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Bone Mineral Density Changes and Bone Turnover in Thyroid Carcinoma Patients Treated with Supraphysiologic Doses of Thyroxine

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Abstract: The aim of this one-year prospective study was to determine whether longterm thyroxine treatment is a risk factor for elevated bone turnover, loss of bone mass and subsequent development of osteoporosis.

Premenopausal women (N = 19), and men (N = 9) suffering from differentiated thyroid gland carcinoma in the mean age of 39.0 ± 8.0 years and 41.8 ± 10.0 years were investigated. All of them had undergone a total thyreoidectomy and subsequent thyroxine therapy. The duration of the TSH-suppressive therapy prior to the the beginning of our study was 9.4 ± 6.4 years in the female and 8.1 ± 6.0 years in the male group. The prospective observation was performed by dual X-ray absorptiometry (DXA) at the spine and the femoral neck and by single-photon absorptiometry (SPA) at the distal radius. Laboratory testings included thyroid hormones T_3, T_4 and TSH, serum calcium, phosphate and PTH, and urinary calcium and phosphate from spontaneous and 24-hour urine samples. Markers of bone formation (osteocalcin, alkaline phosphatase and PICP) and resorption (Ca/Cr and ICTP) were determined.

Statistically significant loss of bone mass was observed only on the distal radius in males ($p<0.05$). At the lumbar spine and femoral neck, only a minor bone loss was registered in a small number of patients. Almost 50 % of the females showed values above the reference range. In more than 30 % of the females, and smaller number of male patients, ICTP values ranged above the reference range, corresponding to elevated bone turnover. These two variables exhibited a slight correlation with bone density at the measured skeletal areas, mostly considering the male group. The results are a proof that accelerated bone turnover and subsequent bone loss occurs during TSH-suppressive thyroxine therapy.

In future prospective studies a prolonged time of observation will be necessary, as well as to increase the number of studied patients, in order to better assess the relative risk of osteoporosis in patients undergoing TSH-suppressive treatment more precisely.

Introduction

Bone remodeling is enhanced by thyroid hormones. Although both osteoclastic and osteoblastic activities increase with elevated concentration of thyroid hormones, the osteoclastic activity with consequential bone loss prevails [1]. Thyrotoxicosis is associated with elevated serum concentration of osteocalcin and alkaline phosphatase [1, 2]. Increased bone resorption is accompanied by elevated urine concentration of hydroxyproline and type I collagen crosslinks. Elevated concentration of these biochemical markers of bone turnover seems to correlate with serum concentration of thyroid hormones. Slightly increased serum calcium concentration was also found in patients with hyperthyroidism. 20 % of patients with thyrotoxicosis were found to have mild hypercalcemia, while the serum concentration and bioactivity of parathyroid hormone (PTH) and 1,25 $(OH)_2D_3$ as well as intestinal calcium absorption were decreased. Hypercalciuria is also a common finding in patients with thyrotoxocis. The increased urinary secretion normalizes after the treatment of thyrotoxicosis [1-3].

Decreased bone mineral density in patients with thyrotoxicosis is reversible with efficient treatment. The administration of high doses of thyroid hormones to suppress the secretion of thyroid-stimulating hormone (TSH) in patients submitted to radical treatment for differentiated carcinoma of the thyroid is considered as an appropriate therapy for these diseases. In patients prone to osteoporosis, however, this therapy may increase the risk of fractures. Supppressive doses of thyroid hormones have been reported to reduce or to have no effect on bone mineral density (BMD) in women. A meta-analysis of the reports in which BMD was assessed in women receiving TSH-suppressive doses of thyroxine concluded that treatment led to a 1 % increase in annual bone loss in postmenopausal women. In contrast, thyroid hormonereplacement therapy without TSH suppression does not appear to have detrimental effects on BMD [1].

Suppressive therapy may accelerate the development of osteoporosis. Many authors point out that the

substitution therapy with thyroxine in hypothyroidism is also a risk factor for the development of osteoporosis [11], while others report that suppressive doses of thyroxine have no major impact on the bone mineral loss [12-18].

According to some authors, the bone health improves with appropriate therapy for thyrotoxicosis, i.e. bone resorption decreases, and calcium absorption and $1,25(OH)_2D_3$ concentration increase, in parallel with an increase in the new bone formation within the first year of treatment. However, others consider the lost bone restoration in thyrotoxicosis as a still unresolved clinical issue.

In patients with thyrotoxicosis, BMD increases with sufficient treatment as compared to pretreatment values. Patients with accelerated bone loss can be detected by BMD measurements over 1-2 years.

In this prospective study, the effect of suppressive therapy with levothyroxine after total surgical thyroidectomy for differentiated thyroid carcinoma was investigated. A small number of longitudinal and several prospective studies on the effect of suppressive therapy with thyroid hormones on bone mass have been conducted worldwide to date.

Patients and Methods

Patients

A cohort study of 28 patients with differentiated thyroid carcinoma was included in the study. There were 19 premenopausal women with regular menstruation, to avoid the possible bone loss due to estrogen deficiency, and nine men, aged <50 years. All patients had undergone total surgical thyroidectomy with subsequent radioiodine ablation (^{131}I), followed by TSH-suppressive therapy with levothyroxine. None of the patients had metastases in the skeleton or other organs, according to thyroid tumour classification (TNM classification). The patients were treated at the Department of Nuclear Medicine, Radiation Protection and Pathophysiology, Osijek Clinical Hospital, and were selected according to the file diagnosis and were asked in writing to present for an interview, when they gave their written informed consent for the study.

Methods

Patient history data were taken, and anthropometric measurements and laboratory tests were performed before the initial BMD measurement. Laboratory tests included determination of thyroid hormones to assess the efficacy of supressive thyroxine therapy, and determination of calcium metabolism and biochemical parameters of bone metabolic activity during follow-up to assess the treatment effect on bone.

Collection of Biological Material Samples

Samples of biological material were collected on an outpatient basis, while the patients received regularly their thyroxine therapy. Twenty four hour urine was collected in special containers, drawn from 6 a.m. on the first day to 6 a.m. on the following day. In the morning after the 24 hour urine collection, fasting blood samples were taken from the patients and submitted immediately to the laboratory.

In addition, two hour urine samples were collected in the morning, over a 2 hour period, from 7 to 9 a.m., after a 15 hour fasting period. Before urine collection, the patients drank destilled water to enhance diuresis. The amount of water was determined according to patient's weight, a rate of 6 ml water per kg body weight was administered.

Anthropometric Measurements

Standard techniqes were used for anthropometric measurements [19]. Body height was measured by a portable stadiometer, and body weight on a medical decimal balance with sliding weight.

Laboratory Tests

Materials and reference values of laboratory tests used in the study are presented in Table 1. Data were provided by manufacturers, and data from the Department of Medical Biochemistry and of Nuclear Medicine, Radiation Protection and Pathophysiology, Osijek University Hospital, were used as reference values.

Thyroid Hormones

Serum levels of triiodothyronine (T_3), thyroxine (T_4) and TSH were determined by means of a commercial competitive immunoassay, the Kodak Clinical Diagnostic Ltd. kit (Amersham, UK). T_3 ,T_4 and TSH test sensitivity was <0.25 nmol/L, >8.0 nmol/L, and 0.006 mIU/L, respectively.

Calcium and phosphorus in serum and urine samples were determined by a colorimetric method, whereas creatinine and alkaline phosphatase were analyzed by the enzymatic PAP method on a Hitachi 737 autoanalyzer.

PTH analysis (intact PTH) was performed by the immunoradiometric method and osteocalcin by radioimmunoassay using CIS biointernational analytic kits. Type I collagen carboxytermal propeptide (PICP) and type I collagen carboxytermal cross-linked telopeptide (ICTP) were determined by radioimmunoassay using the Orion Diagnostica analytical kit.

BMD Measurement

BMD was measured twice during an one year period. The measurements were performed at the Institute of Medical Research and Occupational Medicine, Rebro, Zagreb. A DXA device (GE Lunar Radiation Corporation, Madison, WI), operating on the principle of dual energy x- ray absorptiometry, was used for the measurement of the lumbar spine (L1 –L4 segment) and promixal femur (neck) bone mineral content. Bone mineral density in the lower third of the non dominant forearm was determined on a Lunar SP2 (GE Lunar Radiation Corporation, Madison, WI), operating on the principle of single-photon absorptiometry and using an americium isotope (241Am) as a source of energy. Results were expressed as bone den-

Table 1. Patterns and reference parameters for laboratory analysis enabled in processing.

LAB. ANALYSIS	PATTERNS	REFERENCE PARAMETERS
T_3	serum	1.0 - 3.0 nmol/l
T_4	serum	60 - 160 nmol/l
TSH	serum	0.17 - 2.8 mIU/l
Ca	serum	2.12 - 2.75 mmol/l
P	serum	0.77 - 1.45 mmol/l
AP	serum	78 - 217 U/l
Cr	serum	44 - 115 μmol/l
PTH	serum	10 - 65 pg/ml
OSTEOCALCIN	serum	m 2.3 - 13.8 ng/ml
		f 2.5 - 6.9 ng/ml
PICP	serum	m 50 - 170 μg/l
		f 38 - 202 μg/l
ICTP	serum	1.8 - 5.0 μg/l
Ca/cr	2 hour morning urine after nightly fasting	< 0.425
Ca	24 – hour urine	m < 7.5 mmol/24 h
		f < 6.25 mmol/24 h
P	24 – hour urine	12.9 - 42.0 mmol/24h

Table 2. General characteristics of the study patients.

	PATIENTS		
Parameter	**Female (N = 19)**	**Male (N = 9)**	**Statistical significance***
Age (year)	39.0 ± 8.0	41.8 ± 10.0	NS
Height (cm)	165.2 ± 4.5	180.8 ± 7.6	p<0.0001
Weight (kg)	69.8 ± 10.9	93.8 ± 18.4	p<0.001
Doses of thyroxine (μg/kg)	2.5 ± 0.5	2.2 ± 0.6	NS
Daily doses of thyroxine (μg)	171.0± 30	200.0± 50	NS
Duration of suppressive therapy (years)	9.4 ± 6.4	8.1 ± 6.0	NS

NS – not statistical significant; *t-test

sity, i.e., gram of mineral per square cm (g/cm^2). Measurement precision expressed as coefficient of variation was 0.8 % - 1.5 % for the lumbar spine, 1.5% - 3% for the femoral neck, and 3.0% for the radius.

Statistical Analysis

The Statistics for Windows Version 4.0 was used in statistical processing of the results. The results are presented in figures for each variable and for each individual patient, separately for gender. Mean values and standard deviations (X ± SD) are presented in tables according to groups and gender.

The t-test for dependent samples was employed to assess statistical significance of differences in BMD means, whereas t-test for independent samples was used for other results. The test of correlation was used to assess the level of correlation between the variables. Statistical significance was considered at a level of 5 % (5<0.05).

Results

Patient Characteristics

General characteristics of the study patients are presented in Table 2. Male and female patients were matched by age, and there was no statistically significant difference in the duration of suppressive thyroxine therapy between the two sexes. Body height and weight were significantly greater in men, as expected. Thyroxine dose, expressed as micrograms per kg body weight, was higher in women, whereas total daily dose was higher in men due to their greater body weight, however, these differences were not statistically significant.

Thyroid Hormones

Serum concentrations of T_3 ,T_4 and TSH are depicted in Table 3. T_3 concentrations were within the reference range and reached the upper reference limit in one female patient only. In male patients, T_3 concentrations were also within the reference range. The mean T_3 value was almost identical in the two sexes. T_4 values exceeded the upper reference limit in 11 female and two male pateints. The mean T_4 values were otherwise lower in male than in female patients, which could be contributed to the higher T_4 dose (μg/kg) received by female patients. In most study patients, TSH was considerably below the lower reference limit. Mean TSH values were equally low in both sexes, indicating efficient suppression of TSH secretion during levothyroxine therapy.

Table 3. Thyroid hormones concentraion in serum for patients treated with thyroxine (X ± SD).

	PATIENTS		
Hormones	**Female (N = 19)**	**Male (N = 9)**	**Statistical significance***
T_3 (nmol/l)	2.0 ± 0.4	1.9 ± 0.5	NS
T_4 (nmol/l)	181.0 ± 34	147.0 ± 24	p<0.05
TSH (mIU/l)	0.07 ± 0.62	0.06 ± 0.09	NS

NS – not statistical significant; *t-test

Table 4. Parameters of calcium metabolism and PTH in patients (X ± SD).

	PATIENTS		
Parameter	**Female (N = 19)**	**Male (N = 9)**	**Statistical significance***
Serum			
Ca (mmol/l)	2.52 ± 0.15	2.41 ± 0.19	NS
P (mmol/l)	1.17 ± 0.17	1.08 ± 0.20	NS
cr (mmol/l)	69.0 ± 10.0	88.0 ± 11.0	p<0.0001
PTH (pg/ml)	35.0 ± 20.0	27.0 ± 15.0	NS
24- hour urine test			
Ca (mmol/vol)	3.3 ± 2.1	4.3 ± 2.1	NS
P (mmol/vol)	18.30 ± 10.4	22.9 ± 7.3	NS
2 - hour urine test			
Ca (mmol/l)	0.70 ± 0.51	0.82 ± 0.51	NS
cr (mmol/l)	3.90 ± 2.84	7.35 ± 7.8	NS

NS – statistical significant not found ; *t-test

Biochemical Parameters of Calcium Metabolism

Calcium metabolism parameters are shown in Table 4. Serum calcium levels approaching or slightly exceeding the upper reference limit were found in six female and two male patients. Thus, a higher mean serum calcium concentration was recorded in women than in men, however, the difference did not reach statistical significance. Serum phosphorus was at the upper and lower reference limit in one female and one male patient, respectively. Other patients had serum phosphorus values within the reference range. The mean value of this variable was only slightly higher in women then in men. The values of serum creatinin were within the reference range in both sexes, with a significantly higher mean in men compared to women, a known sex difference for this variable. Serum PTH level was below the mean reference value in 12 female and seven male patients, below the lower reference limit in one female patient, and above the upper reference limit in two female patients. Total calcium in 24 hour urine was increased in one female patient and near the upper reference limit in two male patients. The mean of this variable was slightly higher in men than in women. Considerable variations were recorded in 24 hour urine phosphorus levels, which were below the lower reference limit in six women and above the upper reference limit in one woman. In men, the mean urine phosphorus level was slightly higher than in women. The amount of calcium in the 2 hour urine sample ranged up to 1.4 mmol/L in both sexes and exceeded this limit in one man and one woman only. Calcium levels were higher in male subjects, however, the difference was not statistically significant. The level of creatinin in the 2 hour sample ranged up to 10 mmol/L, exceeding this limit in one female patient and two male patients. The values of this variable were also higher in men than in women, due to some extreme high values that increased the standard deviation, however, the difference was not statistically significant.

Biochemical Markers of Bone Turnover

Generally, the values of total alkaline phosphatase activity were within the reference range in both sexes and there was no significant difference between men and women (Table 5). The values of osteocalcin exceeded the upper reference limit in eight female patients, whereas in men they were all within the reference range. There was no statistically significant difference in this variable between the two sexes. PICP was found to be a less sensitive marker of bone formation than osteocalcin. Increased PICP values were recorded in one male patient, whereas in women they were within the reference range. The mean value of this variable was significantly higher in men than in women, which is a known sex difference. The calcium/creatinine ratio was found to be a less sensitive biochemical marker of bone resorption than ICTP in both sexes. An increased calcium/creatinine ratio was

Table 5. Parameters biochemical markers of bone turnover (X ± SD)

	PATIENTS		
Parameter	**Female (N = 19)**	**Male (N = 9)**	**Statistical significance***
Bone formation			
AP	116.0 ± 57.0	102.0 ± 32.0	NS
Osteocalcin (ng/ml)	6.7 ± 1.8	7.6 ± 2.6	NS
PICP (μg/l)	75.0 ± 21.0	110.0 ± 41.0	$p<0.05$
Bone resorption			
Ca/cr	0.192 ± 0.10	0.156 ± 0.92	NS
ICTP (μg/l)	4.3 ± 1.1	4.3 ± 1.6	NS

NS – not statistical significant; *t-test

Table 6. Bone mineral density of different regions in both male and female patients treated with thyroxine at the beginning of observation and after one year (X ± SD).

	Bone mineral density (g/cm^2)		
	FEMALE PATIENTS (N = 19)		
	Start value	**After 1 year**	**Statistical significance***
Lumbal spine (L_2-L_4)	1.252 ± 0.141	1.259 ± 0.148	NS
Femoral neck	1.070 ± 0.114	1.058 ± 0.113	NS
Distal radius	0.668 ± 0.035	0.663 ± 0.034	NS
	MALE PATIENTS (N = 19)		
Lumbal spine (L_2-L_4)	1.329 ± 0.197	1.333 ± 0.199	NS
Femoral neck	1.123 ± 0.155	1.094 ± 0.149	NS
Distal radius	0.748 ± 0.086	0.732 ± 0.083	$p<0.05$

NS – not statistical significant; *t-test.

observed in one female patient only, whereas increased ICTP concentrations were found in eight female and two male patients. The values of calcium/creatinine ratio were higher in women than in men, but this difference did not reach statistical significance. The concentrations of ICTP were almost identical in both sexes (Table 5).

Bone mineral Density Measurements

The initial measurement, performed at the beginning of the study, when female patients had already been treated with thyroxine for more than nine years, revealed osteopenia in the lumbar spine and femoral neck in two patients each, and in the lower third of the radius in four patients. On occasion of the second measurement performed one year later, no statistically significant BMD loss was detected in any of the skeletal regions, as shown by t-test analysis of the mean values of the first and second measurement (Table 6). Analysis of the one year individual values showed the presence of osteopenia in the lower third of the radius in six women, whereas the bone loss in the regions of the lumbar spine and femoral neck, detected in several women, did not reach osteopenic values. As most BMD values remained unchanged on the second as compared to the initial measurement, there was no significant difference between these two values, as also shown by t-test (Table 6). At the beginning of the study, the male patients had been treated with thyroxine for about eight years. In men, osteopenia was found in the lumbar spine, femoral neck and lower third of the radius in one, two and three patients, respectively. At the timepoint of the second measurement performed a year later, the number of male patients with osteopenia in these particular regions was increased by one each. There was no significant difference in BMD values observed on the first and second measurement for lumbar spine and femoral neck, whereas the difference between the two BMD measurements at the radius was statistically significant (Table 6).

Correlations

As mentioned above, study patients had been treated with thyroxine for 8-9 years before entering the study. The first laboratory tests were perfomed at the beginning of the study, according to the study protocol. BMD values and other variables obtained during the study (second measurement) were correlated with the respective values recorded at the beginning of the study. In female patients, a weak to moderate correlation was found between daily doses of thyroxine, and spinal and femoral neck BMD, whereas the lower third of the radius showed a statistically significant negative correlation with daily thyroxine dose (Table 7). In male patients, daily dose of thyroxine was found to poorly

Table 7. Correlation between serum thyroxine, dose and treatment duration with thyroxine at the beginning of the study in male and female patients.

	Bone mineral density (g/cm^2)		
	Lumbal spine (L2-L4)	**Femoral neck**	**Distal radius**
FEMALE PATIENTS (N = 19)			
T_4	-0.163	0.002	-0.079
Daily thyroxine dose	0.095	0.300	-0.368*
Thyroxine treatment length	0.002	-0.172	-0.077
MALE PATIENTS (N = 19)			
T_4	0.293	-0.119	-0.076
Daily thyroxine dose	0.344	-0.017	0.241
Thyroxine treatment length	-0.083	-0.433	-0.360

• Coefficient of correlation ; *p<0.05

Table 8. Correlation between parameters of calcium metabolism and biochemical markers of bone turnover and bone mineral density at the beginning of the study in female patients (N = 19).

	Bone mineral density (g/cm^2)		
Parameter	**Lumbal spine (L2-L4)**	**Femoral neck**	**Distal radius**
Ca (serum)	-0.010	0.110	0.494**
Ca 24-h urine	-0.080	-0.160	0.088
PTH	-0.266	-0.524***	0.048
AP	0.237	0.399*	0.255
Osteocalcin	-0.073	0.030	0.121
PICP	0.043	0.098	-0.174
Ca/Cr	0.001	0.338*	-0.130
ICTP	0.043	-0.210	0.084

• Coefficient of correlation : *p<0.05; **p<0.01; ***p<0.001.

Table 9. Correlation between parameters of calcium metabolism and biochemical markers of bone turnover and bone mineral density at the beginning of the study in male patients (N = 9).

	Bone mineral density (g/cm^2)		
Parameter	**Lumbal spine (L2-L4)**	**Femoral neck**	**Distal radius**
Ca (serum)	0.341	0.622**	0.475*
Ca 24-h urine	-0.180	0.060	-0.200
PTH	0.532*	0.259	0.518*
AP	0.534*	0.062	0.222
Osteocalcin	-0.013	-0.064	0.266
PICP	-0.049	0.010	-0.301
Ca/Cr	-0.260	-0.040	-0.440
ICTP	0.293	0.043	0.350

• Coefficient of correlation : *p<0.05; **p<0.01.

correlate with BMD of the lumbar spine region and the lower third of the radius. There was a slight correlation between the serum thyroxine concentration and BMD in both sexes, only a weak negative correlation with BMD was found on the femoral neck and radius in men (Table 7). In women, there was no correlation between this variable and BMD (Table 7).

In women, a weak to strong positive correlation was found between alkaline phosphatase and BMD at all three measuring sites on the skeleton, and was statistically significant for the femoral neck (Table 8). A weak to strong negative correlation was observed between PTH and BMD of the spine and femoral neck, and was highly statistically significant for the femoral neck region. There was a statistically significant negative correlation between serum calcium and radius BMD as well as between calcium/creatinine ratio and femoral neck BMD. Other calcium metabolism parameters and bone turnover biochemical markers showed only a slight correlation with BMD (Table 8).

In men, there was a weak to strong correlation of serum calcium and PTH with BMD at all three measuring sites on the skeleton, and a statistically significant correlation of serum calcium with the BMD at the femoral neck and radius, and with PTH and the BMD measured at the spine and radius (Table 9). Alkaline phosphatase, calcium/creatinine ratio and ICTP showed a weak to strong correlation (positive for alka-

line phosphatase and ICTP, aud negative for calcium/creatinine ratio) with BMD in two measuring regions of the skeleton, whereas the correlation between alkaline phosphatase and spinal BMD was statistically significant (Table 9). Other laboratory variables showed a poor correlation (negative for calcium in 24 hour urine and PICP, and positive for osteocalcin) with BMD in one skeletal region only (Table 9).

Discussion

According to the small numer of patients in former longitudinal [7, 10, 29] as well as a few prospective [2, 10, 20] studies, the aim of the present one-year-prospective-study was to contribute to the understanding of the effect of longterm use of thyroid hormones in TSH-suppressive doses on BMD and bone turnover. In most patients, BMD decreased mainly statistically non-significant at all measuring sites during the one year study period. The highest statistically significant bone loss was recorded at the male radius. In their longitudinal study, Cvijetic et al, [11] also found a significant bone loss only in the distal third of the radius in women with hypothyroidism under substitution therapy with thyroxine, which was explained by the stronger resorptive effect of thyroxine on the cortical than on the trabecular bone. In another longitudinal study, Diamond et al. [10] found a BMD reduction at the femoral neck, associated with TSH-suppressive therapy in pre- and postmenopausal women, and at the lumbar spine in postmenopausal women only. There was a significant negative correlation between femoral neck BMD and cumulative dose of thyroxine. Female patients in this study [10] had been on suppressive therapy for about ten years, which is almost identical to the duration of suppressive treatment in our study (about nine years). Unlike to our findings, as reported recently by Larijani and collegues [29], a one year suppressive levothyroxine therapy seems not to be associated with a significant increase of osteoporosis risk in premenopausal women.

The values of T_3, T_4 and TSH recorded in the present study were consistent with the literature [6, 10]. The values of parameters of calcium metabolism (serum calcium, total calcium in 24 hours urine, serum PTH, serum phosphorus, total phosphorus in 24 hours urine) were within the expected range. According to the literature, which describes a mild hypercalciuria in 20% of patients on suppressive thyroxine therapy [1], were our findings of serum calcium levels. Serum PTH was below the mean due to slightly increased serum calcium levels [21]. As expected the mechanisms of calcium metabolism induced the following aftereffects: mild hypercalcaemia lead to lower serum PTH, therefore the urinary calcium concentration increased. Further aftereffect can be seen in increased values of total phosphorus in 24-h urine, which was explained by the increased phosphorus reabsorption in renal tubules due to reduced PTH secretion [21]. Sufficient kidney function was demonstrated by normal serum creatinine values, however, statistically significantly higher in men than in women, which is a known sex difference [22].

The most widely used biochemical markers of bone formation (osteocalcin, alkaline phosphatase and PICP) and bone resorption (calcium/creatinine ratio and ICTP) were employed in the assessment of bone turnover [1, 23, 24]. Osteocalcin and bone alkaline phosphatase have been most commonly used, followed by PICP and serum alkaline phosphatase [2, 10, 20]. Elevated levels of osteocalcin and bone alkaline phosphatase activity were recorded in nearly all studies investigating the effect of supraphysiologic thyroid hormone levels on bone metabolism, whereas PICP and serum alkaline phosphatase were found to be less specific parameters [2, 10, 20]. Due to the elevated osteocalcin values, which were found in 8 of 19 women in our study, we concluded osteocalcin to be the most sensitive parameter of bone formation in female subjects. Identical with the findings of other investigations [1, 2, 10, 20, 23, 24] were the levels of serum osteocalcin, serum alkaline phophatase and PICP in males in our study. There was a slight positive statsistically non-significant correlation between osteocalcin and radius BMD in male subjects and a positive statistically significant correlation between the levels of alkaline phosphatase and BMD of femoral neck and the lumbar spine in female and male patients. These findings are contrary to the results of other authors [25], an observation, which is at present quite difficult to explain.

A variety of biochemical markers of bone resorption have been used. Thus, e.g., only ICTP was used in a recently reported prospective study on the effect of an antiresorptive treatment in patients with hyperthyroidism [20]. These authors demonstrated an increased bone turnover at the beginning of the study, and a significant BMD increase after 18 month follow-up with intranasal administration of calcitonin as antiresorptive therapy. The values of ICTP needed six months and those of bone alkaline phosphase some more to return to the reference range, pointing to an enhanced bone formation, i.e. indicating the process of bone loss in patients with hyperthyroidism to be reversible [20]. In another longitudinal study on patients with thyrotoxicosis, osteocalcin and bone alkaline phosphatase were used to assess osteoblastic activity, and serum pyridinolin and desoxypyridinolin and 24-hour urinary pyridinolin and desoxypyridinolin were used to assess osteoclastic activity. At the beginning of the study, reduced BMD was recorded in the femoral neck region and lumbar spine, while biochemical markers of bone formation were increased in more than half of patients, and urine desoxypyridinolin was increased in all study subjects. In most patients, one year administration of carbimazole to thyrotoxic patients resulted in a BMD increase at the femoral neck and the lumbar spine, whereas urinary desoxypyridinolin and serum osteocalcin returned into the reference range after eight weeks of therapy. The levels of bone alkaline phosphatase decreased at a slower rate, also indicating an enhanced bone turnover and osteoblastic activity. This longitudinal study has also shown that elevated serum thyroxine concentrations play a pivotal role in bone loss [2].

In the present study, ICTP and calcium/creatinine ratio were measured as biochemical markers of bone resorption. ICTP was found to be a more sensitive

marker of bone resorption. ICTP values exceeded the reference range in more than one third of female patients and in a somewhat smaller proportion of male patients, which is consistent with previous literature data [1, 20, 23, 24]. Calcium/creatinine ratio and ICTP showed a poor correlation with BMD at the skeletal regions measured, generally in men only. The correlation was positive for ICTP and negative for the calcium/ creatinine ratio. Differences among various studies on the site on the skeleton most severely affected during the thyroid hormone treatment as well as variabilities of the correlation between thyroid hormone levels and BMD remain to be explained by further studies requiring more study subjects. A small number of patients included in the studies as well as the inhomogenicity of study groups must be taken into account. In addition, a one-year prospective period is obviously inadequate to detect BMD changes in all study patients, considering an acceleration of 1% bone loss per year [10, 26], an amount detectable by current devices used for DXA BMD measurement. The main results of our study are consistent with those reported from other prospective studies mentioned above [2, 10, 20].

Using biochemical markers of bone turnover and BMD measurement, our prospective study demonstrated clearly the presence of increased bone remodeling and bone loss at most of the measured skeletal regions in patients on TSH-suppressive thyroxine therapy over the one-year period. However, a statistically significant bone loss was only found in the distal third of the radius in men. Results of our study are in part inconsistent with those reported by other authors who found, mostly in longitudinal studies, no loss of bone in patients on TSH-suppressive thyroxine therapy [8, 15, 17, 18, 26, 27, 28].

The duration of patient follow-up in future prospective studies should be prolonged and number of study subjects increased to better define the relative risk of TSH-suppressive thyroxine treatment for development of osteoporosis and osteoporotic fractures. Prior to the introduction of TSH-suppressive therapy, patients should be referred for osteodensitometry, with successive control BMD measurements after a one-year period, in order to identify high risk patients for osteoporosis.

Conclusion

This prospective study included a group of premenopausal women (n = 19) and men (n = 9) on longterm TSH-suppressive thyroxine therapy after total thyroidectomy for differentiated thyroid carcinoma. One year measurements of BMD and assessment of biochemical markers of bone turnover pointed to the following conclusions:

1) a statistically significant bone loss was only found in the lower third of the forearm in male subjects ($p<0.05$),
2) in the lumbar spine and femoral neck, mild bone loss was detected in some patients, and did not reach statistical significance in either sex; and
3) changes in the parameters of calcium metabolism were comparable to those reported from other studies, and generally included mild hypercalcemia in some and low PTH levels in most study patients.

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