Differentiated thyroid carcinoma : treatment and clinical consequences of therapy

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Thyroid hormone rather than TSH decreases bone turnover during hypothyroidism in athyroid patients with differentiated thyroid carcinoma

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Submitted

Abstract

Background

Primary hypothyroidism affects bone metabolism. It is not clear whether this has to be attributed to decreased serum thyroid hormone levels per se or to increased TSH levels.

Objective

To document the effects of primary hypothyroidism on bone metabolism and to discriminate between effects mediated by decreased thyroid hormone levels *versus* those mediated by increased TSH levels.

Patients and methods

We studied the effects of recombinant human TSH (rhTSH) in 11 athyroid DTC patients on thyroxine substitution. In addition, we included 11 age-, gender- and BMI-matched athyroid patients previously treated for differentiated thyroid carcinoma (DTC), who were studied after 4 weeks of thyroxine withdrawal and during thyroxine replacement therapy. We measured plasma levels of PTH, 25-OH-vitamin D, procollagen type 1 aminoterminal propeptide levels (P1NP), C-cross-linking terminal telopeptide of type I collagen (CTX), receptor activator for nuclear factor κ B ligand (RANKL) and osteoprotegerin (OPG).

Results

No differences were observed on parameters of bone turnover after rhTSH administration. During thyroxine withdrawal, levels of C-cross-linking terminal telopeptide of type I collagen were significantly lower, whereas levels of osteoprotegerin were significantly higher compared to thyroxine replacement therapy.

Conclusion

Hypothyroidism results in decreased bone turnover. As rhTSH had no impact on bone turnover, it seems that low thyroid hormone levels instead of the increased TSH levels are responsible for the changes in bone turnover during hypothyroidism in DTC patients.

Introduction

The effects of thyroid hormone on bone are established and the conventional view is that hyperthyroidism result in bone loss (1).

But the consequences of hypothyroidism on bone metabolism remain unclear (**Table 1**). Some studies document low bone turnover as evidenced by decreased markers of bone resorption and formation (2-5), whereas others report normal bone turnover (6-8). Most studies, however, included patients with Hashimoto thyroiditis, in whom the duration and the extend of hypothyroidism is not known (3-5,7). Moreover, it is not clear, given the recent suggestion that TSH may be a negative regulator of bone remodelling through direct effect on bone independent of thyroid hormone levels (9-11), if the effects of hypothyroidism must be attributed to increased TSH levels or decreased thyroid hormone levels. It has been reported that TSHR knockout and haploinsufficient mice with normal thyroid hormone levels have decreased bone mass, suggesting that TSH might directly influence bone remodelling (10,12,13). However, other studies question the role of TSH in bone metabolism (14,15).

Three studies in humans have investigated the effect of recombinant human TSH (rhTSH) on bone metabolism, but their results are inconclusive. They showed either no impact on bone turnover (16), increased markers of bone formation (17,18) or decreased markers of bone resorption (18). In hypothyroidism the relative importance of decreased thyroid hormone levels or increased TSH levels on bone remains thus to be established.

The present study was designed in an attempt to discriminate between potential effects mediated by decreased thyroid hormone levels from those mediated by increased TSH levels in a human model in which the reciprocal relationship between thyroid hormone and TSH was interrupted. To this effect, we studied parameters of bone metabolism after parenteral administration of recombinant human TSH (rhTSH) resulting in exogenously increased TSH levels while preserving normal thyroid hormone levels by uninterrupted thyroid hormone substitution in athyroid differentiated thyroid carcinoma (DTC) patients. We studied the same parameters in age-, gender- and BMI matched athyroid DTC patients during short-term thyroxine withdrawal, resulting in decreased thyroid hormone levels and endogenously increased TSH levels and after reestablishment of thyroid hormone substitution.

Table 1: Overview of the literature on the effects of hypothyroidism and rhTSH on parameters of bone turnover

Article	Number of patients	Diagnosis	Control group	Design	Outcome								
					AF	*OC*	*P1NP*	*P1CP*	*OPG*	*CTX*	*U-DPD*	*U-PD*	*RANKL*
Effects of Hypothyroidism													
Botello-Carretero *et al.* (2)	19	DTC Thyroxine withdrawal	18 controls	Prospective		↓			↑		↓	↓	
Toivonen *et al.* (32)	14	DTC Thyroxine withdrawal	38 controls	Prospective		↓	↓	↑		↓			
Sabancu *et al.* (7)	27 20	Hypothyroidism HT + 3 months T4	5 controls	Cross-sectional	=						=		
Sekeroglu *et al.* (8)	16	Hypothyroidism heterogeneous)	15 controls	Cross-sectional	=	=					=		
Nakamura *et al.*(5)	8	Hypothyroidism (heterogeneous)	-	Prospective							↓	↓	
Guang-Da *et al.* (3)	20	Hashimoto thyroiditis	20 controls	Prospective					↑				
Nagasaki *et al.*(4)	53	Hashimoto thyroiditis	53 controls	Prospective					↑				
Effects of Recombinant human TSH													
Mazziotti *et al.* (18)	66	DTC + rhTSH	71 controls	Prospective	↑[B*]				=	↓[*]			
Giusti *et al.* (16)	24	DTC + rhTSH	Reference population	Prospective					=		=		=
Martini *et al.* (17)	30	DTC + rhTSH	80 controls	Prospective			↑[*]		=	=			↓[**]

AF= Alkaline Phosphatase, OC= osteocalcin, P1NP= procollagen type 1 aminoterminal propeptide, P1CP= procollagen type 1 carboxyterminal, OPG= osteoprotegerin (inhibits bone resorption), CTX= C-terminal telopeptide of collagen I, U-PD= Urinary Pyridinium crosslinks, U-DPD= Urinary excretion of Deoxypyridinoline, RANKL= receptor activator nuclear factor κB ligand [B] = Bone specific Alkaline Phosphatase, [*] = in postmenopausal women, [**]= in men and postmenopausal women

Patients and Methods

Subjects

Patients were recruited from the outpatient clinic of the Department of Endocrinology & Metabolic Diseases of Leiden University Medical Center, which is a tertiary referral center for differentiated thyroid carcinoma (DTC). Patients included in the study had a diagnosis of DTC for which they had been treated with near-total thyroidectomy, followed by routine postoperative I-131 radioiodine ablation therapy. Only patients cured for DTC were included, documented by the absence of measurable serum thyroglobulin (Tg) levels during TSH stimulation as well as by negative total-body RaI scintigraphy. Patients with DTC planned for a TSH-stimulated diagnostic protocol were asked to participate in the study. High TSH levels were achieved either by recombinant human TSH stimulation or by 4 week withdrawal of thyroxine. Patients with diabetes mellitus, body mass index (BMI) >35 kg/m^2 or other endocrine diseases were excluded. Patients who used any drugs known to influence bone turnover, such as bisphosphonates, corticosteroids or thiazide diuretics, were also excluded.

The local Ethics Committees of the Leiden University Medical Center approved the study, and written informed consent was obtained from all subjects.

Two groups matched for age, gender and BMI were studied. The first group consisted of 11 athyroid DTC patients who received uninterrupted thyroxine replacement therapy and underwent a TSH stimulation test in the course of monitoring disease state by injections of rhTSH. This resulted in exogenously increased TSH levels with unchanged normal FT4 levels (rhTSH group). The second group also consisted of 11 athyroid DTC patients with short-term thyroxine withdrawal resulting in decreased FT4 levels and endogenously increased TSH levels (thyroxine withdrawal group).

Study Design

Patients in the rhTSH group continued to receive thyroxine substitution and were evaluated prior to recombinant human TSH (Thyrogen, 0.9 mg) which was injected intramuscularly once daily for two consecutive days and patients were also evaluated 1 and 3 days after the last injection of rhTSH.

Patients in the thyroxine withdrawal group were evaluated four weeks after withdrawal of thyroxine substitution and again 8 weeks after restoration of thyroxine replacement therapy.

All patients were assessed at 8.00 a.m. after a 12 hour fast. Height (meters [m]) and weight (kilograms [kg]) were measured and BMI (weight [kg]/lenght^2 [m]) was calculated. Plasma samples were obtained for measurement of FT4, TSH, T3, PTH,

25-OH-vitamin D, procollagen type 1 aminoterminal propeptide levels (P1NP), C-cross-linking terminal telopeptide of type I collagen (CTX), receptor activator for nuclear factor κ B ligand (RANKL) and osteoprotegerin (OPG). Plasma samples were handled immediately and stored at –20° C in Sarstedt tubes.

Biochemical parameters

All plasma and serum samples were measured in one batch. Serum free thyroxine (FT4) and TSH were measured using an electrochemiluminescent immunoassay with a Modular Analytics E-170 system with an intra-assay CV of 1.6-2.2 % and 1.3-5.0 % respectively (Roche Diagnostics, Almere, The Netherlands). Serum T3 was measured using a fluorescent polarisation immunoassay on an AxSYM system (Abbott, Abbott Park, IL, USA CV 2.5-9.0 %). Plasma Parathyroid Hormone (PTH) was measured by an immunoradiometric assay (Nichols Diagnostic Institutes, Wijchen, The Netherlands), calcium and alkaline phosphatase activity by colorimetry on a fully automated Modular P800 system (Roche, Almere, The Netherlands) and 25(OH) vitamin D by RIA (Incstar/DiaSorin, Stillwater, MN, USA). CTX and P1NP were measured by electrochemiluminescent immunoassays using a Modular Analytics E-170 system (Roche Diagnostics, Almere, The Netherlands). RANKL was measured using the ampli sRANKL human kit (Biomedica, Vienna, Austria), an enzyme linked immunoassay with a detection limit of 0.02 pmol/l (intra-assay CV 8-9%, interassay CV 3-6%). Osteoprotegerin was measured by ELISA (Meso Scale Discovery, Gaithersburg, Maryland, USA) with a detection limit of 5.9 pg/ml. In our hands, the range was 206 to 404 pg/ml; CVs were 0.6-16.2%, with an average of 4.6%. All samples were measured in triplo in single batches for the levels of RANKL and osteoprotegerin.

Statistical Analyses

SPSS 15.0 for windows was used for statistical analyses (SPSS. Inc., Chicago, IL, USA). Values are expressed as mean ± SE. Data within subjects were analysed with the paired samples t-test or the ANOVA for repeated measures. Data between subjects were measured with the Mann-Whitney test. Differences were considered statistically significant at $P<0.05$.

Results

Patient demographic characteristics are shown in **Table 2**. Patients in the thyroxine withdrawal group and rhTSH group were well matched and there were no differences in age, gender, BMI, thyroxine dose or duration of follow-up between groups.

Table 2: Patient characteristics

	Thyroxine withdrawal-study (n=11)	rhTSH stimulation study (n = 11)	P-value
Age (years)	45.5 ± 3.0	47.0 ± 2.8	0.65
Sex (m/f)	4 : 7	4 : 7	0.67
BMI (kg/m^2)	28.1 ± 1.3	29.7 ± 2.6	0.75
Thyroxine dose (µg/day)	197 ± 13	200 ± 12	0.70
Duration of TSH suppression (years, (range))	5.0 ± 2.1 (0.6-24.3)	6.7 ± 2.4 (1.2 -25.3)	0.33

Data are expressed as mean ± SE (range) or number of patients

Eleven patients (4 male and 7 female patients) were included in the rhTSH group. Mean thyroxine dose at time of the evaluation was 200 ± 12 µg/day. TSH levels were significantly increased without any changes in FT4 levels 1 and 3 days after rhTSH was administered (**Table 3**).

There were no differences in the levels of calcium, PTH, 25-OH-vitamin D, alkaline phosphatase activity, P1NP, CTX, OPG, RANKL and in the RANKL/OPG ratio between baseline and time points after rhTSH administration.

Eleven patients (4 male and 7 female patients) were included in the thyroxine withdrawal group. Mean thyroxine dose prior to withdrawal was 197 ± 13 µg/day. Four weeks after thyroxine withdrawal, TSH levels were significantly increased at 142.4 ± 10.4 mU/L (normal laboratory reference range 0.3-4.8 mU/L) and FT4 levels were significantly decreased at 1.4 ± 0.2 pmol/L (normal laboratory reference range 10-24 pmol/L). Eight weeks after restoration of thyroxine replacement therapy, six patients had TSH levels within the normal laboratory reference range and five patients had suppressed TSH levels.

There were no significant differences in levels of calcium, PTH, 25-OH-vitamin D, alkaline phosphatase activity, P1NP, RANKL and the RANKL/OPG ratio between thyroxine withdrawal status and 8 weeks after reintroduction of thyroxine replacement therapy (**Table 3**). Serum concentrations of CTX were significantly lower and OPG levels significantly higher during hypothyroidism compared to 8 weeks after reintroduction of thyroxine replacement therapy. There was no significant difference between endogenously and exogenously increased TSH levels respectively obtained 4 weeks after thyroxine withdrawal and 1 day after rhTSH-administration. As expected,

Table 3: Effects of hypothyroidism and rhTSH injections in 11 matched athyroid patients on parameters of bone turnover

	Thyroxine withdrawal group			rhTSH group				
	Thyroxine replacement therapy	**Hypothyroidism**	**P-value[$]**	**Thyroxine replacement therapy**	**rhTSH day 1**	**rhTSH day 2**	**P-value[@]**	**P-value difference hypothyroidism *vs.* difference rhTSH**
TSH (Mu/L)	0.8 ± 0.3[&]	142.4 ± 10.4[#]	0.000	0.06 ± 0.2	143.4 ± 13.6	19.3 ± 2.5	0.00	0.90
FT4 (pmol/L)	24.8 ± 1.2	1.4 ± 0.2[# *]	0.000	23.4 ± 0.8	24.0 ± 0.9	24.3 ± 1.0	0.13	0.00
T3 (pmol/L)	1.3 ± 0.1	0.3 ± 0.1	0.000	1.8 ± 0.1	1.8 ± 0.1	1.8 ± 0.1	0.25	0.00
Parameters of Bone turnover								
Calcium (mmol/L)	2.20 ± 0.02	2.18 ±0.03[*]	0.68	2.25 ± 0.04	2.28 ± 0.3	2.21 ± 0.06	0.17	0.43
PTH (pmol/L)	3.4 ± 0.5	4.0 ± 0.6	0.13	3.7 ± 0.6	3.5 ± 0.6	3.4 ± 0.6	0.55	0.12
25(OH)Vit D (nmol/L)	59 ± 6	57 ± 6	0.52	68 ± 8	67 ± 8	65 ± 9	0.27	1.00
P1NP (ng/ml)	28 ± 5	29 ± 6	0.27	38 ± 4	36 ± 4	37 ± 4	0.18	0.28
CTX (mg/ml)	0.28 ± 0.5	0.24 ± 0.4	0.00	0.33 ± 0.06	0.35 ± 0.06	0.33 ± 0.06	0.23	0.00
OPG (pg/ml)	193 ± 17	246 ± 22	0.00	174.4 ± 11.8	210.4 ± 21.9	198.7 ± 16.5	0.47	0.01
RANKL (pg/ml)	1.1 ± 0.3	1.1 ± 0.3	0.59	1.1 ± 0.3	1.1 ±0.3	1.0 ± 0.3	0.27	1.00
RANKL/OPG ratio	0.006 ± 0.002	0.005 ± 0.002	0.61	0.006 ± 0.002	0.006 ± 0.002	0.006 ± 0.002	0.74	0.44
Alk. Phosphates (U/L)	66 ± 5	66 ± 5	0.81	76 ± 8	77 ± 8	75 ± 7	0.60	0.70

Data is expresses as mean ± SD. [$] Paired samples t-test. [@] ANOVA for repeated measurements, [*] Significantly different *vs.* rhTSH day 1, [#] Significantly different *vs.* rhTSH day 3, [&] Significantly different *vs.* rhTSH thyroxine replacement therapy

FT4 levels were significantly decreased during thyroxine withdrawal compared to the normal levels attained by thyroxine substitution therapy 1 and 3 days after rhTSH administration. The differences in CTX levels and OPG levels were significantly different between the thyroxine withdrawal group and rhTSH group. Calcium levels were significant lower during hypothyroidism compared to rhTSH administration. There were no significant differences observed in any other parameters measured between groups.

Discussion

In this study, we have attempted to dissect the effects of increased TSH levels from those of decreased thyroid hormone levels on bone by studying athyroid DTC patients in which the relationship between thyroid hormone levels and TSH is disrupted. Our findings suggest that acute changes in TSH in the presence of stable thyroid hormone levels obtained by rhTSH administration do not significantly affect skeletal metabolism. The data from our second model suggest that hypothyroidism results in decreased bone turnover rather by decreased plasma thyroid hormone concentrations than by increased TSH concentrations, because rhTSH did not impact on bone turnover in DTC patients. To our knowledge, this is the first study comparing thyroxine withdrawal *versus* rhTSH-injection in age-, gender- and BMI matched DTC patients.

It has been proposed that TSH may modulate bone remodelling independently of thyroid hormones through binding to the TSH receptor on osteoblasts and osteoclasts (10). However, other studies question these findings. Bassett *et al.* reported that Pax $^{-/-}$ mice and hyt/hyt mice, two mouse models of congenital hypothyroidism in which the feedback between TSH and thyroid hormones was intact or disrupted, both displayed delayed ossification, reduced cortical bone, trabecular bone remodelling defects and reduced bone mineralization, indicating that the effects of congenital hypothyroidism on bone are independent of TSH (14). Moreover, Bassett *et al.* showed that osteoblasts and osteoclasts express TSH-receptors, but TSH did not affect a cAMP response or the differentiation or function (14). We used the model of athyroid DTC patients in whom a rhTSH simulation test was performed in an attempt to discriminate between the effects of TSH and those of FT4 on bone metabolism. These patients have no endogenous thyroid hormone production and are therefore an excellent model to study the effects of TSH without interfering effects of changes in thyroid hormone concentrations. However, acute treatment with rhTSH did not affect bone turnover. This is in keeping with a study using the same model (16), but ad odds with two others studies (17,18).

Mazzioti *et al.* found significantly increased levels of bone specific alkaline phosphatase with decreased levels of cross-linking terminal telopeptide of type I collagen in postmenopausal women after rhTSH administration (18). They found no changes in premenopausal women. Martini *et al.* found significantly increased levels of P1NP and RANKL after rhTSH administration (17). These differences were only significant in postmenopausal women for P1NP levels and in postmenopausal women and men for RANKL levels after stratification for gender and menopausal state. We studied only 2 postmenopausal women. This might explain the differences in outcome. We found no differences in osteoprotegerin levels, which is consistent with previous studies (16-18) and in agreement with the finding that TSH regulates bone turnover by different mechanisms than OPG (10,14).

Osteoprotegerin is a member of the TNF receptor superfamily. It inhibits osteoclastogenesis by interrupting the cell-to-cell interaction (19-21). Osteoprotegerin binds to RANKL (22), which is important for osteoclast differentiation. RANKL binds to its receptor, RANK, which is expressed on dendritic cells, T cells, osteoclast precursors and mature osteoclasts (23,24). RANKL increases the survival of RANK positive T cells (23), promotes osteoclast differentiation (22,25-28), stimulates the activity of mature osteoclasts (26,29,30) and promotes survival of osteoclasts by preventing apoptosis.

We also studied the effects of thyroxine replacement therapy after short-term hypothyroidism due to thyroxine withdrawal in age-, gender and BMI matched athyroid DTC patients. Levels of C-cross linking terminal telopeptide of type 1 collagen were lower during hypothyroidism after thyroxine withdrawal compared to 8 weeks after restoration of thyroxine replacement therapy. This is consistent with most reports on hypothyroidism (2,3,31), although Sabancu *et al.* reported no differences in markers of bone turnover during hypothyroidism in a heterogeneous patient population including patients with Hashimoto thyroiditis (7). A disadvantage of the inclusion of patients with Hashimoto thyroiditis may be that the duration and extent of hypothyroidism are not known. OPG levels were also significant higher during hypothyroidism compared to thyroxine replacement therapy. This is consistent with previous studies (2-4) and strengthens our finding that thyroxine withdrawal decreases bone turnover.

In summary, bone turnover is decreased during hypothyroidism after thyroxine withdrawal in DTC patients. We conclude that the low thyroid hormone levels instead of the increased TSH levels are responsible for the decreased bone resorption during hypothyroidism in DTC patients. However, these results must be confirmed in a wider population of men and pre-and postmenopausal women.

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