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Bone and Calcium Metabolism in Subclinical Autoimmune Hyperthyroidism and Hypothyroidism

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Abstract. Bone turnover is reported to increase in favour of resorption in overt hyperthyroidism and the rate of resorption is associated with the levels of thyroid hormones. Hypothyroidism, on the other hand, was shown to cause no disturbance of calcium kinetics and found to associate lower trabecular resorption surfaces and increased bone cortical thickness. Similar studies are very rare in subclinical thyroid disorders and consequently we aimed to examine calcium and bone metabolism in subclinical thyroid disorders. Thirteen patients with subclinical hyperthyroidism secondary to untreated Graves' disease, 20 patients with subclinical hypothyroidism and 10 healthy subjects participated in this survey. Briefly calcium, phosphorus, and creatinine (Cre), urinary deoxypyridinoline (U-DPD) and serum osteocalcin (OC) were measured as biochemical markers for calcium metabolism. Concerning serum Ca and phosphorus levels, there were no differences between three of the groups, but urinary Ca excretion was higher in subclinical hyperthyroid patients compared to control and hypothyroid subjects. Hypothyroid patients had similar U-DPD levels with control subjects ($p = 0.218$). Serum OC and U-DPD were higher in subclinical hyperthyroid compared to control subjects ($p<0.001$ and $p<0.001$ respectively). We demonstrated a higher bone turnover and greater calcium excretion in subclinical hyperthyroid patients. Additionally, we found that subclinical hypothyroidism is not associated with disturbed calcium metabolism. As persistent increase in bone turnover is responsible for accelerated bone loss, patients with Graves' disease may have increased risk for osteoporosis.

Key words: Subclinical hyperthyroidism, Subclinical hypothyroidism, Bone markers, Bone metabolism, Bone turnover
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THE combination of an undetectable serum thyrotropin concentration, as measured by an assay with a threshold of detection 0.1 mU per liter or less, and normal serum triiodothyronine and thyroxine concentrations (usually at the upper end of the normal range) is known as subclinical hyperthyroidism. It is not a rare finding; rates between 0.2% and 11.8% have been reported in different groups, according to age, sex, etc [1]. The etiology is usually the same as that of overt hyperthyroidism [1]. The health implications include general symptoms, effects on the cardiovascular system, and decreased bone density. Subclinical hypothyroidism is defined as a state where the patient is eumetabolic but has a modest increase in the serum TSH level (5 to 15 mU/L), a normal serum T_3 concentration, and low-normal or slightly decreased serum fT_4 levels. The overall prevalence has been reported to range from 4–10% in large general population screening surveys [2].

There are a few studies performed to examine calcium and bone metabolism in subclinical thyroid disorders with conflicting results. Bone turnover is increased in favour of resorption and the rate of resorption is associated with the serum levels of thyroid hormones in hyperthyroidism [3]. Thyroid hormone exerts its effect on osteoblasts via nuclear receptors to stimulate osteoclastic bone resorption [4, 5]; hyperthyroidism is thus one of the major causes of secondary osteoporosis [6]. Correction of hyperthyroid state also reverses metabolic outcomes of hyper-

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thyroidism on bone [7]. Hypothyroidism, on the other hand, was shown to cause no abnormality in calcium levels and found to be associated with lower trabecular resorption surfaces and increased bone cortical thickness [8].

There are studies reporting that subclinical hyperthyroidism secondary to thyroxine suppression for nodular goiter does not lead to bone loss [9]. There are also other studies with opposite results [10]. In a recent study, Kumeda *et al.*, reported that patients with Graves' disease (GD) who exhibited normal fT_3 and fT_4 levels with maintenance of serum TSH suppression long after antithyroid treatment exhibited a significant increase in bone turnover [11]. Consequently, we aimed to examine calcium and bone metabolism in patients with subclinical thyroid disorders.

Patients and Methods

This study was conducted in accordance with the Declaration of Helsinki and approved by the local Institutional Human Research Committee. Thirteen patients (8 women, 5 male; aged 41.92 ± 1.491 SE yrs) with untreated subclinical hyperthyroidism secondary to GD, 20 patients (14 women, 6 male; aged 45 ± 1.98 SE yrs) with untreated subclinical hypothyroidism secondary to Hashimoto's thyroiditis and 10 healthy subjects (7 women, 3 male; aged 39.6 ± 2.918 SE yrs) participated in this study. The diagnosis of subclinical GD was defined by the presence of a diffuse goiter with normal fT_3 and fT_4 associated with decreased TSH concentrations in serum and increased thyroid uptake of radioactive 99 m Tc. Diagnosis of subclinical hypothyroidism was made by the presence of low 99 m Tc uptake by thyroid, inhomogenous thyroidal ultrasound appearance and high titres of antithyroid antibodies. Patients had serum TSH levels as follows: a subclinical hyperthyroid group with normal fT_3 and fT_4 levels and serum TSH lower than 0.1 mU/L, and a subclinical hypothyroid group who had serum TSH higher than 4.5 mU/L and normal fT_3 and fT_4 levels. Sex and age-matched control group had normal TSH levels between 0.4–4 mU/L. Height, body weight, and body composition were similar in all three of the groups. All female patients and control subjects were premenopausal. No patient had a history of hepatic or renal disorders, alcoholism, or other major medical conditions or had taken any medications that might affect calcium metabolism.

Blood and urine samples

Blood and urine samples were drawn after an overnight fast and were kept frozen at –20°C until assayed for determination of biochemical markers.

Thyroid functions

fT_3 (n: 1.8–4.2 pg/ml), fT_4 (n: 0.8–1.9 ng/dl), TSH (n: 0.4–4 μU/ml) were measured using commercially available kits (DPC, Diagnostics Products Corporation, Los Angeles, CA, USA).

Biochemical parameters for calcium and bone metabolism

Biochemical markers for calcium metabolism were serum levels of calcium and phosphorus and urinary excretion of calcium (Ca), phosphorus and deoxypyridinoline. Ca, phosphorus, and creatinine (Cre) were measured in serum and urine using standard laboratory methods. As bone resorption marker, urinary deoxypyridinoline (U-DPD) was determined by solid phase chemiluminescent enzyme-labeled immunoassay method (DPC, Diagnostics Products Corporation, Los Angeles, CA, USA) and was corrected for urinary creatinin measured by the automatic analyzer. Inter and intra assay CVs for U-DPD were 8% and 10.1%, respectively. As a bone formation marker, serum osteocalcin was measured by an immunometric assay method (DPC, Diagnostics Products Corporation, Los Angeles, CA, USA). Inter and intra assay CVs for OC were 4.5% and 8.1%. In our central laboratory, normal ranges for U-DPD are 3–7.4 nM DPD/mM creatinin for females, 2.3–5.4 nM DPD/mM creatinin for males. Normal range for OC is 3.1–13.7 ng/ml.

Statistical analysis

Results are expressed as mean ± SEM unless otherwise indicated. Differences between groups were analyzed using the Mann-Whitney U test for assessment of mean values. $P<0.05$ was considered significant. Statistical analysis was performed with the SPSS for Windows, 9.05.

Results

Patients' and control subjects' profiles, laboratory characteristics and statistical differences between groups are shown in Table 1.

Bone metabolic parameters for subclinical GD, subclinical hypothyroid patients and TSH-normal subjects

Concerning serum Ca and phosphorus levels, there were no differences between three of the groups; but 24-hour urinary Ca excretion was higher in subclinical GD group compared to control and hypothyroid subjects (GD 0.45 ± 0.017 μmol/mmol; control group 0.34 ± 0.008 μmol/mmol, hypothyroid group 0.34 ± 0.008 μmol/mmol, p<0.001). U-DPD excretion was higher in subclinical hyperthyroid group compared to both control and hypothyroid groups, (9.52 ± 0.872 nmol/mmol, 5.17 ± 0.573 nmol/mmol; 4.32 ± 0.424 nmol/mmol respectively; p<0.01). Statistical difference for U-DPD between hypothyroid patients and control group was not significant (p = 0.218). Osteocalcin was significantly lower in subclinical hypothyroid subjects in comparison to control group (6.62 ± 0.52 vs.10.81 ± 0.975 nmol/L; p<0.001).

Table 1. Characteristics of healthy subjects and subclinical thyroid patients

	Mean ± SEM	P
Age (yrs)		
Control	39.6 ± 2.918	
Subclinical hypothyroid	41.55 ± 1.982	NS
Subclinical GD	41.92 ± 1.491	NS
fT_3 (pg/ml)		
Control	3.10 ± 0.102	
Subclinical hypothyroid	3.19 ± 0.110	NS
Subclinical GD	4 ± 0.122	NS
fT_4 (ng/dl)		
Control	1.30 ± 0.075	
Subclinical hypothyroid	1.09 ± 0.035	NS
Subclinical GD	1.46 ± 0.070	NS
TSH (μIU/ml)		
Control	1.31 ± 0.319	
Subclinical hypothyroid	7.72 ± 0.564	0.001
Subclinical GD	0.03 ± 0.009	0.001
Calcium (mg/dl)		
Control	9.32 ± 0.241	
Subclinical hypothyroid	9.2 ± 0.141	NS
Subclinical GD	9.49 ± 0.175	NS
Phosphorus (mg/dl)		
Control	3.49 ± 0.192	
Subclinical hypothyroid	3.62 ± 0.130	NS
Subclinical GD	3.76 ± 0.186	NS
Urinary calcium (μmol/mmol)		
Control	0.34 ± 0.008	
Subclinical hypothyroid	0.34 ± 0.008	NS
Subclinical GD	0.45 ± 0.017	0.01
U-DPD (nmol/mmol)		
Control	5.17 ± 0.573	
Subclinical hypothyroid	4.32 ± 0.424	NS
Subclinical GD	9.52 ± 0.872	0.001
OC (nmol/L)		
Control	10.81 ± 0.975	
Subclinical Hypothyroid	6.62 ± 0.520	0.001
Subclinical GD	14.26 ± 0.864	0.014

P: Statistical difference between subclinical thyroid patients and healthy subjects.

Correlation between thyroid hormones and bone markers

In subclinical hyperthyroid group, neither fT_3 nor fT_4 was correlated with bone markers and Ca excretion; fT_3 did not correlate with OC (r = –0.032; $P = 0.919$), U-DPD (r = –0.226; $P = 0.458$), urinary calcium (r = 0.037; $P = 0.937$) nor did fT_4 correlate with OC (r = –0.413; $P = 0.161$), U-DPD (r = 0.234; $P = 0.441$), urinary calcium (r = 0.197; $P = 0.520$).

Discussion

In this cross-sectional study, we found that bone turnover is increased in subclinical hyperthyroidism, as evidenced by increased U-DPD and OC levels. The subclinical hyperthyroid group had higher urinary calcium excretion than the control group. On the other hand, calcium excretion rate of the hypothyroid group was not different from the control group. Even though their serum levels of fT_3 and fT_4 were in the normal range, the hypothyroid group had osteocalcin levels lower than control subjects, but their U-DPD levels were not significantly different from the control group.

There are several reports on bone and calcium metabolism in overt hyperthyroid patients. Bone turnover in hyperthyroid patients was found to be increased and serum calcium elevated compared to euthyroid state [11].

To our knowledge, only a few studies to date have focused on the effect of a subclinical hyperthyroid or hypothyroid state on bone metabolism with inconsis-

tent results. Our demonstration of higher bone turnover in subclinical GD group is in accordance with the findings of Kumeda *et al*. [11] and other authors [12]. However, in contrast to our results, Kumeda could not demonstrate a higher calcium excretion in subclinical hyperthyroid group. Mosekilde *et al.* [13] reported that bone turnover decreases early during antithyroid treatment, and the mineral balance is converted to positive. This may be the reason for the lower calcium excretion in Kumeda's study as their patients were treated. Pantazi and Papapetrou also reported that bone turnover, although decreasing early during antithyroid treatment, increases thereafter [14]. In our study, the subclinical GD patients were not treated yet and a long time might have elapsed before the diagnosis hence the bone metabolism might have been disturbed for a longer time, leading to higher calcium excretion. However the reason for the difference in terms of calcium excretion between our results and Kumeda's results is still not clear.

We could not demonstrate a correlation between bone markers and thyroid hormones, and this situation might be explained by Kumeda's proposal that Trab is a marker of bone metabolism in subclinical GD patients independent of the thyroid hormone status since TSH receptor is claimed to be expressed in certain osteoblast-like rat osteosarcoma cells [15]. However, as we do not routinely perform Trab measurement in our laboratory, we could not examine the association between Trab and bone markers.

Faber *et al*. reported that subclinical hyperthyroid patients with nodular goiter, in whom serum TSH was suppressed, exhibited accelerated bone loss at a rate of about 2%/yr [12]. In contrast to these studies, De Menis *et al*. reported that patients with overt hyperthyroidism displayed a significant enhancement of both bone resorption (increased serum calcium and urinary excretion of hydroxyproline) and bone formation (increased serum levels of osteocalcin and alkaline phosphatase) when compared both to controls and to patients with subclinical hypothyroidism, and that no significant alterations of bone metabolism parameters were found in patients with subclinical hyperthyroidism in comparison with healthy controls [16]. Similarly, Gurlek and Gedik reported that endogenous subclinical hyperthyroidism is not associated with increased bone turnover, and bone mineral density is not reduced in premenopausal women, at least in the short term [17]. Although the results of studies on bone metabolism in subclinical hyperthyroidism are inconsistent, the difference between our results of bone metabolism in subclinical GD and the studies reporting maintenance of normal bone metabolism in subclinical hyperthyroidism secondary to T4 overdosage might be explained again by independent influence of Trab on bone.

Subclinical hypothyroidism was reported to be associated with somewhat higher bone density compared to healthy subjects [18]. In our study, mean value of U-DPD in subclinical hypothyroid patients was not different from that of the control group and they had lower osteocalcin levels than control group. This can be attributed to a decreased bone turnover in hypothyroid group. Overt hypothyroidism causes elevated parathyroid hormone levels that might result from mild resistance to PTH. In turn, increased PTH leads to increased 1,25-dihydroxyvitamin D, causing the relative increase in calcium absorption [19]. Subclinical hypothyroidism likewise might lead to decreased bone turnover and a higher bone density by the same mechanisms. The clinical significance of this situation needs to be investigated more extensively.

In conclusion, depending on the results of this and previous studies, we may propose that bone metabolism is strongly affected by thyroid hormone status such that even a slight change in thyroid hormones to a level of subclinical hyperthyroidism results in accelerated bone turnover and calcium excretion. As persistent increase in bone turnover is responsible for accelerated bone loss, TSH-suppressed patients with Graves' disease may have increased risk for secondary osteoporosis. Even though their serum levels of fT_3 and fT_4 are in the normal range, further study is needed to elucidate the significance of increased bone turnover on the rate of bone loss in patients with subclinical GD. It might be recommended to observe these patients from the point of accelerated bone metabolism, since evidence of increased turnover may be a criterion for antithyroid treatment. On the other hand, bone turnover in subclinical hypothyroidism is decreased and calcium excretion is not affected.

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