Bone turnover in hyperthyroidism before and after thyrostatic management

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ABSTRACT. Hyperthyroidism is associated with enhanced osteoblastic and osteoclastic activity, and patients frequently have low bone mineral density and high bone turnover. The aim of this study was to examine the bone formation and resorption markers trend in 12 female patients, before and after normalization of thyroid activity. The following measurements were made at baseline and 1 and 6 months after hormone normalization induced by methimazole treatment: total alkaline phosphatase (ALP), bone alkaline phosphatase (BALP), collagen type C-terminal propeptide (PICP), osteocalcin (BGP), telopeptide (ICTP), urinary-hydroxyproline/urinary creatinine (uOHP/uCreat), urinary calcium/urinary creatinine (uCa/uCreat) and deoxypyridinoline crosslinks (D-Pyr). Compared with controls, all of these parameters were significantly increased (ALP *p*=0.014; BALP *p*=0.0001; PICP *p*=0.013; BGP *p*=0.009; ICTP *p*=0.0001; uOHP/uCreat *p*=0.002; uCa/uCreat *p*=0.044; crosslinks *p*=0.0001). After treatment the values of ALP, BALP and PICP in hyperthyroid patients showed an initial slight increase and then a significant downwards trend (ALP *p*=0.008, BAP *p*=0.001, PICP *p*=0.026). Furthermore, resorption markers showed a significant decrease (uOHP/uCreat *p*<0.005 and D-Pyr *p*<0.008). As regards lumbar BMD patients, measurements were significantly reduced in comparison with the control group (*p*=0.005). Six months after serum thyroid hormones level normalization, we observed a significant increase (*p*=0.014 *vs* baseline). Both neoformation and resorption markers are useful to assess pathological bone turnover and bone involvement in hyperthyroidism. They could also be employed to monitor the effect of antithyroid treatment on bone and to indicate if bone antiresorption therapy should be considered.

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INTRODUCTION

Thyrotoxicosis enhances bone turnover by increasing the number of osteoclasts and resorption sites. Both cortical and trabecular bone thus display a substantial increase in both superficial osteoclastic resorption and osteoblastic bone formation (1, 2). This increased resorptive-formation ratio significantly reduces bone mineral density (BMD) (3, 4), and makes thyrotoxicosis a risk factor for osteoporosis and its associated fractures (5, 6).

Normalization of thyroid hormone levels with antithyroid drugs is followed by significant BMD increments after 1-2 years (7-10).

Morphometric and dynamic studies have shown that increased secretion results in a significant excess of resorption that markedly disturbs the balance between resorption and formation (1, 11, 12). The bone remodeling changes observed in hyperthyroidism have been attributed to increased cAMP production, and the probably prostaglandin-related release of resorption-stimulating factors (13-15). In fact, an increase of IL (interleukin)-6 and IL-8 was found in hyperthyroid patients (16), even though the precise pathogenetic meaning of cytokines on bone metabolism is unclear; yet, data obtained on osteoblasts stimulated *in vitro* by T3 (17) seem to show that osteoporosis in hyperthyroidism is closely associated with serum IL-6 levels.

Functional and structural bone changes in hyperthyroidism are normally associated with elevated levels of serum alkaline phosphatase (ALP) and osteocalcin (BGP), two biological bone formation

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markers (9, 18, 19). Direct stimulation by thyroid hormone of osteoblasts possessing nuclear T3 receptors has been demonstrated (20). The resorption markers urinary hydroxyproline (uOHP) and pyridinoline are also elevated and their levels significantly correlate with the circulating thyroid hormone concentration (19, 21, 22). Garnero *et al*. have shown that urinary pyridinoline cross-link excretion is a highly sensitive marker for altered bone metabolism, whereas BGP osteocalcin and bone alkaline phosphatase (BALP) increments are less impressive (22).

Since biochemical bone turnover markers are of particular interest in the investigation of bone disorders associated with thyroid diseases, in this study we measured their plasma and urinary levels to determine their changes in hyperthyroid patients before and during treatment and to define the effects of hyperthyroidism on bone turnover.

MATERIALS AND METHODS

We examined 12 female patients aged 20-68 (5 post-menopausal) with a clinically evident and biologically confirmed (high thyroid hormone level and TSH inhibition) hyperthyroidism due to Graves'disease (3 patients) and multinodular goiter (9 patients). None of them had been treated with antithyroid drugs or other drugs known to influence bone calcium metabolism (including hormonal replacement therapy), and they were all free from acute or chronic diseases.

The controls were 12 age-matched, clinically healthy with normal plasma TSH and thyroid hormones women; the 5 post-menopausal controls did not differ from the 5 post-menopausal hyperthyroid patients for what regards years since menopause.

The following parameters were evaluated at the baseline in both groups:

A) blood FT3, FT4, TSH, calcium and phosphorus, ALP, intact PTH, medium molecule PTH, BGP, BALP, C1 procollagen (PICP), telopeptide (ICTP);
B) urinary deoxypyridinoline (D-Pyr) crosslinks, urinary hydroxyproline/urinary creatinine (uOHP/uCreat), urinary calcium/urinary creatinine (uCa/UCreat) and urinary phosphorus (2-hour morning urine);
C) L2-L4 bone density.

All of the patients received for 2 months a mean dose of 17.5 mg/day methimazole (range 10-20 mg) and their thyroid hormones levels were measured monthly; then the mean dose was of 10 mg (range 5-15 mg).

All of the parameters were reassessed 1 month and 6 months after thyroid compensation.

The Amerlex-mAb FT3 and FT4 kits were used to assay the free hormone fractions (CV within assay was 3.5% for fT3 and 3.7% for fT4; CV between assay was 6.5% for fT3 and 5.0% for fT4). TSH levels were quantified by the Biocode TSH IRMA kit (CV within assay 6.2% and between assay 7.1%; sensitivity 0.05 (μU/ml). Blood phosphorus and calcium were evaluated enzymatically and ALP by the IFCC method (ALP CV 2.6%).

uCa was assayed by photonic absorption, while uOHP was tested by HPLC with a fluorometric detector. Results of both markers were expressed as a ratio to creatinine.

PTH was determined by RIA methods using Incstar Corporation kits (CV within assay 5%; CV between assay 7.3%). Also BGP was measured on serum after centrifugation and storage at -20 C by RIA methods (Incstar Corporation kits) (CV within assay 3.8% and between assay 4.8%).

The bone isoenzyme of ALP was tested by an IRMA method from Hybritech (CV within assay 4.2%; CV between assay 7.2%). PICP and ICTP were determined using the RIA method provided by Orion Diagnostica (CV within assay 2.1% and between assay 4.1%; CV within assay 4.4% and between assay 4.9%, respectively).

The urinary D-Pyr crosslinks were measured by an enzyme immunoassay provided by Metra Biosystems (CV within assay 4.3% and between assay 4.6%).

Lumbar BMD was measured by means of the DXA technique (Hologic QDR 1000 system); our CV is 1.2% *in vivo* and 0.5% *in vitro*.

Statistics were obtained by using Student's *t* test for paired and unpaired data.

RESULTS

The blood calcium, phosphorus and PTH levels of hyperthyroid patients were not significantly different from those of the controls and remained within the normal limits throughout the study.

Patients' TSH, thyroid hormone, bone marker and BMD are compared with those of the controls in Table 1.

Figure 1 shows the percent variations of all of the parameters in patients *vs* controls in basal conditions.

No significant correlation between FT3 and FT4 and the turnover markers was found in the patient group. The temporary trend of bone formation markers (ALP, BALP, BGP, PICP) are shown in Figure 2, those of the resorption markers (ICTP, OHP/uCreat and D-Pyr crosslinks) in Figure 3. Total ALP baseline was higher (p=0.014) in patients than in controls (Table 2) and strongly increased, although not

Table 1 - *TSH, FT3, and FT4 basal values in hyperthyroid patients.*

Patients	TSH (μU/ml)	FT3 (pg/ml)	FT4 (pg/ml)
N. 1	0.01	19.7	48.9
N. 2	0.00	21	52.1
N. 3	0.00	19	49.3
N. 4	0.00	21.4	52.7
N. 5	0.01	22.3	56.4
N. 6	0.02	20	47.2
N. 7	0.00	17	43.5
N. 8	0.00	20.3	49
N. 9	0.01	21	51
N. 10	0.00	25.2	59.7
N. 11	0.00	18.8	47.3
N. 12	0.00	20.9	50.1
Mean values	0.00±0.01	20.55±2.02	45.97±13.54
Normal range	0.3-5	2.2-4.7	8-20

significantly, one month after compensation before decreasing (Fig. 2).
Similar patterns were displayed by the other markers: the patients' baseline BALP was significantly higher than in the controls ($p<0.0001$) (Table 2) and rose one month from normalization before falling within the normal range after six months (Fig. 2).
The patients' higher baseline PICP ($p=0.013$ *vs* the controls) displayed a slight significant change during the study ($p=0.026$ *vs* one month) (Fig. 2).
The patients' BGP baseline was slightly higher than in the controls ($p=0.009$) (Table 2). The decrease after normalization was not significant (Fig. 2).
The ICTP baseline was significantly higher than in the controls ($p=0.0001$) (Table 2). Substantial decreases after compensation resulted in levels significantly below the baseline ($p=0.0001$) (Fig. 3).
The uOHP/uCreat ratio was higher than in the controls ($p=0.002$) (Table 2) and fell significantly after normalization (Fig. 3).
Similar patterns were displayed by uCa/uCreat and the D-Pyr crosslinks (Table 2). The latter was significantly higher ($p=0.0001$) than in controls (Table 2) and both fell significantly ($p=0.003$) one month after compensation and mantained this decrease after six months ($p=0.008$) (Fig. 3).
Lastly, the baseline lumbar BMD was significantly lower in the patients ($p=0.005$) and rose slightly, but significantly, six months after compensation (Fig. 4).

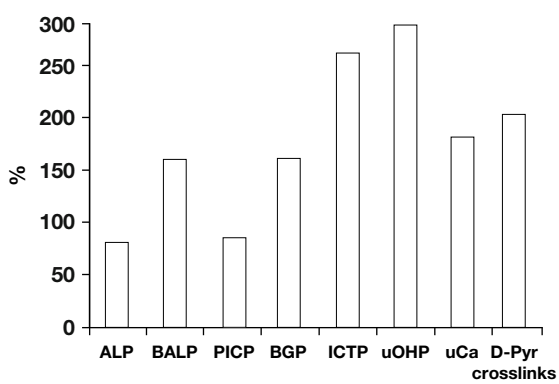

Fig. 1 - *Percent differences between patient and control bone turnover marker baselines.*

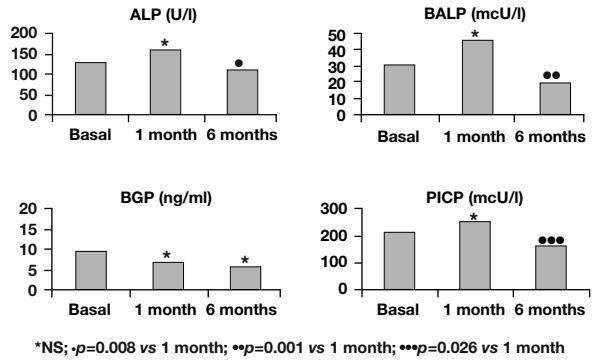

Fig. 2 - *Bone formation marker patterns in hyperthyroid patients: baseline and one and six months after thyroid hormone normalization.*

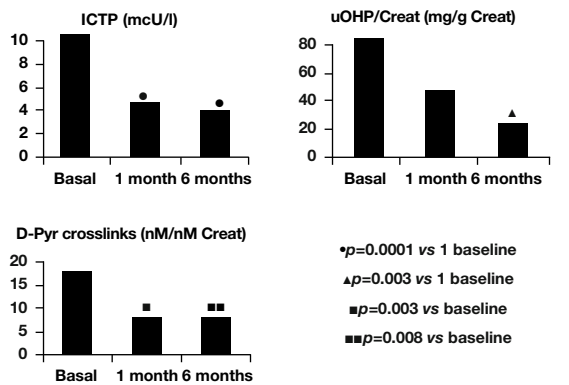

Fig. 3 - *Bone resorptions marker patterns in hyperthyroid patients: baseline and one and six months after thyroid hormone normalization.*

Table 2 - *Mean±SD in controls and hypertension patients.*

	Controls	Basal hyperthyroid patients	
TSH (µU/ml)	3.53±0.46	0.00±0.00	$p<0.0001$
FT3 (pg/ml)	2.82±0.49	20.66±2.17	$p<0.0001$
FT4 (pg/ml)	10.45±0.9	50.69±4.73	$p<0.0001$
ALP (U/l)	77±26.9	139.4±64.19	$p=0.014$
BALP (µU/l)	12.21±7.08	33.23±14.07	$p=0.0001$
PICP (µU/l)	122.8±36.67	234.5±104.09	$p=0.013$
BGP (ng/ml)	3.7±1.74	9.68±5.50	$p=0.009$
ICTP (µU/l)	2.71±1.02	10.20±3.22	$p=0.0001$
uOHP/Creat (mg/g Creat)	19.99±6.65	80.30±48.85	$p=0.002$
uCa/Creat (mg/mg Creat)	0.07±0.05	0.19±0.17	$p=0.44$
D-Pyr Crosslinks/Creat (nMol/mMol Creat)	6.5±1.42	19.65±8.91	$p=0.0001$
BMD (g/cm^2)	1.010±0.18	0.85±0.2	$p=0.05$

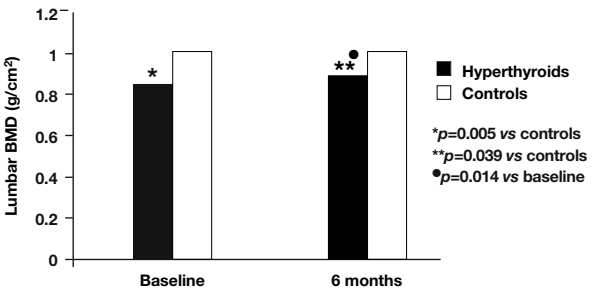

Fig. 4 - *BMD patterns in controls and in hyperthyroid patients (baseline and six months after thyroid hormone normalization).*

DISCUSSION

Hyperthyroidism is accompanied by marked changes in bone remodeling that may lead to substantial BMD reduction.

In our patients the transient increase in specific bone formation markers (ALP, BALP and PICP) observed one month after the achievement of euthyroidism was evidence of enhanced osteoblastic activity, probably reflecting an attempt of bone turnover to become positive, in response to increased resorption; alternatively it can be explained by the persistent slight increase in cortical bone turnover (23).

In contrast, BGP concentrations did not change significantly and, at least with the method used in the present study, are of little assistance in the evaluation of bone turnover. Other workers, however, have reported significant BGP increases in hyperthyroid patients and a significant correlation between FT3 and BGP baseline (9, 11, 18, 24). This discrepancy may be due to the variability of different BGP assays and of measuring samples from the same subject in different analytical batches (25). Our data suggest that BGP, at least measured by this method, is less able than BALP to indicate changes in bone metabolism.

It is clear that hyperthyroidism shifts the balance of bone remodeling from formation to resorption (13, 19, 21, 22).

The bone resorption markers, on the other hand, fell in step with the reduction in thyroid hormones and quickly became normal when compensation was obtained (21, 22).

D-Pyr displayed the most rapid and significant reduction. This behavior as observed by other authors (16) is different from that of bone formation markers and suggests the persistence of the increase of bone formation ever after attainment of the euthyroid state.

Also the L2-L4 BMD, significantly lower in hyperthyroid patients than in controls, raised significantly in a very short time (26) and so we can conclude that specific bone management therefore is not required.

As pointed out by Siddiqi *et al.* however, the exact role of bone markers in hyperthyroidism remains to be defined (9).

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