

Research paper

Recombinant human thyroid stimulating hormone does not acutely change serum osteoprotegerin and soluble receptor activator of nuclear factor- κ B ligand in patients under evaluation for differentiated thyroid carcinoma*

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ABSTRACT

OBJECTIVE: Some extra-thyroid actions of thyroid stimulating hormone (TSH), such as an *in vitro* action on bone, have been described. Our aim was to evaluate *in vivo* the acute effect of a recombinant human TSH (rhTSH)-induced TSH surge on Osteoprotegerin (OPG) and receptor activator of the nuclear factor- κ B (RANK-L) levels in patients under levo-thyroxine (L-T4) therapy. **DESIGN:** 24 patients with differentiated thyroid carcinoma (DTC) were studied. Standard rhTSH testing was performed. OPG, RANK-L, TSH, thyroid hormones, thyroglobulin and several parameters of bone metabolism were evaluated. **RESULTS:** Baseline OPG and RANK-L levels were in the range of our reference population. An inverse correlation between OPG and spinal Z-score ($p=0.029$) and between RANK-L and age ($p=0.018$) or urinary calcium/creatinine ratio ($p=0.011$) was detected. After rhTSH administration, a significant ($p<0.001$) increase in TSH was found. No significant increase in OPG or RANK-L levels after rhTSH was observed. No correlation was detected between TSH peak value after rhTSH and maximal percentage change in OPG or RANK-L. A slight increase in urinary cross-links after rhTSH was found. **CONCLUSIONS:** In a small group of subjects with a history of DTC on L-T4 regimen, our study did not support an acute direct effect of TSH on OPG and RANK-L.

Key words: Differentiated thyroid carcinoma, Osteoprotegerin, RANK-L, rhTSH

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1. INTRODUCTION

Bone homeostasis is the result of coordinated interactions among local and systemic factors that regulate the genesis, activity and survival of osteoblasts and osteoclasts. Recent experimental studies have shown that osteoclastogenesis is mainly determined by an endogenous cytokine system, which regulates the maturation of preosteoclasts into active osteoclasts.¹⁻⁴ Osteoprotegerin (OPG)^{1,2} and receptor activator of the nuclear factor- κ B (RANK)/RANK-ligand (RANK-L) system^{3,4} are now thought to explain the mechanisms of the cross-talk between osteoblasts and osteoclasts. OPG is considered to be a soluble receptor secreted by several types of cells, including osteoblasts^{1,5}. Thyroid follicular cells also seem to produce OPG, and this synthesis may be modulated by thyroid autoimmunity.⁶ OPG works as a decoy receptor for RANK-L, thereby acting as an inhibitor of osteoclastogenesis.⁷ Many cell types, including osteoblasts, express RANK-L.⁸ The cell-bound form of RANK-L is the most common, although a soluble form exists, which is produced by the cleavage of an ectodomain truncated by a protease and primary secretion from cells.^{4,7} By binding to RANK, RANK-L promotes osteoclast differentiation, activation, survival and adherence to bone surface.^{9,10}

In humans, recent studies have indicated that serum OPG can be regarded as a marker of cardiovascular risk¹¹ and that the OPG/RANK/RANK-L system is involved in several diseases characterized by bone loss, such as osteoporosis – either in the elderly or glucocorticoid-induced¹² – bone metastasis,¹³ and rheumatoid arthritis.¹⁴ On the other hand, a pilot study in which OPG was administered to post-menopausal women showed that it might have a therapeutic role in reducing bone loss.¹⁵ Overt hyperthyroidism is another condition in which bone loss occurs.¹⁶ In patients with hyperthyroidism, an increase in serum OPG levels has been reported which can be reversed by treating the disease, even in the presence of persistent microstructural deterioration of bone as determined by ultrasonography.¹⁷ An increase in serum OPG has also been found in sub-clinical and overt hypothyroidism before substitutive therapy, probably as a consequence of vascular damage.¹⁸ Recently, in patients operated on for differentiated thyroid carcinomas (DTC) and on long-term suppres-

sive levo-thyroxine (L-T4) treatment, an increase in serum OPG in comparison with age-matched normal subjects has been reported.¹⁹ Finally, in a cross-sectional study performed on a random sample of community-dwelling adults, serum OPG levels were associated with a profile of bone turnover markers favouring bone formation, suggesting that OPG may be protective against age-related bone loss.²⁰

To our knowledge, very few data are available on soluble RANK-L in humans, owing to technical problems in the assay.⁸ In a group of endurance runners, a significant decrease in RANK-L was observed during running, the extent of which seems to be correlated with the distance covered.²¹ Moreover, reduced soluble RANK-L levels in DTC in comparison with healthy euthyroid age-matched controls have recently been reported.¹⁹

Our knowledge of the OPG/RANK/RANK-L system in humans is still scant. The future role of its evaluation in clinical practice remains to be clarified,⁸ and the controversial extra-thyroid action of TSH on bone must be considered within this scenario.²² TSH might exert a direct role in bone remodelling by interacting with specific receptors expressed on bone cells.²² The increase in serum TSH levels produced by recombinant human TSH (rhTSH) administration can be exploited in an *in vivo* model to evaluate the possible acute effect exerted by TSH on the OPG/RANK/RANK-L system. To our knowledge, only one paper has been published on this topic. Mazziotti et al²³ showed that the acute increase in TSH levels in DTC patients after rhTSH is accompanied by a decrease in serum C-telopeptide of type 1 collagen and an increase in bone-alkaline phosphatase, without any significant effect on circulating OPG concentration.

Our study was scheduled to further evaluate, in a well characterized group of DTC patients, the acute effect of rhTSH on OPG and to extend evaluations to soluble RANK-L and urinary products of bone collagen metabolism.

MATERIALS AND METHODS

Subjects

The study was conducted during scheduled yearly

rhTSH testing of 24 outpatients (mean age \pm SD: 50.7 ± 12.2 years) with a history of stage 1-4 DTC. Pertinent clinical data on the subjects studied are reported in Table 1. The majority of patients were pre-menopausal ($n=7$; 38.0 ± 7.5 years) and post-menopausal ($n=10$; 59.6 ± 6.2 years) females. Our study group also included 7 males (mean age 50.7 ± 11.6 years). DTC had been diagnosed 1 to 25 years prior to the study (median 3 years); total thyroid ablation by near-total thyroidectomy and subsequent radioiodine therapy was our standard treatment for all subjects. Histology revealed papillary cancer, follicular variant of papillary

cancer, follicular cancer and insular cancer in 18, 4, 1 and 1 subjects, respectively (Table 1). All subjects were on an L-T4 regimen at the time of examination. L-T4 therapy was TSH-suppressive in the majority of patients. In 6 patients (cases 1, 7, 12, 20-22) rhTSH testing was performed 2 months after their scheduled L-T4 regimen had been reduced by about 25%, in accordance with our protocol of sensitizing thyroid to rhTSH administration.²⁴ Cases 23 and 24 did not show suppressed TSH despite receiving high L-T4 doses, probably because of inadequate absorption of the drug (Table 1).

Table 1. Pertinent clinical and hormonal data of the subjects studied.

Case	Age yrs	Age at diagnosis yrs	Histology (1)	Stage (2)	BMI kg/m ²	L-T4 μ g/week	FT3 pmol/l	FT4 pmol/l	TSH mIU/l	Tg μ g/l
Pre-menopausal women										
1	28	18	PTC	1	20.7	700	3.5	16.3	1.52	<0.5
2	31	28	PTC	1	22.1	875	7.1	29.6	<0.03	<0.5
3	33	29	F var PTC	1	19.2	850	4.8	17.4	0.03	<0.5
4	40	38	PTC	1	18.5	1050	5.4	22.5	0.12	<0.5
5	42	41	PTC	1	25.7	1100	5.5	28.6	0.10	<0.5
6	43	18	PTC	1	35.6	1212	4.6	22.4	<0.03	<0.5
7	49	45	PTC	4	35.1	700	4.4	14.3	1.64	<0.5
Post-menopausal women										
8	53	51	PTC	2	27.7	875	3.4	15.1	0.60	<0.5
9	53	44	F var PTC	2	26.8	700	3.1	19.8	0.21	<0.5
10	55	54	F var PTC	2	24.8	787	3.7	23.8	0.92	<0.5
11	57	56	F var PTC	1	22.9	1000	5.2	18.8	1.13	0.5
12	58	56	PTC	1	25.6	700	3.1	13.2	2.31	<0.5
13	58	54	PTC	2	26.9	700	4.1	16.9	0.10	<0.5
14	58	46	PTC	2	25.9	875	4.4	19.0	0.05	<0.5
15	65	62	PTC	1	27.6	1050	4.3	21.9	<0.03	<0.5
16	68	66	PTC	1	36.9	1050	5.2	30.0	<0.03	0.5
17	71	68	Insular	3	32.4	875	4.6	21.4	0.12	<0.5
Males										
18	32	30	FTC	1	22.2	925	6.0	25.2	0.05	0.5
19	38	35	PTC	1	29.4	1500	3.4	14.3	0.62	<0.5
20	50	47	PTC	2	34.1	350	3.1	14.9	3.90	<0.5
21	54	50	PTC	3	23.3	525	5.1	14.4	2.83	<0.5
22	58	56	PTC	3	25.6	700	3.1	13.2	2.31	<0.5
23	61	58	PTC	1	26.2	925	4.1	22.1	9.34	<0.5
24	62	60	PTC	1	25.6	1100	4.3	30.4	2.85	<0.5

¹PTC = papillary thyroid carcinoma, F var PTC = follicular variant of PTC, FTC = follicular thyroid carcinoma. Thyroid staging was evaluated according to the TNM 2002 classification.

Protocol

All patients underwent clinical examination and, specifically, neck palpation and body mass index (BMI) evaluation. Pharmacological history was recorded and ultrasonography was carried out. Following overnight fasting and, after 12hrs abstinence from smoking, a blood sample was obtained for serum OPG, RANK-L, thyroid hormones, TSH and thyroglobulin (Tg) determination. Fasting urine spot was collected for the determination of urinary cross-links: pyridinoline (PD)/creatinine ratio and deoxypyridinoline (DPD)/creatinine ratio. Samples were collected before and 3, 6 and 9 days after rhTSH administration. Patients received two consecutive daily (days 1 and 2) i.m. doses of rhTSH (0.9; Genzyme Co., Cambridge, MA, USA). Baseline (day 0) serum PTH, osteocalcin, total calcium, phosphorus, creatinine, alkaline phosphatase (ALP) and urinary 24-h calcium, calcium/creatinine ratio and hydroxyproline (HP)/creatinine ratio were also evaluated. Anti-Tg autoantibody (TgAb) was evaluated to identify sera in which a Tg-recovery test was necessary to obtain reliable Tg values.²⁵ Serum and urine were frozen at -70°C before assays, which were performed within 6 months of collection. Except for cases 3 and 18, bone mineral density (BMD; gr/cm²) of four regions of the spine (lumbar vertebrae L1-L4) and total femur was also measured in the antero-posterior projection by dual energy X-ray absorptiometry (DXA).

Assays

Serum OPG and RANK-L were assayed by means of a commercial ELISA sandwich (Biomedica Medizinprodukte, Wien, Austria). Samples from the same subject were evaluated in duplicate in the same assay. In our laboratory, in a cohort of 80 DTC patients (68 females and 12 males; age range 27-81 years) and 55 subjects with a history of thyroid surgery for benign thyroid disease (51 females and 4 males; age range 39-73 years), the median value observed for OPG was 2.4 pmol/l (range 0.6-5.7 pmol/l) and 2.8 pmol/l (range 1.0-5.9 pmol/l), respectively. In the same cohorts of subjects, soluble RANK-L presented a median value of 0.24 pmol/l (range <0.08-1.28 pmol/l) and 0.25 pmol/l (range <0.08-0.91 pmol/l), respectively. In our laboratory the detection limits were 0.1 pmol/l and 0.08 pmol/l for OPG and RANK-L. Intra-assay

variation expressed as coefficients of variation (CV) was 5% for OPG and 3% for RANK-L. Serum Tg was assayed by chemiluminescence immunoassay (Roche Diagnostics, Mannheim, Germany). Assays were standardized against the certified reference for human Tg (CRM 457) of the Community Bureau of References of the European Commission. The lower analytic limit and functional sensitivity of the method are 0.1 µg/l and 0.5 µg/l, respectively. In our laboratory the intra-assay CVs were 5% and 8%. On the basis of the functional sensitivity of the methods, we selected 0.5 µg/l as the cut-off value to discriminate undetectable from detectable Tg levels. Tg-antibodies were measured by commercial assay (Dia Sorin, Saluggia, Italy). A concentration of 100 mIU/l IgG to Tg was taken as the cut-off value. Serum free-thyroid hormones and TSH were measured by ultra-sensitive chemiluminescence immunoassay (Roche Diagnostics). Normal ranges are 0.3-4.2 mIU/l for TSH, 3.9-6.8 pmol/l and 12.0-22.0 pmol/l for free-T3 (FT3) and free-T4 (FT4), respectively. PTH was analysed by chemiluminescence immunoassay (Immulite 2000; Diagnostic Products Co., San Juan Capistrano, CA, USA). Intra-assay CV was 4% and assay sensitivity was 5 ng/l. In our laboratory the normal range of PTH is 15-65 ng/l. Osteocalcin was measured by chemiluminescence immunoassay (Nichols Advantage, San Juan Capistrano, CA, USA). The normal range is 0.5-7.0 µg/l and the intra-assay CV was 5%. Serum creatinine (normal range: 44-115 µmol/l), calcium (2.12-2.70 mmol/l), phosphorus (0.80-1.45 mmol/l), and total alkaline phosphatase (ALP; 98-280 U/l) were determined by Modular Roche. Urinary calcium concentration was measured by standard procedures in 24-h samples (normal range: 1.25-7.50 mmol/24h), or in spot urine samples and expressed as the molar ratio to creatinine (normal range: 0.0-0.2). Urinary HP, PD and DPD were measured by high-performance liquid chromatography (HPLC, BioRad, Milan, Italy). Methods were optimised by using the CDC (Centre for Disease Control and Prevention, Atlanta, GA, USA) standards. Data are expressed as the molar ratio to creatinine. In our laboratory normal ranges were 6-22, 25-63 and 5-14, respectively. CVs were 4% for HP, 6% for PD and 7% for PDP. BMD was evaluated by DXA instruments (Hologic QDR 1500 and QDR 4500, Waltham, MA, USA). The so-

called standard deviation scores, which indicate the deviation from normal values, were calculated by means of the following equations: T score = BMD measured – BMD young normal population/SD; Z score = BMD measured – BMD sex- and age-matched population/SD.

Statistical analysis

Data were analysed by means of the Prism 4.0 software (GraphPad Software, San Diego, CA, USA). Changes in OPG, RANK-L, TSH, Tg, FT3, FT4, and cross-links under rhTSH testing were analysed by means of the non-parametric Kruskal-Wallis test, followed, when appropriate, by Dunn's multiple comparison test. Baseline data were compared with those of the reference population by Mann-Whitney test. Correlation analyses between variables were carried out by Spearman correlation. All values quoted are means \pm SEM. Data below the functional sensitivity of the assay were analysed for statistical purposes by means of the functional sensitivity value. Exact p values ranging from 0.200 to 0.001 are reported. Significance was taken as $p < 0.050$. At least 1 year after primary therapies, the best predictor of cure in DTC patients was considered to be an undetectable Tg ($< 0.5 \mu\text{g/l}$) level after rhTSH testing, combined with negative neck ultrasonography.^{26,27} According to the WHO classification system, osteoporosis was defined as T-scores lower than 2.5 at any of the major

measurement sites.²⁸

RESULTS

L-T4 dosages ranged from 350 to 1500 $\mu\text{g/week}$ (median 875 $\mu\text{g/week}$; $880.2 \pm 48.7 \mu\text{g/week}$). Free-T3 was in the normal range in all subjects, while 9 out of 24 subjects showed slight hyperthyroxinemia at the time of rhTSH testing (Table 1). Under L-T4, TSH ranged from undetectable ($< 0.03 \text{ mU/l}$) to 9.34 mU/l (median 0.40 mU/l; $0.53 \pm 0.03 \text{ mU/l}$). The inverse correlation between L-T4 dosage and TSH suppression was significant ($r_s -0.41$; $p = 0.044$). Undetectable baseline Tg levels were found in all patients (Table 1). Baseline OPG (median 2.2 pmol/l; $2.2 \pm 0.2 \text{ pmol/l}$) and RANK-L (median 0.24 pmol/l; $0.26 \pm 0.04 \text{ pmol/l}$) levels were in the range of our cohort of reference subjects who had undergone surgery for malignant or benign thyroid disease (Table 2). Baseline OPG has shown only a significant inverse correlation with spinal Z-score ($r_s -0.46$, $p = 0.029$), but no other significant correlation was observed. By contrast, soluble RANK-L was negatively related with age ($r_s -0.48$, $p = 0.018$) and urinary Ca/creatinine ratio ($r_s -0.51$, $p = 0.01$). Other serum parameters were found in the normal range, but PTH was slightly increased in four patients (cases 4, 13, 14, 24), osteocalcin in four (cases 10, 11, 14, 15), phosphorus in one²¹ and ALP in two,^{7,12} while creatinine and ALP were slightly reduced in cases 12

Table 2. Serum biochemical data of patients with differentiated thyroid carcinoma studied at baseline at the time of the rhTSH test.

	OPG pmol/l	RANK-L pmol/l	PTH ng/l	Osteocalcin $\mu\text{g/l}$	Ca mmol/l	P mmol/l	Creatinine $\mu\text{mol/l}$	ALP U/l
Pre-menopausal women (n=7)								
median	2.5	0.34	43	4.0	2.4	1.0	70.8	185
25% percentile	2.2	0.26	38	3.3	2.3	0.9	70.8	141
75% percentile	4.1	0.78	65	5.1	2.5	1.1	70.8	296
Post-menopausal women (n=10)								
median	2.2	0.19	47	4.4	2.5	1.1	62.0	193
25% percentile	1.3	0.10	37	2.7	2.4	0.9	53.1	158
75% percentile	2.7	0.29	61	8.1	2.5	1.2	88.5	217
Males (n=7)								
median	1.9	0.31	44	2.9	2.3	1.0	79.6	182
25% percentile	1.4	0.22	37	2.2	2.1	0.9	66.4	131
75% percentile	2.8	0.39	65	4.2	2.7	1.6	110.6	188

(See Assay section in Materials and Methods for normal ranges)

and 3, respectively (Table 2). Urinary calcium excretion was increased in 24-h samples in cases 7, 15 and 22, and reduced in case 21; however, when corrected for creatinine excretion in spot samples, only case 8 showed an abnormal value (Table 3). Urinary HP and cross-links were increased in several post-menopausal subjects. More than one of these parameters of bone

resorption were simultaneously increased in four subjects (cases 9, 12, 14 and 15) (Table 3). A decrease in BMD was found at the spinal and total-hip level in 48% and 43% of subjects, respectively. In addition, spinal osteoporosis was found in six subjects (cases 1, 8, 9, 12 14 and 15) and total-hip osteoporosis in only one (case 9) (Table 4).

Table 3. Pertinent urine biochemical data of DTC patients studied at baseline of the rhTSH test. The ratios between the urinary excretion of calcium, hydroxyproline (HP), pyridinoline (PD) and deoxypyridinoline (DPD) and that of creatinine were calculated on morning diluted urine collected according to methodology described in the Materials and Methods section.

	24h U-Ca mmol/24h	Ca/creatinine nmol/nmol	HP/creatinine nmol/nmol	PD/creatinine nmol/nmol	DPD/creatinine nmol/nmol
Pre-menopausal women (n=7)					
median	4.1	0.08	19.4	45.7	10.0
25% percentile	2.7	0.04	17.6	36.2	8.5
75% percentile	6.7	0.14	23.2	53.6	14.2
Post-menopausal women (n=10)					
median	3.5	0.09	22.1	55.9	11.6
25% percentile	2.5	0.06	18.1	38.3	9.4
75% percentile	6.4	0.14	27.7	71.6	17.1
Males (n=7)					
median	4.5	0.04	16.5	28.2	5.5
25% percentile	2.6	0.03	15.9	26.0	5.0
75% percentile	7.1	0.12	20.0	32.5	6.7

See Assay section in Material and Methods for normal ranges.

Table 4. Bone mass density evaluated at the spinal (L2-L4) and total-hip sites by DXA equipment. The SD from the peak bone mass (T score) and the age- and sex-matched controls of instrument (Z score) are reported.

Case	Spinal BMD			Total hip BMD		
	BMD g/cm ²	T score	Z score	BMD g/cm ²	T score	Z score
Pre-menopausal women (n=6)						
median	1.087	-0.8	-0.8	0.993	0.2	0.0
25% percentile	0.820	-2.5	-2.3	0.836	-1.2	-1.1
75% percentile	1.218	1.0	0.5	1.098	1.0	1.5
Post-menopausal women (n=10)						
median	0.827	-2.2	-0.5	0.743	-1.5	-0.6
25% percentile	0.788	-2.7	-1.2	0.643	-2.2	-1.5
75% percentile	1.009	-1.1	0.1	0.853	-0.9	0.4
Males (n=6)						
median	1.138	0.2	0.0	0.927	-0.9	-0.4
25% percentile	0.901	-1.3	-1.5	0.810	-1.5	-1.0
75% percentile	1.300	2.1	1.7	1.012	-0.1	0.5

Free T3 and FT4 concentrations were unaffected by rhTSH administration (data not reported). Twenty-four hours after the second administration of rhTSH, the level of serum TSH was significantly increased (median 192.2 IU/l; 217 ± 20.9 mIU/l; $p < 0.001$ vs baseline). After rhTSH, all but three patients had undetectable Tg levels. Tg detectable levels were noted in cases 14 (1.5 $\mu\text{g/l}$), 16 (0.9 $\mu\text{g/l}$) and 18 (1.1 $\mu\text{g/l}$).

Figure 1 shows the values of OPG and RANK-L after rhTSH administration. No significant variation in OPG and RANK-L was found following rhTSH administration. No correlation was observed between the peak value of TSH on day 3 and the maximal percentage change in OPG (median 109.0%; $126 \pm 14.2\%$) and RANK-L (median 80.0%; $175.8 \pm 58.9\%$) attained after rhTSH administration. A slight increase in urinary cross-links was observed during rhTSH testing (Figure 2), but no correlation between TSH peak and maximal percentage change in PD/creatinine (median 131%; $124 \pm 11.2\%$) or DPD/creatinine (127%; $131.5 \pm 13.1\%$) ratio was observed.

DISCUSSION

It has been shown that besides the thyroid many tissues express TSH receptors (TSHRs).²⁹ Therefore, several biological roles for TSH have been speculated. In recent years, TSH has been considered to exert extra-thyroid effects, such as the production of nitric oxide *in vivo*³⁰ and of cyclic adenosine monophosphate *in vitro*.³¹ A recent paper has also suggested that rhTSH may stimulate thyroid angiogenesis *in vivo*.³² Moreover, it has been shown that interleukin-6 (IL-6) release from human abdominal adipose cells in culture is regulated by TSH.³³ Furthermore, rhTSH increases serum IL-6 response in thyroidectomized patients, indicating an extra-thyroid site of TSH action.³³

It has also been claimed that TSH may exert a critical effect on components of skeletal remodelling by interacting with a specific receptor expressed on osteoblast and osteoclast precursors.²² *In vitro*, TSH promotes osteoclastogenesis, while in experimental animals, reduced expression of the TSH receptor leads to the development of osteoporosis. These results suggest that TSH could have an inhibitory effect on bone resorption,²² though its physiological relevance

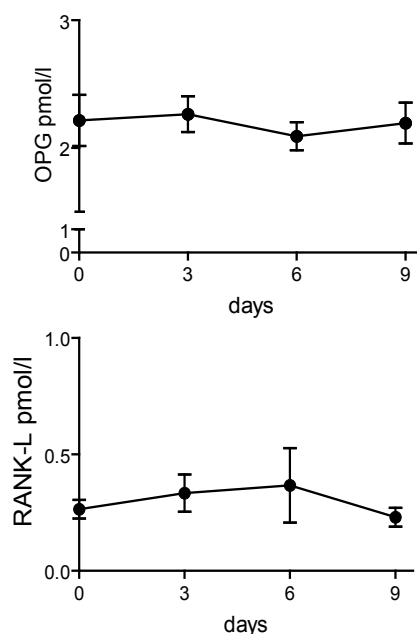


Figure 1. Time-course of serum OPG and RANK-L before and after rhTSH administration.

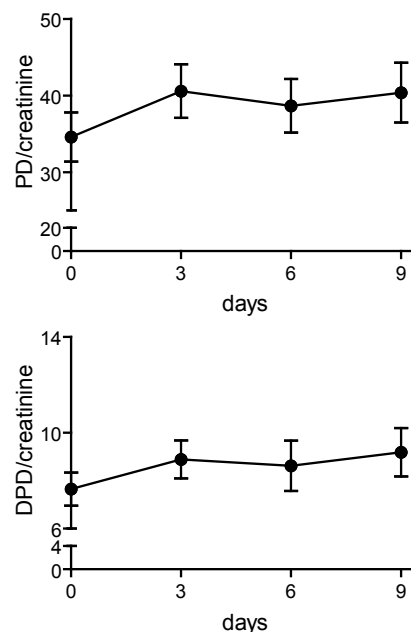


Figure 2. Time-course of urinary cross-links, PD/creatinine and DPD/creatinine ratios evaluated in 18 DTC subjects before and after rhTSH administration.

seems to be slight because of the weak expression of the TSH receptor on human osteoblasts.³⁴ However, it is possible that extreme conditions, such as the TSH increase in hypothyroidism or TSH suppression in

hyperthyroidism, may activate additional regulatory pathways of bone remodelling which are normally not operative. The link between the pituitary-thyroid axis and bone metabolism is not fully understood, as abnormal serum thyroid hormone levels act as confounding factors. Indeed, it is well known that thyroid hormones have a direct effect on bone cells through specific receptor binding³⁵ and subsequent enhancement of mRNA expression,³⁶ thereby producing the generally acknowledged feature of bone loss and osteoporosis.¹⁶

Whether the link between the pituitary-thyroid axis and bone remodelling involves a TSH action on the OPG/RANK/RANK-L system is a controversial issue. Alterations in serum OPG levels have been described in both hyper- and hypothyroidism^{17,18} but, in our experience, OPG and RANK-L levels are comparable in patients with a history of DTC and suppressed endogenous TSH on L-T4, and in those with a history of non-malignant thyroid diseases.³⁷ By contrast, other authors have recently reported an increase in OPG and a decrease in RANK-L levels in DTC patients in comparison with age-matched normal subjects.¹⁹

In our study, rhTSH was administered both to subjects with slight hyperthyroxinemia and to those in whom L-T4 had previously been reduced for diagnostic purposes.²⁴ This procedure was intended to sensitize the Tg response to rhTSH according to a protocol currently under evaluation. However, a significant increase in serum TSH was seen following rhTSH administration without any change in OPG. This finding is in agreement with that reported by Mazziotti et al.²³ In addition, our study documents no significant rhTSH-stimulated variation in soluble RANK-L levels. No correlation was observed between the peak value of TSH on day 3 and the maximal percentage change in OPG and RANK-L after rhTSH administration. Therefore, our data do not support an acute direct effect on OPG and RANK-L in patients with a history of DTC on L-T4 regimen, thus confirming the view that the inhibitory role of TSH on osteoclastogenesis is achieved through mechanisms that do not involve osteoblast-osteoclast cross-talk via the OPG/RANK/RANKL system. Our results are in agreement with the recent observation

that TNF α is the critical cytokine in mediating the downstream anti-resorption effects of TSH on the skeleton.³⁸

There is a substantial difference between Mazziotti's study²³ and ours in the sequential profile of the biochemical markers of bone metabolism observed during rhTSH administration. These authors found that the acute increase in serum TSH levels was accompanied by a reversible decrease in serum C-terminal cross-linking telopeptide of type I collagen (cross-laps) in postmenopausal thyroidectomized women on L-T4 suppressive therapy. In our study, however, we did not observe an analogous change in the urinary excretion of DPD during rhTSH administration. As discussed by Mazziotti et al,²³ the temporal relationship between the increase in TSH and the transient decrease in cross-laps would suggest that bone resorption is affected by TSH itself. However, the potential change in glucose handling due to the fasting condition and TSH-insulin interactions at the receptor level in Mazziotti's model might have contributed to the decrease observed in cross-laps, as the marker is strongly affected by feeding.³⁹ The absence of a fall in cross-laps in premenopausal women,²³ as well as the absence of significant changes in DPD excretion, indirectly supports the view that the observed drop in cross-laps might have a non-osseous origin. The absence of acute changes in the biochemical markers of osteoclast activity in our study do not rule out a long-term skeletal effect of TSH in humans. Indeed, while collagen degradation products reflect osteoclast activity, they might not be sensitive to the sudden change in osteoclastogenesis induced by the sharp increase of TSH concentration in the plasma.

In conclusion, this study confirms that OPG is not involved in the skeletal effect of TSH, and also excludes any involvement of RANKL or of the OPG-to-RANKL ratio. While the skeletal effects of TSH in humans require further analysis, this study indicates that any changes in serum OPG observed in acute alterations of thyroid function should be regarded as compensatory attempts to enhance the activation frequency of bone remodelling via mechanisms independent of the OPG/RANK/RANKL system.

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