Decreased serum IGF-I and dehydroepiandrosterone sulphate may be risk factors for the development of reduced bone mass in postmenopausal women with endogenous subclinical hyperthyroidism

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Abstract
Postmenopausal women with endogenous subclinical hyperthyroidism seem to have reduced bone mass, which does not correlate with serum thyroid hormone levels. Relative insufficiencies of IGF-I and dehydroepiandrosterone sulphate (DHEAS) might be additional risk factors for low bone density in these patients. We measured IGF-I, IGF-binding protein-3 (IGFBP-3) and DHEAS levels together with bone mineral density (BMD) of the femoral neck and lumbar spine in women with an autonomously functioning thyroid nodule. Sixty-three women were classified as subclinical hyperthyroid (31 pre- and 32 postmenopausal) and 39 as overt hyperthyroid (16 pre- and 23 postmenopausal) and results were compared with data obtained from 41 age-matched euthyroid healthy women. In premenopausal women BMD was reduced only in the overt hyperthyroid group, and only in the spine, to 92% (P < 0.05). Serum IGF-I as well as IGFBP-3 were increased in the manifest hyperthyroid group, to 157% (P < 0.001) and 129% (P < 0.05) respectively, whereas DHEAS levels did not change in either premenopausal patient group. In postmenopausal women BMD was significantly reduced both in the subclinical hyperthyroid group (spine to 90% and femoral neck to 88%; P < 0.05), as well as in the hyperthyroid group (spine to 78% and femoral neck to 86%; P < 0.01). In contrast to premenopausal women, serum IGF-I and IGFBP-3 did not change in the two groups who were postmenopausal and serum DHEAS levels were reduced to 58% (P < 0.001) in both postmenopausal groups with subclinical as well as overt hyperthyroidism. In the same two groups of patients, serum IGF-I and DHEAS levels correlated with BMD (femoral neck; both r = 0.50, P < 0.05). In conclusion, women with a solitary autonomous thyroid nodule with subclinical hyperthyroidism have reduced BMD only if they are postmenopausal. This is probably due to the effect of subtle increases in thyroid hormone production together with lack of oestrogen protection of the skeleton. But additional risk factors for the development of enhanced bone loss might be a state of relative IGF-I and DHEAS insufficiency in these patients as well as in postmenopausal women with overt hyperthyroidism.

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Introduction
In a previous study (1) we found reduced bone mineral density (BMD) in postmenopausal women with endogenous subclinical hyperthyroidism. As BMD did not correlate with thyroid hormone levels it was assumed that some additional risk factors might play a role in these cases.

Growth hormone (GH)-dependent insulin-like growth factor-I (IGF-I) is involved in bone formation and maintenance (2, 3). However, the relationship between IGF-I levels, BMD and the development of osteoporosis is still not clear (4, 5).

Recently, a relationship was suggested between serum dehydroepiandrosterone (DHEA) and DHEA sulphate (DHEAS) levels and the production of IGF-I (6), raising the possibility that DHEA represents a physiological regulator of IGF-I and IGF-binding protein-3 (IGFBP-3). It has been argued that especially adrenal-derived DHEAS plays an important role in preserving bone mass and decreased production of DHEAS may contribute to the development of postmenopausal osteoporosis (7–10).

Serum levels of IGF-I and DHEAS decline with age (3, 4, 11–12); thus it is possible that reduced production of IGF-I and DHEAS acts as an additional risk factor for the development of reduced bone mass in postmenopausal women with subclinical hyperthyroidism. We have undertaken the present study to elucidate the following. (A) Are serum IGF-I, IGFBP-3 and DHEAS levels changed in subclinical and in overt hyperthyroidism due to a solitary autonomously functioning thyroid nodule? (B) Are there any differences due to menopausal...
state? (C) Does a relationship exist between the serum IGF-I and/or DHEAS level and BMD in postmenopausal endogenous subclinical hyperthyroidism?

Materials and methods

We studied 102 patients with a solitary autonomously functioning thyroid nodule. Patients were divided into two groups. Group 1 patients had subclinical hyperthyroidism \( (n = 63) \): height 164 ± 5 cm; weight 63 ± 8 kg. Patients were clinically euthyroid, their serum thyrotrophin (TSH) was reduced \(<0.1\, \text{mU/l}\), whereas serum levels of free thyroxine \((\text{FT}_4)\) and free triiodothyronine \((\text{FT}_3)\) were still within normal range. The hormone determinations were performed twice within 1 month to ensure strict inclusion criteria for this group. Group 2 patients had a toxic solitary autonomous thyroid nodule \( (n = 39) \): height 161 ± 5 cm; weight 58 ± 7 kg. Patients were clinically hyperthyroid, TSH levels were suppressed and \(\text{FT}_4\) and/or \(\text{FT}_3\) levels were elevated. In both groups a scintiscan demonstrated a solitary 'hot' nodule with suppression of the extranodular tissue, and a thyroid nodule was known to the patients at least 2 years before the investigation.

Subjects were assigned as premenopausal (mean age 41 years; range 35–46 years) and postmenopausal (60 years; 53–64 years) groups. All premenopausal subjects had regular menstruation with no history of amenorrhea. In postmenopausal patients the menstrual periods had ceased at least 2 years before the study.

Age-matched healthy females served as controls \( (n = 41) \): height 163 ± 6 cm. weight 64 ± 6 kg: premenopausal \( (n = 20) \): 40 years (33–45 years) and postmenopausal \( (n = 21) \): 58 years (52–65 years). The control subjects were healthy and euthyroid with no history of thyroid disease and without goitre. A physical and laboratory examination of all subjects studied excluded diseases that may alter bone metabolism. All postmenopausal subjects had had a natural menopause at least 2 years before the study. None of the subjects had received oestrogens, glucocorticoids, calcitonin or thiazides. Subjects with known alcohol abuse or smoking more than ten cigarettes per day were excluded. All subjects gave informed consent for their participation in the study.

Serum \(\text{FT}_4\), \(\text{FT}_3\), and TSH levels were measured by commercially available kits (DYNOTest; Henning, Berlin, Germany). The lower detection limit of the immuno-radiometric TSH assay was 0.05 mU/l (defined as mean + 2 s.d. of the zero standard) and the working-functional sensitivity (the lowest TSH value having an interassay coefficient of variation (C.V.) of 10%) was 0.1 mU/l.

Serum IGF-I was measured after acid-ethanol extraction by a radioisotopic assay (IGF-I by extraction; Nichols Institute, San Juan Capistrano, CA, USA). The intra-assay C.V. values were 6.3% and 3.7% at analyte levels of 45–5 and 19.5 nmol/l respectively, with an interassay C.V. of 9.1% at 19.5 nmol/l.

Serum IGFBP-3 levels were measured by RIA (Diagnostic System Lab. Inc., TX, USA). The intra- and interassay C.V. values at 12.2 ± 5 nmol/l were 6.1% and 10.2% respectively.

Serum DHEAS levels were determined by an in-house RIA employing a \(^{13}\text{H}\)DHEA tracer and a polyclonal antibody raised in rabbits against DHEA-3-hemisuccinate-BSA in our laboratory (titre 1:10 000) (13, 14). Cross-reactivity between DHEAS and oestradiol, progesterone, testosterone and cortisol was less than 0.3%. Intra- and interassay C.V. values were below 7.8% and 13.3% respectively for both low, normal and elevated serum concentrations.

Serum oestradiol levels were measured by RIA (15). The intra-assay C.V. was 8.9% at normal oestradiol levels. Serum osteocalcin levels were assayed by a kit produced by the Isotope Institute of the Hungarian Academy of Sciences, Budapest, Hungary (code number: RK-36; the intra-assay C.V. was 5.6%). Urinary free deoxypyridinoline \((\text{Dpd})\) was measured in the first morning void by Pyrlinks-D kit (METRA Biosystems, Mountain View, CA, USA) and expressed as \(\mu\text{mol Dpd/mmol creatinine}\). The intra-assay C.V. was <10%. The oestradiol, osteocalcin and \(\text{Dpd}\) were analysed in the same assay.

Spine (lumbar vertebrae 2–4) and femoral neck BMD \((\text{g/cm}^2)\) were measured by dual energy X-ray absorptiometry (Norland, XR-26 type) with a C.V. of 0.42% for repeated short-term phantom measurements. The \(\text{in vivo}\) precision was 1.37% (lumbar spine) and 0.86% (femoral neck) respectively.

For statistical analysis differences between groups were evaluated by ANOVA. Paired comparisons were performed by Student's \(t\)-test. Data are given as means ± s.d.

Results

Serum \(\text{FT}_4\), \(\text{FT}_3\), and TSH values of the patients and controls are given in Table 1. No difference was found between pre- and postmenopausal women in any of the

Table 1 Serum \(\text{FT}_4\), \(\text{FT}_3\) and TSH levels in euthyroid subjects and in patients with endogenous subclinical hyperthyroidism or toxic nodular goitre. Values are means ± s.d. Ranges for \(\text{FT}_4\), and \(\text{FT}_3\) are given in brackets.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>(\text{FT}_4) ((\text{pmol/l}))</th>
<th>(\text{FT}_3) ((\text{pmol/l}))</th>
<th>TSH ((\text{mU/l}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Euthyroid ((n = 41))</td>
<td>14.3 ± 2.5</td>
<td>6.9 ± 0.9</td>
<td>1.1 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>((11.5–20.9))</td>
<td>((4.8–8.0))</td>
<td></td>
</tr>
<tr>
<td>Subclinical hyperthyroid ((n = 63))</td>
<td>16.9 ± 3.3</td>
<td>7.3 ± 0.9</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td></td>
<td>((12.5–21.6))</td>
<td>((5.7–8.1))</td>
<td></td>
</tr>
<tr>
<td>Toxic nodular goitre ((n = 39))</td>
<td>35.9 ± 14.5**</td>
<td>12.8 ± 3.0**</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td></td>
<td>((19.8–75.3))</td>
<td>((9.6–19.1))</td>
<td></td>
</tr>
<tr>
<td>Reference levels</td>
<td>10–25</td>
<td>3.5–8.5</td>
<td>0.3–4.0</td>
</tr>
</tbody>
</table>

\(** P < 0.01; *** P < 0.001\) vs age-matched euthyroid controls.
Table 2 BMD and serum IGF-I, IGFBP-3 and DHEAS levels in pre- and postmenopausal euthyroid subjects and in patients with subclinical hyperthyroidism or toxic nodular goitre. Values are means ± s.d.

<table>
<thead>
<tr>
<th></th>
<th>Premenopausal women</th>
<th>Postmenopausal women</th>
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<tbody>
<tr>
<td></td>
<td>Euthyroid (n = 20)</td>
<td>Subclinical hyperthyroid (n = 31)</td>
</tr>
<tr>
<td></td>
<td>Euthyroid (n = 21)</td>
<td>Subclinical hyperthyroid (n = 32)</td>
</tr>
<tr>
<td>Lumbar spine (g/cm²)</td>
<td>1.04 ± 0.10</td>
<td>1.01 ± 0.11</td>
</tr>
<tr>
<td>Femoral neck (g/cm²)</td>
<td>0.89 ± 0.08</td>
<td>0.88 ± 0.09</td>
</tr>
<tr>
<td>IGF-I (nmol/l)</td>
<td>21.0 ± 6.9</td>
<td>25.2 ± 8.1</td>
</tr>
<tr>
<td>IGFBP-3 (nmol/l)</td>
<td>110.3 ± 23.5</td>
<td>121.1 ± 27.0</td>
</tr>
<tr>
<td>DHEAS (μmol/l)</td>
<td>4.3 ± 2.2</td>
<td>4.0 ± 1.4</td>
</tr>
</tbody>
</table>

* P < 0.05, ** P < 0.01, *** P < 0.001 vs age-matched euthyroid controls.

three groups. Patients with subclinical hyperthyroidism had, by definition, normal FT₄ and FT₃ levels, while the serum TSH concentration was already less than 0.1 mU/L. In patients with a toxic solitary nodule the mean FT₄ and FT₃ values were unequivocally elevated, while serum TSH level was suppressed.

BMD in premenopausal women was reduced only on lumbar spine in overt hyperthyroid patients. In contrast, in postmenopausal women both lumbar spine and femoral neck BMD were reduced in both subclinical and overt hyperthyroid patients (P < 0.05 at least).

IGF-I levels were increased to 120% (not significant) in premenopausal subclinical hyperthyroidism as compared with age-matched control subjects, and to 157% (P < 0.001) in the overt hyperthyroid group (Table 2). In contrast, no increase was demonstrated in IGF-I levels in the postmenopausal subclinical hyperthyroid group, whereas a modest but insignificant rise to 125% was noted in the postmenopausal toxic nodular goitre group. Similarly IGFBP-3 levels were significantly increased only in the premenopausal toxic group (Table 2) as compared with age-matched euthyroid subjects. Mean DHEAS levels did not change in premenopausal women with subclinical or overt hyperthyroidism (Table 2). Conversely, in post-menopausal women with either subclinical hyperthyroid or toxic nodular goitre, DHEAS levels were lower than in age-matched euthyroid subjects (P < 0.001).

Serum oestradiol levels were almost identical in the three groups of postmenopausal women (Table 3). Measurement of markers of bone turnover demonstrated an increase in osteocalcin to a mean 139% (not significant) in subclinical hyperthyroidism and to 239% (P < 0.001) in overt hyperthyroid postmenopausal women. Urinary Dpd was increased in both patient groups (Table 3).

BMD of the femoral neck correlated positively to serum IGF-I levels in the 32 postmenopausal subjects with endogenous subclinical hyperthyroidism (r = 0.499, P < 0.05; Fig. 1a), as well as to serum DHEAS levels (r = 0.503, P < 0.05; Fig. 1b). In these patients there was a trend, but not a significant correlation, between the BMD of the spine versus IGF-I and DHEAS levels.

Table 3 Serum oestradiol and osteocalcin, as well as urinary Dpd values in postmenopausal euthyroid subjects and in patients with endogenous subclinical hyperthyroidism and toxic nodular goitre. Values are means ± s.d.

<table>
<thead>
<tr>
<th></th>
<th>Oestradiol (nmol/l)</th>
<th>Osteocalcin (µg/l)</th>
<th>Dpd (nmol/ mmol creatinine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Euthyroid (n = 21)</td>
<td>0.35 ± 0.10</td>
<td>8.71 ± 2.74</td>
<td>6.26 ± 1.74</td>
</tr>
<tr>
<td>Subclinical hyperthyroid (n = 32)</td>
<td>0.34 ± 0.09</td>
<td>12.11 ± 2.48</td>
<td>9.58 ± 1.98</td>
</tr>
<tr>
<td>Toxic nodular goitre (n = 23)</td>
<td>0.35 ± 0.11</td>
<td>20.84 ± 7.05</td>
<td>18.88 ± 9.37</td>
</tr>
</tbody>
</table>

a P < 0.001, b P < 0.05 vs age-matched euthyroid controls; c P < 0.01 vs subclinical hyperthyroidism.

Discussion

In previous studies (16) it was anticipated that preserved oestrogen production is the main reason for the minimal loss of bone mass in premenopausal women. It was suggested therefore that a slight increase in bone turnover induced by subclinical hyperthyroidism (identified with a subnormal serum TSH level) would have greater impact on the postmenopausal bone mass than on the premenopausal bone mass. We therefore investigated pre- and postmenopausal women independently.
The present study extends and supports our previous findings (1) that bone mass in postmenopausal women with endogenous subclinical hyperthyroidism is reduced compared with sex- and age-matched controls. Markers of bone metabolism also indicate a slightly increased bone turnover in postmenopausal subclinical hyperthyroidism. Since BMD did not correlate with the serum thyroid hormone levels, it was suggested that other risk factors for low bone density with complex interactions play an additional role in the subclinical hyperthyroid state in the development of osteopenia in these women. Different oestrogen levels could not account for the reduced BMD in subclinical hyperthyroidism, since serum oestradiol levels were almost identical in postmenopausal controls and women with subclinical hyperthyroidism.

IGF-I is a bone cell mitogen, it has potent stimulatory effects on the function of osteoblasts and increases bone formation (2). IGF-I present in the bone may be derived from local production from osteoblasts (17), which seems GH-independent, or hepatic production, which seems GH-dependent (17). Serum levels, as well as skeletal content of IGF-I (18) seem to decline with age, and in the present study serum IGF-I levels were lower in euthyroid postmenopausal women than in premenopausal ones. Serum IGF-I levels in patients with osteoporosis have been reported to be decreased (5, 19, 20), or unaltered (4, 21).

Thyroid hormones stimulate IGF-I production of bone cells in vitro (22). Recently increased IGF-I levels were found in thyrotoxic patients (23) and it might be speculated that hyperthyroid patients who are unable to synthesize increased amounts of IGF-I during the disease are at higher risk of developing reduced bone mass (24). In accordance, we found increased IGF-I levels in the toxic nodular goitre group compared with the sex- and age-matched controls, but only in the premenopausal group, postmenopausal women with hyperthyroidism did not have increased IGF-I levels. Similarly, among women with subclinical hyperthyroidism, only those who were premenopausal seemed to have increased IGF-I levels. In postmenopausal subclinical hyperthyroid patients a positive correlation between serum IGF-I levels and BMD of the femoral neck was found.

In the circulation IGF-I is bound to binding proteins (25). IGFBP-3 is the major circulating binding protein, the production of which is regulated by GH (26). IGFBP-3 probably acts as a reservoir for circulating IGF-I. It correlates with IGF levels, and seems less age-dependent than IGF-I (27). In the present study IGFBP-3 showed similar changes as IGF-I in pre- and postmenopausal women with subclinical or overt hyperthyroidism, only premenopausal hyperthyroid women having elevated IGFBP-3. These findings together may suggest that in women with postmenopausal subclinical hyperthyroidism as well as overt hyperthyroidism a state of relative IGF-I deficiency is present and might contribute to reduced bone mass and ultimately osteoporosis in these women.

A progressive decline of adrenal secretion of DHEA and DHEAS has been demonstrated with ageing parallel with that of IGF-I level (28), and considerable individual differences were demonstrated in the rate of decline. Testosterone or DHEAS deficiency might be another risk factor for the development of low bone mass in postmenopausal women with subclinical or overt hyperthyroidism, independent of the effect from thyroid hormones. This hypothesis was supported by a reduction in DHEAS levels in these two groups of patients in the present study. Increased levels of thyroid hormones could not explain this finding, since DHEAS levels were equally reduced in these groups, with quite different thyroid hormone levels. DHEAS levels in serum seem to correlate positively to bone density of different sites of the skeleton (29) and, in accordance with this, we found a correlation between DHEAS levels and BMD of the femoral neck in the postmenopausal patient group. The exact mechanism of action of DHEAS on bone is unclear. Recently, a relationship was suggested (6) between serum

Figure 1 Relationship between the BMD of the femoral neck and (a) serum IGF-I levels and (b) serum DHEAS levels in 32 postmenopausal patients with endogenous subclinical hyperthyroidism: (a) \( r = 0.499; P < 0.05 \) and (b) \( r = 0.503; P < 0.05 \).
DHEA and DHEAS levels and the production of IGF-I, raising the possibility that DHEAS represents a physiological regulator of the IGF-I/IGFBP-3 system. However, in the present study no correlation was found between the serum IGF-I and DHEAS levels.

In summary, we have found reduced BMD in both lumbar spine and femoral neck in overt hyperthyroidism due to a solitary adenoma, in both pre- (only spine) and postmenopausal women. Patients with subclinical hyperthyroidism also had reduced BMD, but only if they were postmenopausal. This is probably an effect of thyroid hormones, but might also be due to a relative insufficiency of IGF-I which, in parallel with IGFBP-3, failed to increase in post- but not premenopausal women with subclinical or overt hyperthyroidism. Also a relative DHEAS insufficiency seems to be an additional factor responsible for reduced BMD in postmenopausal women with subclinical hyperthyroidism as well as overt hyperthyroidism, since DHEAS levels were reduced only in these two patient groups.

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