CLINICAL STUDY

Thyroid hormone deficiency and postmenopausal status independently increase serum osteoprotegerin concentrations in women

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Abstract

Objective: To study the impact of thyroxine (T4) withdrawal on serum osteoprotegerin concentrations in women, using a healthy euthyroid control group matched for age and postmenopausal status as reference.

Subjects and design: Nineteen women with differentiated thyroid carcinoma were studied the last day on T4 suppressive treatment, 4–7 days after withdrawal and the day before whole body scanning. Eighteen women matched for age and postmenopausal status served as controls. Serum thyroid hormones, urinary bone markers and serum osteoprotegerin concentrations were measured. Statistical methods included repeated measures analysis of variance and one-way analysis of variance. **Results:** Patients progressed from subclinical or mild hyperthyroidism at baseline to normal free T4 and triiodothyronine levels 4–7 days later, ending in overt hypothyroidism before scanning. Serum osteoprotegerin increased, and urinary deoxypyridolines/creatinine and pyridolines/creatinine ratios decreased, with acute hypothyroidism ($P=0.026$, $P=0.003$, and $P<0.001$ respectively). Urinary deoxypyridolines/creatinine ratio, pyridolines/creatinine ratio, and serum osteocalcin during hypothyroidism were lower compared with those of healthy controls ($P=0.023$, $P=0.019$, and $P=0.011$ respectively). Serum osteoprotegerin concentrations were higher in postmenopausal patients when compared with premenopausal ones, irrespective of the changes in thyroid function ($P=0.001$).

Conclusion: Serum osteoprotegerin concentrations increase following acute hypothyroidism after T4 withdrawal in women with differentiated thyroid carcinoma, and also with postmenopausal status.

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Introduction

Osteoprotegerin, generally considered to be a soluble receptor which is secreted by different tissues and cell types, functions as an inhibitor of osteoclastogenesis (1, 2). This function is mediated through its binding and posterior neutralization of the receptor activator of nuclear factor- κ B ligand (3). The osteoprotegerin knockout mouse develops severe osteoporosis, whereas its overexpression in transgenic mouse models leads to osteopetrosis (4).

Serum osteoprotegerin concentrations are increased in women with osteoporosis when compared with age-matched controls, especially in women with higher bone turnover (5, 6). It has been suggested that the increase in serum osteoprotegerin levels may represent a compensatory response to the enhanced osteoclastic bone resorption observed in osteoporosis (4).

Recent studies suggest that thyroid disorders may influence serum osteoprotegerin concentrations. First, endogenous hyperthyroidism increases serum

osteoprotegerin concentration, which return to the normal range after ensuring euthyroidism by medical treatment (7, 8). Secondly, the administration of thyrotropin (TSH)-suppressive doses of thyroxine (T4) may also increase serum osteoprotegerin levels in men (9). Finally, chronic autoimmune hypothyroidism also increases serum osteoprotegerin concentrations and hormone replacement therapy with T4 normalizes its level (10–12).

However, these studies were performed in groups of patients presenting with several etiologies, degrees and duration of hypo- or hyperthyroidism, and submitted to different therapeutic measures that might have acted as confounding factors on the results.

To provide a more precise estimation of the effects of thyroid function on serum osteoprotegerin levels, whilst avoiding the potential confounders described above, we studied the changes in serum osteoprotegerin concentrations in a single cohort of women before and after T4 withdrawal. Furthermore, we also used a healthy euthyroid control group of women matched for age

and postmenopausal status to provide an estimation of the magnitude and possible clinical relevance of these changes.

Patients and methods

Twenty-two women with differentiated thyroid carcinoma referred for a routine whole body scanning during follow-up, after initial total thyroidectomy and ^{131}I ablation, were recruited prospectively.

Patients were studied during T4 withdrawal at three time points: the last day on T4 at their usual TSH suppressive doses, 4–7 days after withdrawal (mean $\pm$ s.d., 5.2 ± 1.0 days) and the day before TSH-stimulated thyroglobulin measurement and whole body scanning (29.8 ± 4.3 days after T4 withdrawal). Thyroid function in these patients was expected to change from subclinical or mild hyperthyroidism at the first visit, to a situation of normal circulating levels of free T4 (FT4) and triiodothyronine (T3) at the second, ending in a state of overt hypothyroidism at the last visit.

The indication for whole body scanning after T4 withdrawal (recombinant human TSH was not available in Spain at the time of the study), as well as the degree of suppression of endogenous TSH secretion (one patient had a TSH level between 0.1 and $0.5 \mu\text{U/ml}$, while the remainder had TSH levels below $0.1 \mu\text{U/ml}$), and the doses of T4 used during follow-up, were decided by the physicians referring these patients and were not influenced by any of the authors of the study. Two patients were excluded from the study because their baseline TSH levels were above $0.5 \mu\text{U/ml}$ and therefore did not fulfill the requisite of subclinical or mild hyperthyroidism at the first visit. Another was excluded because of the presence of hypoparathyroidism, treated with oral calcium and 1,25-dihydroxyvitamin D. None of the patients had cardiovascular disease or diabetes mellitus.

The age of the remaining 19 patients was 41 ± 12 (mean $\pm$ s.d.) years. All these patients were considered in remission from their thyroid cancer, as indicated by previous thyroglobulin measurements and whole body scanning (the latter was still performed in the follow-up of low risk patients at the time of the study).

Eighteen healthy female volunteers, matched for age (40 ± 16 years) and without any thyroid disorder, served as controls. They were not taking any drug known to influence thyroid function or calcium metabolism, and were not using bone anti-resorptive medications or estrogen-replacement therapy. Data regarding other pathophysiological aspects of most of the patients and controls have been reported previously (13–16).

Patients and controls were matched for the percentage of postmenopausal women (47.4 and 50% respectively). The ethics committee of the Hospital

Ramón y Cajal approved the study and written informed consent was obtained from all the participants.

Study protocol and analytical procedures

Patients and controls reported early in the morning after a 12 h fast, when blood samples were obtained and the urine samples collected by the subjects during the previous 24 h were processed. Patients were advised to take their usual T4 dose just after waking up, before reporting to the hospital on the day of the first visit, and medication was withdrawn thereafter. Evaluation was repeated in the patients 4–7 days later and the day before whole body scanning was performed. Controls were evaluated only at one time point.

Serum osteoprotegerin concentrations were measured in duplicate using a commercial enzyme-linked immunosorbent assay (RayBiotech Inc., Norcross, GA, USA). The sensitivity of the assay was 1 pg/ml (0.05 pmol/l) and the mean intra- and interassay coefficients of variation were below 15%.

Serum osteocalcin concentrations were measured in duplicate using a commercial enzyme-linked immunosorbent assay (BioSource International Inc., Camarillo, CA, USA). The sensitivity of the assay was 0.4 ng/ml and the mean intra- and interassay coefficients of variation were below 15%.

Twenty-four hour urine samples were assayed for creatinine by the alkaline sodium picrate method using an Abbott Aeroset Automated Instrument Analyzer (Abbott Laboratories) with mean intra- and interassay coefficients of variation below 2%. Urinary bone markers (pyridolines and deoxypyridolines) were measured by high-performance liquid chromatography (Bio-Rad Laboratories) and expressed as their ratio to urinary creatinine.

Serum samples were obtained for determination of TSH, FT4 and free T3 (FT3) using commercial immunochemiluminescent assays (Immulite 2000, Diagnostic Products Corporation, Los Angeles, CA, USA). The mean intra- and interassay coefficients of variation were below 10% for all these assays. The normal ranges were $0.4\text{--}4.0 \mu\text{U/ml}$ for serum TSH, $0.8\text{--}1.9 \text{ ng/dl}$ for FT4 and $1.8\text{--}4.2 \text{ pg/ml}$ for FT3 as reported by the Central Laboratory of Hospital Ramón y Cajal.

Statistical analysis

Data are expressed as means $\pm$ s.d. in the tables and means $\pm$ s.e.m. in the figures. The normal distribution of the variables was analyzed using the Kolmogorov–Smirnov test. Logarithmic transformations were applied as needed to ensure normality before applying parametric tests.

Data from the patients were analyzed by repeated measures using general linear model. The data at each time point during T4 withdrawal were used as the

within-subjects effect, postmenopausal status was introduced as the between-subjects effect and age was introduced as a covariate. The interactions of the within- and between-subjects effects and with the covariate were also analyzed.

The comparisons of the values of the patients at each time point with the controls were performed by separate one-way analysis of variance, followed by Dunnett's test for comparison with a control group. A α value of 0.05 was chosen as the level of statistical significance. Statistical analyses were performed using SPSS for Macintosh, version 10 (SPSS Inc., Chicago, IL, USA).

Results

Basal characteristics and thyroid function in patients

The clinical and biochemical characteristics of the patients are shown in Table 1. As expected from chronic treatment with supraphysiological doses of T₄, the mean serum thyroid hormone levels were in the mildly hyperthyroid range at the first evaluation (16 of 19 patients had increased FT₄ levels, whereas all the patients had suppressed TSH levels and only two had increased FT₃ concentrations; Fig. 1).

At the second visit, mean TSH levels were still below the normal range, whereas mean serum FT₄ and FT₃ were within the normal range (Fig. 1). Fifteen of nineteen patients still had decreased TSH levels, but their FT₄ and FT₃ levels were within the normal range except in one who still had minimally increased FT₄ levels (Fig. 1). When considering the patients as a group, these subjects presented with lower mean TSH and FT₃ levels compared with euthyroid controls (Fig. 1).

At the third time point, all the patients presented with increased serum TSH and low serum FT₄ and FT₃ levels, with the exception of two patients who had FT₃ levels in the lower limit of the normal range (Fig. 1). When considering the patients as a group, mean serum TSH was increased and mean FT₄ and FT₃ levels were decreased compared with euthyroid controls and with the normal range established in the Central Laboratory of Hospital Ramón y Cajal (Fig. 1).

Table 1 Basal characteristics of the patients included in the study ($n=19$).

Postmenopausal (n , %)	9 (47.4%)
Thyroxine dose ($\mu\text{g/day}$)	2.5 ± 0.6
Age (years)	41 ± 12
Weight (kg)	67.3 ± 13.8
Height (m)	1.6 ± 0.06
Body mass index (kg/m^2)	26.8 ± 5.4
Serum TSH ($\mu\text{U/ml}$)	0.035 ± 0.069
Serum FT ₄ (ng/dl)	2.21 ± 0.49
Serum FT ₃ (pg/ml)	3.24 ± 0.72

Data are mean $\pm$ s.d. unless otherwise stated.

Figure 1 Thyroid hormone function in patients with differentiated thyroid carcinoma on suppressive thyroxine treatment and during thyroxine withdrawal, as compared to euthyroid controls. Data are represented as mean $\pm$ s.e.m. and the dot scattergram shows the individual data. The shaded areas represent the normal ranges for serum thyroid hormone levels as reported by the Central Laboratory of Hospital Ramón y Cajal. * $P < 0.05$ or less compared with the values from the euthyroid control group, which were $\text{TSH} = 1.87 \pm 0.88 \text{ KU/mL}$, $\text{FT}_4 = 1.29 \pm 0.13 \text{ ng/dl}$ and $\text{FT}_3 = 3.38 \pm 0.77 \text{ pg/ml}$; † $P < 0.05$ or less compared with visit 1; ‡ $P < 0.05$ or less compared with visit 2; § $P < 0.05$ or less compared with both visits 1 and 2.

Serum osteoprotegerin and osteocalcin concentrations and urinary bone markers

Serum osteoprotegerin increased after T₄ withdrawal in patients with differentiated thyroid carcinoma and were higher in the hypothyroid state compared with the time that patients had serum FT₄ and FT₃ within the normal

Figure 2 Serum osteoprotegerin levels and urinary bone resorption markers in patients with differentiated thyroid carcinoma on suppressive thyroxine treatment and during thyroxine withdrawal, and also compared with euthyroid controls. Data are represented as mean $\pm$ S.E.M. and the dot scattergram shows the individual data. The dashed lines represent the means of the control group and the shaded areas the 95% CI. * $P < 0.05$ or less compared with the values from the control group; † $P < 0.05$ or less compared with visit 1; ‡ $P < 0.05$ or less compared with visit 2; § $P < 0.05$ or less compared with both visits 1 and 2.

range (Fig. 2). However, serum osteoprotegerin concentrations in athyreotic women were not different to those observed in the euthyroid controls throughout the study (Fig. 2).

The increase in serum osteoprotegerin levels was paralleled by a decrease in urinary bone markers: the urinary deoxypyridolines/creatinine ratio was lower in the hypothyroid state compared with the time that patients had mild or subclinical hyperthyroidism and when patients' serum FT4 and FT3 were in the normal range; the urinary pyridolines/creatinine ratio was also lower in the hypothyroid state compared with the time that patients had mild or subclinical hyperthyroidism; both urinary deoxypyridolines/creatinine and pyridolines/creatinine ratios were lower in the hypothyroid state compared with those of controls (Fig. 2).

Yet the serum osteocalcin concentration, a marker of bone formation, also decreased after T4 withdrawal in patients with differentiated thyroid carcinoma, and was lower in the hypothyroid state compared with the time that patients had mild or subclinical hyperthyroidism and when patients' serum FT4 and FT3 were in the

normal range, and also compared with euthyroid controls (Fig. 2). Therefore, both bone resorption and formation were apparently inhibited during T4 withdrawal.

When data were analyzed, taking into account the postmenopausal status and the age of patients, serum osteoprotegerin concentrations were higher in postmenopausal patients compared with premenopausal women, irrespective of the moment in which the patients were evaluated (Fig. 3). Age had no statistically significant independent effect on serum osteoprotegerin concentrations when considering the menopausal status of the patients studied here ($F = 2.803$, $P = 0.090$).

However, the changes in serum osteoprotegerin concentration during T4 withdrawal were similar in pre- and postmenopausal women, because the interaction of postmenopausal status and thyroid function was not significant ($F = 0.433$, $P = 0.656$). This result suggests that thyroid function and postmenopausal status have independent effects on serum osteoprotegerin concentrations.

Figure 3 Serum osteoprotegerin levels in patients with differentiated thyroid carcinoma on suppressive thyroxine treatment and during thyroxine withdrawal, classified into postmenopausal (open circles) and premenopausal (closed circles) subgroups. Data are represented as mean $\pm$ S.E.M. and the dot scattergram shows the individual data. The values from the post- and premenopausal euthyroid control subgroups were 183 ± 45 and 137 ± 38 pg/mL respectively. $P < 0.05$ or less were found between post- and premenopausal subgroups.

On the contrary, serum osteocalcin concentration and urinary bone markers showed no statistically significant differences between post- and premenopausal patients, a finding possibly related to the high dispersion observed in these parameters (Table 2).

Finally, when both patients and controls were considered as a whole, postmenopausal women had higher serum osteoprotegerin concentrations than premenopausal women ($F = 7.620$, $P = 0.009$), but there were no differences in serum osteocalcin levels or urinary bone markers (data not shown).

Discussion

Our results show that serum osteoprotegerin levels increase during short-term hypothyroidism after T4 withdrawal in a cohort of women with differentiated thyroid carcinoma. This was accompanied by a decrease in serum osteocalcin and urinary bone markers, which were lower than those values of controls when patients were hypothyroid, as expected (17, 18). We have also shown that, irrespective of thyroid function, menopausal status increased serum osteoprotegerin levels. Therefore, it appears that both thyroid hormones and postmenopausal status independently influence serum osteoprotegerin concentrations.

Our findings confirm those of the previous studies showing that both chronic overt hypothyroidism (10–12) and even subclinical hypothyroidism (11, 12) are associated with elevations in serum osteoprotegerin. However, those studies included patients with autoimmune thyroid disease with unknown duration of hypothyroidism, and therefore the possibility that thyroid autoimmunity may modulate osteoprotegerin production could have been a confounding factor (19). Two of these studies included only premenopausal women (11, 12) and the other one included both men and women, with no information about the postmenopausal status of these women (10). As postmenopausal status is an important regulator of serum osteoprotegerin concentrations (5, 6), those studies did not provide information about the possible interaction of the former with thyroid function. By including only women with surgical non-autoimmune overt hypothyroidism (avoiding possible confounding effects of gender, autoimmunity and of the degree of hypothyroidism) and controlling the influence of menopause on the results, our present experimental design avoided the potential confounding factors present in the previous studies, confirming that thyroid hormone deficiency by itself results in an increase in serum osteoprotegerin concentrations.

Osteoprotegerin is an inhibitor of osteoclastogenesis (1, 2) and therefore may constitute a pathophysiological

Table 2 Serum osteocalcin concentrations and urinary bone markers in patients and controls according to postmenopausal status.

	Patients with differentiated thyroid carcinoma (n=19)			Controls (n=18)
	Visit 1; subclinical or mild hyperthyroidism	Visit 2; normal FT4 and FT3 levels	Visit 3; overt hypothyroidism	
Serum osteocalcin levels (ng/ml)				
Premenopausal	19.7±21.2	16.1±11.5	10.3±6.7	22.2±12.6
Postmenopausal	15.7±7.5	14.9±6.6	9.3±3.4	17.4±5.6
Deoxypyridolines/creatinine (pM/μM)				
Premenopausal	13.2±11.8	11.7±10.9	7.9±7.9	9.6±3.3
Postmenopausal	12.2±7.3	10.7±4.8	7.8±5.2	8.9±1.7
Pyridolines/creatinine (pM/μM)				
Premenopausal	63.1±41.3	57.0±52.6	42.3±33.7	53.1±16.8
Postmenopausal	57.1±23.9	55.9±14.9	39.4±16.9	45.3±3.9

Nine patients (47.4%) and nine controls (50%) were postmenopausal. Data are means $\pm$ S.D.

link between hypothyroidism and the decrease of bone resorption. The precise mechanisms by which thyroid hormone deficiency increases serum osteoprotegerin have not yet been elucidated. It has been proposed that the increased cardiovascular risk associated with hypothyroidism or a reduced renal clearance of osteoprotegerin could be possible explanations (10). Furthermore, we cannot completely exclude the possibility that the elevation in serum osteoprotegerin concentrations during hypothyroidism might represent a response to the inhibition of bone metabolism that results from thyroid hormone deficiency (17, 20), although this mechanism seems unlikely as osteoprotegerin usually increases in response to increased bone resorption (4), and is positively associated with osteocalcin levels (5).

The possibility exists that the changes in serum osteoprotegerin levels observed during hypothyroidism in our series are not solely a result of thyroid hormone deficiency but might also be related to the marked increase in serum TSH concentration reached after T4 withdrawal. TSH may be a direct negative regulator of bone metabolism (21, 22) and it could be hypothesized that, as an inhibitor of osteoclastogenesis, osteoprotegerin could mediate these TSH effects. Of note, osteoprotegerin is up-regulated by TSH in thyroid cells (19) yet, because all the patients in our study had no evidence of residual thyroid tissue, the possibility that the very high concentrations of TSH reached after T4 withdrawal stimulated osteoprotegerin secretion by remnant thyroid cells seems highly unlikely. In conceptual agreement, the acute administration of recombinant human TSH to thyroidectomized patients does not result in significant changes in serum osteoprotegerin concentrations (23), also suggesting that TSH does not induce clinically relevant osteoprotegerin secretion in tissues other than the thyroid. Nevertheless, further research is needed to unravel the precise pathophysiological roles of thyroid hormone deficiency and osteoprotegerin interactions on bone metabolism.

On the other hand, endogenous hyperthyroidism has been shown to increase serum osteoprotegerin concentrations (7, 8), an effect which may be related to the expression of osteoprotegerin mRNA in bone cells induced by T3 observed *in vitro* (24, 25). Furthermore, the administration of suppressive doses of T4 may also increase serum osteoprotegerin levels in both men (9) and women (23).

The absence of increased serum osteoprotegerin concentrations when our patients were receiving TSH-suppressive doses of T4 might be related to the mild or subclinical hyperthyroidism resulting from this practice, considering that most of our patients had serum FT3 levels within the normal range at the initial visit. However, our present results cannot exclude that more severe degrees of thyroid hormone excess might result in changes in serum osteoprotegerin levels.

In conclusion, short-term overt hypothyroidism and menopause result in independent increases in serum osteoprotegerin concentrations in women. The effect of hypothyroidism appears to be related to an overall inhibition of bone metabolism that includes reduced bone resorption and formation. The precise pathophysiological meaning of these findings remain to be established.

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References

- 1 Simonet WS, Lacey DL, Dunstan CR, Kelley M, Chang MS, Luthy R, Nguyen HQ, Wooden S, Bennett L, Boone T, Shimamoto G, DeRose M, Elliott R, Colombero A, Tan HL, Trail G, Sullivan J, Davy E, Bucay N, Renshaw-Gegg L, Hughes TM, Hill D, Pattison W, Campbell P, Sander S, Van G, Tarpley J, Derby P, Lee R & Boyle WJ. Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. *Cell* 1997 **89** 309–319.
- 2 Tsuda E, Goto M, Mochizuki S, Yano K, Kobayashi F, Morinaga T & Higashio K. Isolation of a novel cytokine from human fibroblasts that specifically inhibits osteoclastogenesis. *Biochemical and Biophysical Research Communications* 1997 **234** 137–142.
- 3 Yasuda H, Shima N, Nakagawa N, Yamaguchi K, Kinosaki M, Mochizuki S, Tomoyasu A, Yano K, Goto M, Murakami A, Tsuda E, Morinaga T, Higashio K, Udagawa N, Takahashi N & Suda T. Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL. *PNAS* 1998 **95** 3597–3602.
- 4 Rogers A & Eastell R. Circulating osteoprotegerin and receptor activator for nuclear factor kappaB ligand: clinical utility in metabolic bone disease assessment. *Journal of Clinical Endocrinology and Metabolism* 2005 **90** 6323–6331.
- 5 Indridason OS, Franzson L & Sigurdsson G. Serum osteoprotegerin and its relationship with bone mineral density and markers of bone turnover. *Osteoporosis International* 2005 **16** 417–423.
- 6 Yano K, Tsuda E, Washida N, Kobayashi F, Goto M, Harada A, Ikeda K, Higashio K & Yamada Y. Immunological characterization of circulating osteoprotegerin/osteoclastogenesis inhibitory factor: increased serum concentrations in postmenopausal women with osteoporosis. *Journal of Bone and Mineral Research* 1999 **14** 518–527.
- 7 Mochizuki Y, Banba N, Hattori Y & Monden T. Correlation between serum osteoprotegerin and biomarkers of bone metabolism during anti-thyroid treatment in patients with Graves' disease. *Hormone Research* 2006 **66** 236–239.
- 8 Amato G, Mazziotti G, Sorvillo F, Piscopo M, Lalli E, Biondi B, Iorio S, Molinari A, Giustina A & Carella C. High serum osteoprotegerin levels in patients with hyperthyroidism: effect of medical treatment. *Bone* 2004 **35** 785–791.

- 9 Mikosch P, Igerc I, Kudlacek S, Woloszczuk W, Gallowitsch HJ, Kresnik E, Stettner H, Grimm G, Lind P & Pietschmann P. Receptor activator of nuclear factor kappaB ligand and osteoprotegerin in men with thyroid cancer. *European Journal of Clinical Investigation* 2006 **36** 566–573.
- 10 Nagasaki T, Inaba M, Jono S, Hiura Y, Tahara H, Shirakawa K, Onoda N, Ishikawa T, Ishimura E & Nishizawa Y. Increased levels of serum osteoprotegerin in hypothyroid patients and its normalization with restoration of normal thyroid function. *European Journal of Endocrinology* 2005 **152** 347–353.
- 11 Guang-da X, Hui-ling S, Zhi-song C & Lin-shuang Z. Changes in plasma concentrations of osteoprotegerin before and after levothyroxine replacement therapy in hypothyroid patients. *Journal of Clinical Endocrinology and Metabolism* 2005 **90** 5765–5768.
- 12 Guang-Da X, Hui-Ling S, Zhi-Song C & Lin-Shuang Z. Alteration of plasma concentrations of OPG before and after levothyroxine replacement therapy in hypothyroid patients. *Journal of Endocrinological Investigation* 2005 **28** 965–972.
- 13 Botella-Carretero JI, Alvarez-Blasco F, Sancho J & Escobar-Morreale HF. Effects of thyroid hormones on serum levels of adipokines as studied in patients with differentiated thyroid carcinoma during thyroxine withdrawal. *Thyroid* 2006 **16** 397–402.
- 14 Botella-Carretero JI, Prados A, Manzano L, Montero MT, Escribano L, Sancho J & Escobar-Morreale HF. The effects of thyroid hormones on circulating markers of cell-mediated immune response, as studied in patients with differentiated thyroid carcinoma before and during thyroxine withdrawal. *European Journal of Endocrinology* 2005 **153** 223–230.
- 15 Botella-Carretero JI, Gomez-Bueno M, Barrios V, Caballero C, Garcia-Robles R, Sancho J & Escobar-Morreale HF. Chronic thyrotropin-suppressive therapy with levothyroxine and short-term overt hypothyroidism after thyroxine withdrawal are associated with undesirable cardiovascular effects in patients with differentiated thyroid carcinoma. *Endocrine-Related Cancer* 2004 **11** 345–356.
- 16 Botella-Carretero JI, Galan JM, Caballero C, Sancho J & Escobar-Morreale HF. Quality of life and psychometric functionality in patients with differentiated thyroid carcinoma. *Endocrine-Related Cancer* 2003 **10** 601–610.
- 17 Nakamura H, Mori T, Genma R, Suzuki Y, Natsume H, Andoh S, Kitahara R, Nagasawa S, Nishiyama K & Yoshimi T. Urinary excretion of pyridinoline and deoxypyridinoline measured by immunoassay in hypothyroidism. *Clinical Endocrinology* 1996 **44** 447–451.
- 18 Regalbuto C, Alagona C, Maiorana R, Di Paola R, Cianci M, Alagona G, Sapienza S, Vigneri R & Pezzino V. Acute changes in clinical parameters and thyroid function peripheral markers following L-T4 withdrawal in patients totally thyroidectomized for thyroid cancer. *Journal of Endocrinological Investigation* 2006 **29** 32–40.
- 19 Hofbauer LC, Kluger S, Kuhne CA, Dunstan CR, Burchert A, Schoppet M, Zielke A & Heufelder AE. Detection and characterization of RANK ligand and osteoprotegerin in the thyroid gland. *Journal of Cellular Biochemistry* 2002 **86** 642–650.
- 20 Abu EO, Horner A, Teti A, Chatterjee VK & Compston JE. The localization of thyroid hormone receptor mRNAs in human bone. *Thyroid* 2000 **10** 287–293.
- 21 Abe E, Mariani RC, Yu W, Wu XB, Ando T, Li Y, Iqbal J, Eldeiry L, Rajendren G, Blair HC, Davies TF & Zaidi M. TSH is a negative regulator of skeletal remodeling. *Cell* 2003 **115** 151–162.
- 22 Novack DV. TSH, the bone suppressing hormone. *Cell* 2003 **115** 129–130.
- 23 Mazziotti G, Sorvillo F, Piscopo M, Cioffi M, Pilla P, Biondi B, Iorio S, Giustina A, Amato G & Carella C. Recombinant human TSH modulates *in vivo* C-telopeptides of type-1 collagen and bone alkaline phosphatase, but not osteoprotegerin production in postmenopausal women monitored for differentiated thyroid carcinoma. *Journal of Bone and Mineral Research* 2005 **20** 480–486.
- 24 Varga F, Spitzer S & Klaushofer K. Triiodothyronine (T3) and 1,25-dihydroxyvitamin D3 (1,25D3) inversely regulate OPG gene expression in dependence of the osteoblastic phenotype. *Calcified Tissue International* 2004 **74** 382–387.
- 25 Kanatani M, Sugimoto T, Sowa H, Kobayashi T, Kanzawa M & Chihara K. Thyroid hormone stimulates osteoclast differentiation by a mechanism independent of RANKL–RANK interaction. *Journal of Cell Physiology* 2004 **201** 17–25.

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