Acute effects of recombinant human TSH on bone markers in differentiated thyroid cancer

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Abstract

Patients suffering from differentiated thyroid cancer receive suppressive of TSH thyroxine treatment of long duration. This study was undertaken to determine changes on bone serum markers after administration of recombinant human TSH in differentiated thyroid cancer patients on thyroxine treatment. *Forty-five patients* undergoing diagnostic evaluation of their disease and 48 matched controls were investigated: two injections of 0.9mg of recombinant human TSH were given to the patients (on days 1 and 2). Blood samples were collected the day before first injection (day 0) and days 3, 5 and 10 after recombinant human TSH administration. Blood samples were obtained for serum TSH, bone alkaline phosphatase, osteocalcin, osteoprotegerin, receptor activator of nuclear factor kB ligand and bone tartrate resistant acid phosphatase. *Recombinant human TSH induced* a significant increase in bone alkaline phosphatase on day 3 up to day 10 in postmenopausal women. A statistically significant increase was also observed in serum receptor activator of nuclear factor kB ligand in both men and postmenopausal women on day 3 while on day 10 these values returned to baseline levels. No significant effects were seen in other parameters at any time of the investigation. *In conclusion,* we demonstrated significant increases in receptor activator of nuclear factor kB ligand and bone alkaline phosphatase after TSH stimulation. The changes in these bone indices were more prominent in the group of postmenopausal women.

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Introduction

The clinical effects and the mechanism by which thyroid hormones act on bones have been a subject of discussion for a long time [1]. Most of thyroid function disorders may result in reduced bone density and/or increased fracture rate that should be taken into consideration at clinical evaluation [2]. Thyrotropin (TSH) receptors are present both in osteoclast and osteoblast and TSH can modulate bone remodeling independently of thyroid hormones [3]. The introduction of new diagnostic tools and biochemical bone markers enabled smaller but important changes in bone mineral metabolism to become apparent [4].

Receptor activator of the nuclear factor-kB ligand (RANK-L) and osteoprotegerin (OPG) pathway regulates the production and activity of cells in the osteoclast lineage. RANK-L interacts with RANK, a TNF receptor family member, on cells of the macrophage/monocyte lineage as well as on mature osteoclasts. The soluble protein OPG, released by osteoblasts interrupts the interaction between RANK-L and RANK. In hyperthyroid patients increased levels of OPG have been reported associated with bone loss [5]. Long-term suppressive thyroxine treatment also increases serum OPG levels in patients operated for differentiated thyroid cancer (DTC) [6].

Tartrate-resistant acid phosphatase (TRAP) activity has been suggested as a marker of osteoclast activity in bone resorption [7]. Serum TRAP activity may be elevated in postmenopausal healthy and osteoporotic women, in whom TRAP activity is inversely correlated with bone mineral density [8]. The alkaline phosphatases form a family of isoenzymes [9], and serum bone alkaline phosphatase (bALP) is clearly less affected by nonskeletal disorders and therefore is more specific for changes in bone formation [10]. Osteocalcin (OC) is one of the major noncollagenous proteins of the bone matrix [4, 5], and has been used extensively in bone studies as an index of bone formation [11].

Thyroid cancer patients form an interesting group for bone studies, while they are treated with TSH suppressive thyroxine treatment. With the wider use of recombinant human TSH (rhTSH) in DTC management [12], questions rose regarding the acute effects of TSH on bone metabolism. The aim of this study was to evaluate the effects of acute increases of serum TSH after rhTSH administration on bALP, OC, OPG, RANK-L and TRAP in DTC patients.

Subjects and methods

Forty-five patients were included: 28 women (7 premenopausal / 21 postmenopausal) and 17 men, who were compared with 48 healthy age- and sex-matched controls (11 premenopausal women/ 20 postmenopausal women /17 men). Female controls were also matched for menopausal status.

All patients had differentiated thyroid cancer (DTC) that had been treated with total thyroidectomy, followed by a radioiodine ablative dose administration. Only patients in complete remission were considered for participation in the study (normal neck ultrasound, negative ^{131}I whole body scan and stable low thyroglobulin levels). Except for thyroxine therapy in TSH suppressive doses, patients were not using any drugs and they did not have any other diseases known to affect bone and calcium metabolism. Patients with hypoparathyroidism after thyroid surgery with known osteoporosis or those taking bone-active drugs were not included in the study. None of the postmenopausal women was under oestrogen replacement treatment. Controls did not have thyroid or other known chronic disease and were not receiving any medication. They were recruited from the Osteoporosis and Thyroid disease outpatient clinics of Papageorgiou University Hospital. Controls did not undergo rhTSH stimulation.

Patients and controls gave informed consent and the study was approved by the Institutional Review Board.

Recombinant human TSH (rhTSH (Genzyme Co., Cambridge, MA, USA) was administered at a dosage of 0.9mg intramuscularly once daily, for two days (day 1 and day 2) in DTC patients undergoing periodic staging of their disease and a diagnostic ^{131}I whole body scan and serum thyroglobulin measurement were performed on the 5th day of the protocol. Samples were collected the day before the first injection (day 0) and on days 3, 5 and 10 after rhTSH administration and kept deep frozen, up to the time of analysis.

Every sample was collected following overnight fasting and after 12h abstinence from smoking. In every case, a blood sample was obtained for serum TSH, bALP, OC, OPG, RANK-L and TRAP evaluation in all patients and controls.

Immunoradiometric assay (IRMA) was used for the determination of serum TSH (DIA-Sorin, Italy). The detection limit of the assay, the intra- and inter-assay variations expressed as coefficient of variation (CV%) were 0.05mU/L, 3.1%, and 4.1% respectively. Osteocalcin (OC) was assessed by radioimmunoassay (Myria RIA kit, Technogenetics, Milan, Italy) with detection range of 0-60ng/mL (0.172nmol/L) and sensitivity 0.30ng/mL. Serum OPG was assayed by a commercial enzyme linked immunosorbent assay (ELISA) sandwich (DRG International Inc. USA). The mean value study with serum samples from young healthy donors has been established by the manufacturer at 4.1±0.33 pmol/L. Observed intra-assay and inter-assay variation CV% was 5% and 6% respectively. Solid-phase immunofixed enzyme activity assay (Immunodiagnostic Systems Ltd., Boldon, UK) was used for the determination of osteoclast-derived TRAP-5b activity from human serum samples. Intra- and inter-assay variation CV% were both 6%. The sRANK-L test kit (Demeditec Diagnostics GmbH, Kiel, Germany), an enzyme immunoassay for the detection of soluble, noncomplexed human RANK-L directly in biological fluids, had a detection limit of 0.1pmol/L, intra-assay CV 4.57% and inter-assay CV 6%-8%. Enzyme immunoassay (EIA) (Ostase®, Immunodiagnostic Systems Ltd., Boldon, UK) was used for the quantitative measurement of bone specific alkaline phosphatase (bALP) with detection limit of <1μg/L, inter- and intra-assay CV% lower than 10%.

All samples from the same subject were evaluated in duplicate for any assessment in the same assay. Serum TSH was analyzed on the day of collection.

Statistical analysis

Values are expressed as mean±SD. Group means were compared by analysis of variance (one-way ANOVA) with Bonferroni's correction for multigroup comparison.

The P value<0.05 were considered significant. Analysis was performed using SPSS

software for Windows, version 13.0 (SPSS Inc., Chicago, IL).

Results

Patients' characteristics are shown in Table 1. Differentiated thyroid carcinoma patients' and control groups were not significantly different in age even when analyzed according to sex. Data were also analyzed separately for men, premenopausal and postmenopausal women.

As expected in thyroid cancer patients, basal serum TSH was significantly lower (P<0.01) than in the control group due to suppressive thyroxine dose. Following rhTSH administration, maximum serum TSH was observed a day after the second rhTSH injection (at day 3). Peak serum TSH was lower in males than in females, although significant differences were seen only between males and postmenopausal women (74.75±16.4 vs 92.01±25.10mU/mL).

Patients showed higher OC concentrations than controls (Table 1). rhTSH did not induce significant changes in serum OC levels.

In DTC patients bALP was significantly higher than in the control group (12.78±5.4 vs 8.9±3.1μg/L, P<0.05). However, this difference was not significant between subgroups. rhTSH induced significant increases of serum bALP levels, especially at day 3, only in postmenopausal patients (P<0.005) (Fig. 1), and bALP levels remained high even at day 10.

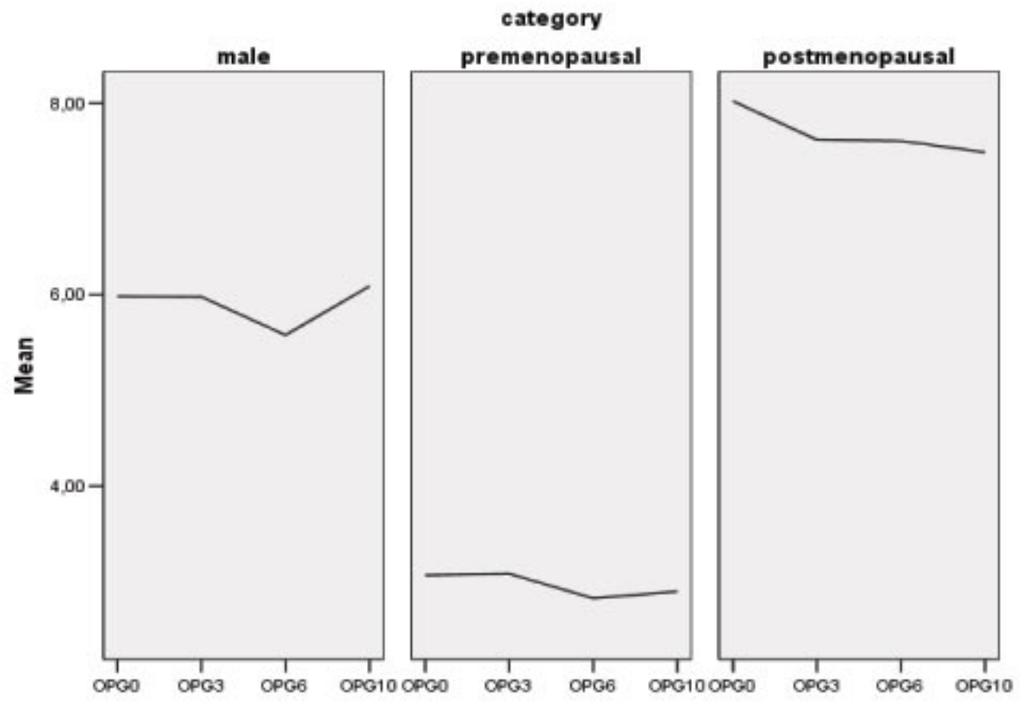

Figure 1. Changes in OPG levels in patients at days 0, 3, 6 and 10.

Lower OPG values were found in premenopausal women and high in postmenopausal women at day 0

(3.07±1.7pmol/L vs 8.01±2.7pmol/L, (P<0.05). No significant changes were seen in each study group at any time point between patients and controls.

Significantly higher serum RANK-L levels were found in male patients. All controls groups showed higher serum RANK-L value compared to patient groups (P<0.01). After rhTSH administration, a significant increase of serum RANK-L was found in both men and postmenopausal women at day 3 while at day 10 the values returned to baseline levels. This increase was not significant in premenopausal women (Fig. 2).

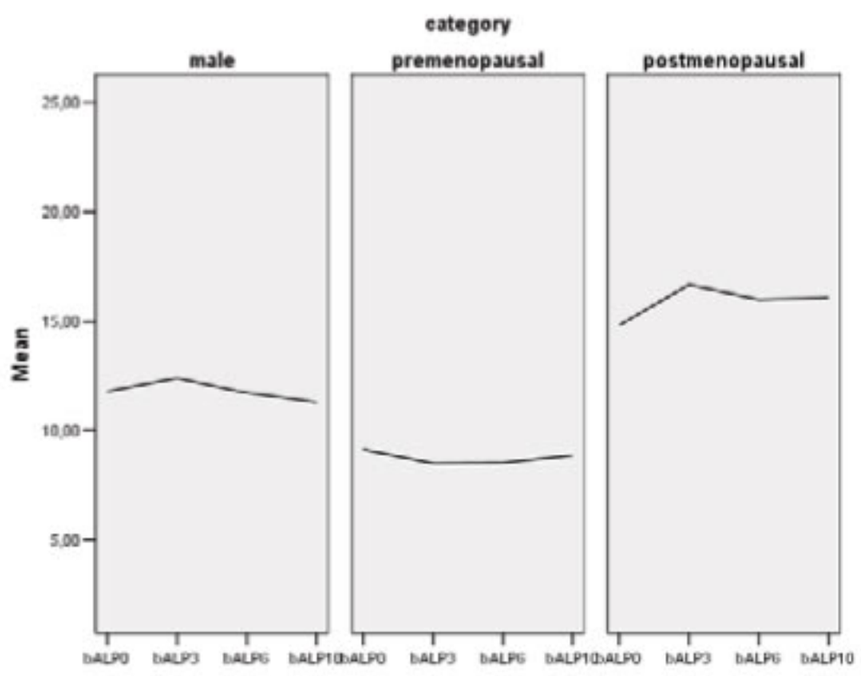

Figure 2. Changes in bALP levels

In all DTC patients a strong, significant positive correlation between OPG baseline levels and age was seen (r^2=0.6 P<0.001), while RANK-L baseline levels were negatively related to age (P=0.054) (Fig. 3).

Results

During follow-up, DTC, patients are given on suppressive thyroxine treatment for a long period of time, which induces subclinical hyperthyroidism in most of these cases. Patients with hyperthyroidism or with a history of thyroid cancer seem to have fractures earlier in life then the general population [12], and therefore it is important to identify the possible negative effects of suppressive thyrotoxine treatment on bone metabolism, in these groups.

Overt [13] and subclinical hyperthyroidism [14] have been associated with increased serum OPG levels, which normalize after introduction of treatment. In patients with thyroid cancer under thyroxine treatment, changes in the OPG/RANK-L system have been reported: a study on male thyroid cancer patients reported increased OPG and decreased RANK-L levels compared to matched controls [15]. Another study with male and female thyroid cancer patients reported that postmenopausal women had significantly lower OPG levels and higher RANK-L concentrations than matched controls [16]. Contrary to the previous study, we observed higher OPG levels in the subgroup of postmenopausal patients. Despite the discrepancies in OPG levels in our and in earlier studies [17], all these studies revealed a positive correlation between OPG levels and age in thyroid cancer patients, similarly to other studies which evaluated patients with benign thyroid diseases [17]. The rise of OPG levels with age cannot solely explain the increased OPG levels found in our postmenopausal patient subgroup, as OPG differences between premenopausal and postmenopausal DTC patients was more marked than in controls. A possible additive effect of age and subclinical hyperthyroidism in our patients compared to our controls (with normal thyroid function) may exist. In accordance with previous reports [16], RANK-L levels were found in our study lower in DTC patients compared to controls. Taken together, the OPG/RANK-L system in DTC patients was found affected, with increased OPG and decreased RANK-L levels at baseline. The effects of recombinant TSH (rhTSH) on bone turnover markers have recently examined in a study of 30 DTC patients [3]: after rhTSH, OPG did not change and RANK-L levels increased in men and postmenopausal women at day 3, returning to baseline levels at day 5. Similarly, we also observed that after rhTSH stimulation OPG levels varied insignificantly, while RANK-L increased in men and postmenopausal women at day 3 and returned to baseline values at day 10. We aimed to see if delayed responses exist for bone markers after rhTSH stimulation and therefore extended our study period up to 10 days. Overall, a transient up-regulation of the OPG/RANK-L system seems to occur after rhTSH. Evidence for an inhibition of bone resorption in this setting has also been given by a recent work [18], showing decreases in calcium and C- and N-terminal telopeptides of type I collagen and increases in PTH at day 5 after stimulation.

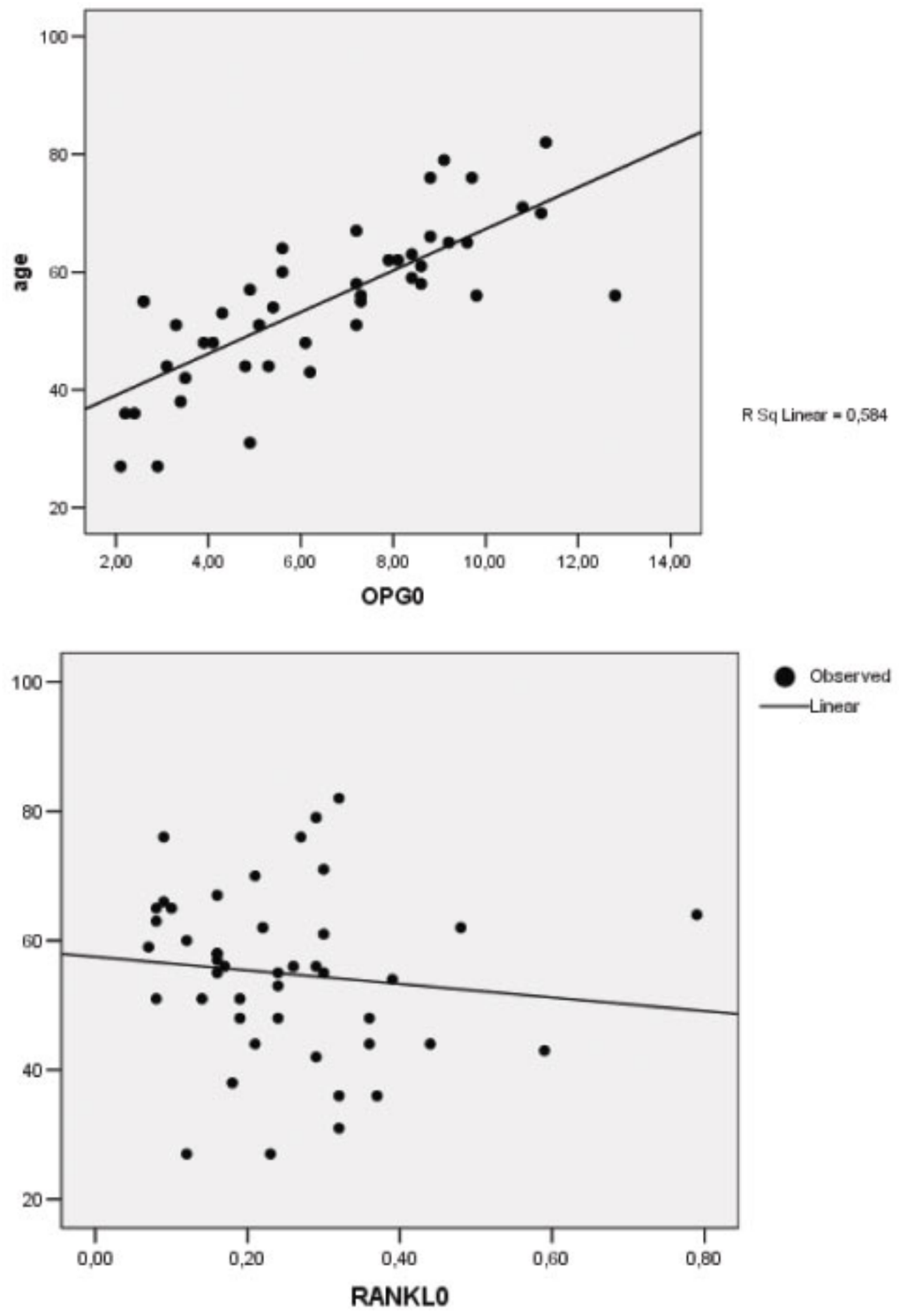

Figure 3. Positive correlation between OPG at baseline and age (r^2=0.6 P<0.001) (above), and negative correlation between RANKL at baseline and age (r=0.3, P=0.054) (below).

Higher TRAP levels were seen in males, both controls and patients (P<0.05). All DTC patients had elevated TRAP with significant results noted only in pre- and postmenopausal women (P<0.05). There was no significant variation of TRAP levels induced by rhTSH.

Table 1. *Characteristics of patients and controls*

	Men		Premenopausal		Postmenopausal	
	Controls	DTC	Controls	DTC	Controls	DTC
Number	17	17	11	7	20	21
Age (yrs)	51.9± 9.4	54.2± 12.7	33±8.2	35.5±6.6	63.2±9.1	61.3±8.6
TSH (mU/mL)	2.2± 0.8	0.3± 0.3 [a]	2.8±0.3	0.1±0.1 [a]	2.5±0.4	0.2±0.3 [a]
FT4 ng/dL	1.1±0.4	1.42±0.3	0.9±0.3	1.34±0.4	1.1±0.3	1.48±0.3
OC (ng/mL)	5.5 ±1.2	7.8 ± 2.1 [a]	4.3 ±2.2	7.9 ±3.5 [a]	7.1 ±3.2[b]	8.1 ±3.7
RANKL (pmol/Lt)	0.92±0.41	0.33± 0.17[a]	0.65±0.32	0.23±0.08[a]	0.75±0.43	0.19±0.08[a]
bALP (μg/Lt)	8.9±3.1	11.78± 4.2[a]	8.5±3.2	9.1±2.8[d]	9.2±1.9	14.82±6.1[a]
TRAP U/Lt	4.8 ±1.6	5.1 ± 0.6	2.2 ±0.1	4.2 ±0.7 [a]	3.0 ±0.5 [b]	4.6 ±0.5[a]
OPG (pmol/Lt)	4.1±1.7	5.98± 2.04	2.7±1.1	3.07±1.7[d]	5.8±1.99	8.01±2.7[a]

Values are expressed as mean□SD. [a]P <0.05 vs controls, [b]P <0.05 vs men & premenopausal (controls), [c]P <0.05 vs controls postmenopausal, [d]P <0.05 vs men and postmenopausal with DTC.

Additionally, rhTSH induced a significant increase in bALP in postmenopausal women (present at days 3, 5 and 10). Increases in baseline bALP and further increments of bALP for 7 days after rhTSH [19] have been observed. The relatively prolonged effect on bALP levels favors the notion of stimulation of bone formation after rhTSH stimulation.

At baseline, serum OC was elevated in premenopausal women and male patients, in agreement with an older study in female patients [20]. Levels of TRAP were also found increased in both premenopausal and postmenopausal women, although a significant correlation with age has been observed [21]. In order to explain the discrepancies seen between studies one has to take into consideration a) the effects of different doses of suppressive thyroxine treatment, as smaller doses induce less striking or even no changes in bone markers [22] and b) the menopausal status of women.

In conclusion, we demonstrated significant increases in RANK-L and bALP after rhTSH stimulation, without effects on serum OPG, OC and TRAP. The changes in bone indices were more prominent in the group of postmenopausal patients. Bearing in mind that these patients are the most vulnerable for suffering from osteoporosis, the need for longitudinal studies to a) examine the optimum thyroxine suppressive dose and the minimum period of time for TSH suppression in these patients and b) to clarify the effects of TSH stimulation are justified.

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