperthyroid patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>x</td>
<td>2.684</td>
<td>1.26</td>
<td>188</td>
<td>1015</td>
<td>3351</td>
</tr>
<tr>
<td>se (N)</td>
<td>0.017 (40)</td>
<td>0.03 (40)</td>
<td>15 (40)</td>
<td>112 (39)</td>
<td>190 (39)</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.001</td>
<td>&lt;0.01</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

¹) Calculated as (dU-calcium (mol)/dU-creatinine (mol)) x 10³.
²) Calculated as (dU-phosphorus (mol)/dU-creatinine (mol)) x 10³.
Fig. 1.
Histomorphometric values in hyperthyroid patients and controls (mean ± se).
RESULTS

Table 1 gives the chemical quantities measured of calcium-phosphorus metabolism in the hyperthyroid patients and in controls. The mean serum concentration and urinary excretion of calcium and phosphorus and the mean serum level of alkaline phosphatase was increased in the hyperthyroid patients.

Fig. 1 shows the results of the morphometric analyses of bone in the hyperthyroid patients and in the controls. AVTB was decreased, the mean cortical thickness was unchanged and the cortical porosity was markedly increased in the hyperthyroid patients. The amount of unmineralized bone (OV) was unchanged, but OS was increased and WOS decreased. The bone mineralization

Table 2.
Correlations between thyroid function, serum calcium, serum phosphorus, serum alkaline phosphatase and bone morphometry in 40 hyperthyroid patients.

<table>
<thead>
<tr>
<th></th>
<th>Unmineralized bone</th>
<th>Bone mineralization</th>
<th>Bone resorption</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OS</td>
<td>OV</td>
<td>WOS</td>
</tr>
<tr>
<td>N</td>
<td>37</td>
<td>37</td>
<td>31</td>
</tr>
</tbody>
</table>

*S-T₄*

| R  | -0.11 | -0.16 | -0.16 | -0.11 | -0.35 | 0.05 | 0.37 | 0.24 | 0.15 |
| P  | n.s.  | n.s.  | n.s.  | n.s.  | n.s.  | n.s. | <0.05 | n.s. | n.s. |

*S-T₃-test*

| R  | -0.26 | -0.36 | -0.14 | 0.22  | -0.45 | 0.05 | 0.19 | 0.41 | 0.03 |
| P  | n.s.  | <0.05 | n.s.  | n.s.  | <0.05 | n.s. | n.s. | <0.02 | n.s. |

*FTI*

| R  | -0.19 | -0.25 | -0.25 | 0.00  | -0.43 | 0.13 | 0.40 | 0.34 | 0.18 |
| P  | n.s.  | n.s.  | n.s.  | n.s.  | <0.05 | n.s. | <0.01 | <0.05 | n.s. |

*S-calcium*

| R  | -0.05 | -0.05 | -0.25 | 0.11  | -0.27 | 0.07 | 0.04 | 0.63 | 0.21 |
| P  | n.s.  | n.s.  | n.s.  | n.s.  | n.s.  | n.s. | n.s. | <0.001 | n.s. |

*S-phosphorus*

| R  | -0.04 | -0.21 | 0.00  | 0.01  | -0.32 | -0.05 | 0.16 | 0.36 | 0.17 |
| P  | n.s.  | n.s.  | n.s.  | n.s.  | n.s.  | n.s.  | n.s. | <0.05 | n.s. |

*S-alkaline phosphatase*

| R  | 0.32  | 0.50  | -0.33 | 0.03  | 0.40  | 0.02  | 0.16 | -0.02 | -0.20 |
| P  | n.s.  | <0.01 | n.s.  | <0.05 | n.s.  | n.s.  | n.s. | n.s.  | n.s.  |
activity was increased as measured by CR and ATCS, whereas the mineralization activity of the osteoid (AOCS) was normal. Bone resorption measured by RS and CAR was considerably increased, POL was slightly but insignificantly increased ($P < 0.1$).

An inverse correlation was found between AVTB and age both in the hyperthyroid patients ($\text{AVTB} = -0.19$ (age in years) + 26.55, $r = -0.39$, $P < 0.05$) and in the controls ($\text{AVTB} = -0.19$ (age in years + 30.50, $r = -0.55$, $P < 0.01$). It was therefore investigated whether the difference, $\Delta \text{AVTB}$, between the observed AVTB in the hyperthyroid patients and the expected AVTB according to the regression line between AVTB and age in the controls was correlated to thyroid function. No significant correlation was found between $\Delta \text{AVTB}$ (or AVTB) and thyroid function tests, duration of hyperthyroid state or the product of these two parameters.

The porosity of cortical bone was increased in the hyperthyroid patients and positively correlated to S-T₃-test ($R = 0.32; P < 0.05$) and FTI ($R = 0.32$, $P < 0.05$) but not to S-T₄ ($R = 0.19, P > 0.10$). No correlation was found between duration of hyperthyroid state and cortical porosity.

Table 2 (upper part) correlates the morphometric data of bone re-modeling to the degree of hyperthyroidism expressed by S-T₄, S-T₃-test and FTI. The amount of unmineralized bone (OV) was inversely correlated to S-T₃-test. CR was not correlated to thyroid activity but a significant inverse correlation was found between ATCS and thyroid activity (Table 2, Fig. 2). The osteoclastic activity in trabecular bone (RS) and in cortical bone (CAR) was positively correlated to thyroid activity (Table 2, Fig. 2) whereas the size of the peri-osteocytic lacunes (POL) was unrelated to thyroid activity.

Correlations between morphometric values of bone resorption and bone mineralization and free thyroxine index in hyperthyroid patients (double logarithmic plots).
Correlations between serum levels and urinary excretions of calcium and phosphorus and relative number of Haversian canals with active resorption in cortical bone in hyperthyroid patients (semi-logarithmic and double-logarithmic plots).

No differences in bone morphometry or in chemical quantities were found between patients with diffuse or nodular toxic goitre.

Table 2 (lower part) correlates the morphometric values of bone re-modeling to serum concentrations of calcium, phosphorus and alkaline phosphatase. The osteoclastic activity in cortical bone (CAR) was positively correlated to serum levels of calcium and phosphorus (Table 2, Fig. 3) and to the urinary excretion of calcium ($R = 0.50, P < 0.01$) and phosphorus ($R = 0.35, P < 0.05$) (Fig. 3). Neither the osteoclastic activity in trabecular bone (RS) nor the osteocytic osteolysis (POL) was correlated to these quantities. Serum alkaline phos-
phatase was positively correlated to the amount of osteoid (OV) and to the mineralization activity in trabecular bone (ACTS) (Table 2).

A highly positive correlation ($R = 0.78$, $P < 0.01$) was found between the osteoclastic activity in cortical bone (CAR) and the cortical porosity (Fig. 4).

**DISCUSSION**

The bone changes in hyperthyroidism are specific and characterized by an increased bone turn-over. The demonstrated increase in bone turn-over is in agreement with kinetic studies using radiocalcium (Krane et al. 1956) and with the finding of increased serum alkaline phosphatase values and increased urinary excretion of hydroxyproline (Kivirikko et al. 1965; Siersbæk-Nielsen et al. 1971) in patients with hyperthyroidism.

In contrast to changes induced by parathyroid hormone (Meunier et al. 1969, 1971) the osteoclastic activity in hyperthyroidism was more pronounced in cortical bone than in trabecular bone and POL did not differ significantly from control values. The bone changes differed from those in osteoporosis (Meunier et al. 1973; Ciroux et al. 1975) by the increased turn-over, and from those in osteomalacia (Vignon & Meunier 1973; Delling 1975) by the decrease in WOS, the normal OV, the increase in CR and the normal AOS.

The present investigation demonstrates that thyroid hormone(s) stimulates the osteoclastic bone resorption both in trabecular bone (RS) and in cortical bone.
bone (CAR). The mean values of RS and CAR were 1.9 and 5.9 times higher, respectively, than those of normal controls. The osteoclastic activity in cortical bone was positively correlated to serum concentrations and urinary excretions of calcium and phosphorus. The osteoclastic activity in trabecular bone was not correlated to these chemical quantities. This indicates that increased cortical osteoclastic activity is mainly responsible for the increased bone mineral mobilization in hyperthyroidism. The increase in cortical osteoclastic activity is followed by increased cortical porosity as demonstrated by the positive correlation between CAR and cortical porosity.

The mean volume of the peri-osteocytic lacunes (POL) was slightly but insignificantly increased ($P < 0.10$) in hyperthyroidism. This might be explained by an abnormal representation of peri-osteocytic lacunes of different sizes in hyperthyroidism. Experimental studies in dogs (Marotti et al., in press) have shown a decrease in the size of the peri-osteocytic lacunes from outer to inner regions in secondary osteons. The increased porosity in hyperthyroidism as a result of increased osteoclastic activity in the canals will result in an exclusion of the small osteocytes in the inner region in the determination of the mean value of POL.

The increase in bone resorption in hyperthyroidism is a direct effect of thyroid hormone(s) on bone and not an effect of parathyroid activation. This is supported by the finding of decreased serum levels of parathyroid hormone inversely correlated to serum calcium in hyperthyroid patients (Mosekilde & Christensen 1977) and by the histomorphometric differences mentioned above between bone changes induced by parathyroid hormone and by thyroid hormone(s). Furthermore, both normal and parathyroidectomized dogs show increased bone resorption after thyroxine feeding (Adams & Jowsey 1967). It has been demonstrated that thyroid hormone(s) stimulates bone resorption in cultured foetal rat bones as measured by the release of previously incorporated $^{45}$Ca, by the hydroxyproline concentration of treated bone and by histologic examination (Munday et al. 1976). The increased resorption occurred at concentrations of thyroid hormone(s) approaching those in hyperthyroidism and could not be inhibited by indomethacin. The findings indicate that the increase in osteoclastic activity in hyperthyroidism is independent of parathyroid hormone, vascular changes or increased prostaglandin synthesis.

Bone mineralization activity was increased in hyperthyroidism as shown by a rise in both the mean daily calcification rate (CR) and in the mean percentage of trabecular surfaces with active mineralization (ATCS). This increased bone mineralization must, however, be accompanied by an increased apposition of osteoid (bone formation) since the amount of osteoid remained normal. Initially bone mineralization may have been slightly greater than bone formation since the mean width of the osteoid seams (WOS) was reduced. Obviously a new equilibrium will be restored later with an equal increase in bone minera-
lization and bone formation rate. The positive correlation between serum alkaline phosphatase and ATCS suggests that the increased mean serum level of alkaline phosphatase in hyperthyroidism reflects the increased bone formation.

It was remarkable that the calcification rate (CR) was unchanged with increasing thyroid activity, where as the percentage trabecular surfaces with active mineralization (ATCS) decreased when thyroid activity increased. This indicates that the rate of mineralization and formation of bone is greater in patients with a mild to moderate hyperthyroidism than in patients with a severe hyperthyroidism. The increase in bone formation may be secondary to the increased bone resorption. Much evidence points to a cellular coupling of bone resorption and bone formation activity in different bone diseases (Takahashi et al. 1964; Rasmussen & Bordier 1974; Aaron 1976) probably so that increased bone resorption leads to increased bone formation. The effect of thyroid hormone(s) on bone formation is unknown. Assuming that the increased bone formation in hyperthyroidism is secondary to increased bone resorption, the inverse correlation between ATCS and thyroid activity could be explained by an inhibiting effect of thyroid hormone(s) on bone formation. Contrary, if the increased bone formation is caused by a direct stimulation of thyroid hormone(s), the inverse correlation between ATCS and thyroid activity must be a result of an inhibiting effect of some other factor on bone formation, e.g. low serum levels of parathyroid hormone (Mosekilde & Christensen 1977) or of 1.25-dihydroxycholecalciferol secondary to hypercalcæmia.

In trabecular bone both bone resorption and bone formation was increased in hyperthyroidism. The decrease in AVTB demonstrates, however, that bone resorption is greater than bone formation. This is in agreement with the positive correlation between trabecular resorption surfaces (RS) and thyroid activity and the inverse correlation between trabecular surfaces with active mineralization (ATCS) and thyroid activity. It was impossible, however, to demonstrate any correlation between the volume of trabecular bone (AVTB) or the calculated decrease in AVTB (A AVTB) and thyroid activity or the duration of the hyperthyroid state.

REFERENCES


524
Mosekilde L. & Christensen M. S.: Acta endocr. 84 (1977) 566.
Nielsen H.: Acta med. scand. 142 (1952) 783.

Received on October 25th, 1976.