Clinical aspects of endogenous hypothyroidism and subclinical hyperthyroidism in patients with differentiated thyroid carcinoma

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Thyroid Hormone Independent Associations between serum TSH levels and Indicators of Bone Turnover in Cured Patients with Differentiated Thyroid Carcinoma

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Abstract

Objective: It has been proposed that thyrotropin (TSH) has thyroid hormone independent effects on bone mineral density (BMD) and bone metabolism. This concept is still controversial and has not been studied in human subjects in detail. We addressed this question by studying relationships between serum TSH concentration and indicators of bone turnover, after controlling for T3, FT4 and non-thyroid factors relevant to BMD and bone metabolism. We also studied the contribution of the TSHR-Asp727Glu polymorphism to these relationships.

Design: We performed a cross-sectional study with 148 patients, who had been thyroidectomized for differentiated thyroid carcinoma.

Methods: We measured BMD of the Femoral Neck and Lumbar Spine. FT4, T3, TSH, Bone Specific Alkaline Phosphatase, procollagen type 1 aminoterminal propeptide levels, C-crosslinking Terminal Telopeptide of Type I collagen and Urinary N-Telopeptide of Collagen Cross-links were measured. Genotypes of the TSHR-Asp727Glu polymorphism were determined by Taqman assay.

Results: We found a significant, inverse correlation between serum TSH levels and indicators of bone turnover that was independent of serum FT4 and T3 levels as well as other parameters influencing bone metabolism. We found that carriers of the TSHR-Asp727Glu polymorphism had an 8.1% higher femoral neck BMD, which was however no longer significant after adjusting for body mass index.

Conclusion: We conclude that in this group of patients, serum TSH was related to indicators of bone remodeling independently of thyroid hormone levels. This may point to a functional role of the TSHR in bone in humans. Further research into this mechanism needs to be performed.

Introduction

The effects of thyroid hormone on bone metabolism are well established, ranging from decreased skeletal development in childhood hypothyroidism to an increased risk for osteoporosis in hyperthyroidism (1,2,3). The pathophysiology of osteoporosis in hyperthyroidism is multifactorial (4,5), including shortening of the bone remodeling cycle (6) and acceleration of bone turnover (7). Thyroid hormone indirectly promotes osteoclast formation and activation by inducing the expression of cytokines, prostaglandins and the receptor activator of nuclear factor κ-B ligand (RANKL) (8,9,10). Apart from overt hyperthyroidism, subclinical hyperthyroidism also appears to be associated with decreased bone mineral density as reviewed by Heemstra *et al*. (11,12,3).

An important development has been the discovery of the thyrotropin (TSH) receptor (TSHR) in bone (13,14,15). 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 remodeling (14,5,16). This is intriguing, because effects on bone metabolism that were previously ascribed to high thyroid hormone levels could also be attributed to suppressed TSH levels (17,5,16). Furthermore, in animal studies, low doses of TSH increased bone volume and improved microarchitecture in ovariectomized rats (18), without increasing serum thyroid hormone levels. However, the concept has been challenged by investigations concluding that bone loss in thyrotoxicosis is mediated predominantly by thyroid hormone receptor (TR) α (19). The functional role of a TSHR in bone has been studied in humans to a limited extent. The relationship between low TSH levels and decreased bone mineral density (BMD) has been documented in humans (20,21) but this could still be attributed to elevated thyroid hormone levels. In a study in patients in follow-up for differentiated thyroid carcinoma (DTC), a supraphysiological dose of recombinant human TSH had thyroid hormone independent effects on bone-metabolism parameters (22). We therefore decided to study the independent relation between serum TSH levels and indicators of bone turnover in thyroidectomized patients for differentiated thyroid carcinoma receiving thyroid hormone substitution. The advantage of this group is that these subjects have more uniform FT4 levels. As it was recently reported that the TSHR-Asp727Glu polymorphism was associated with 2.3% higher BMD in elderly carriers (23), we also decided to study this relationship and BMD in these patients. Although the functional consequences of the polymorphism are debated (24), the lower plasma TSH levels in patients carrying the polymorphism could point toward a higher sensitivity of the variant compared to the wild-type TSHR. (25,26). We hypothesized that a group of thyroidectomized patients receiving thyroid hormone substitution would be optimal to study the relationship between the TSHR-Asp727Glu polymorphism and bone as these subjects are not expected to show compensatory lower serum TSH levels if they carry the TSHR-Asp727Glu polymorphism (26,25).

Patients and Methods

Patients were recruited from the outpatient clinic of the Department of Endocrinology of Leiden University Medical Center. Patients were included in the study who had a diagnosis of DTC, for which they had been treated by near-total thyroidectomy, followed by routine postoperative I-131 radioiodine ablation therapy, in all but 4 cases. All patients were cured as defined by the absence of I-131 accumulation at diagnostic scintigraphy, serum thyroglobulin concentrations below 2 μg/L after TSH stimulation, the absence of Tg antibodies, a normal neck ultrasound and no other indication for disease (27). Patients with tumour relapse were only included if they were subsequently cured.

None of the patients used any drug or had a disease known to influence bone metabolism, including estrogen-replacement therapy. Patients taking calcium or vitamine D supplements were also excluded. The Leiden University Medical Center ethics committees approved the study, and written informed consent was obtained from all subjects.

Study design
After an overnight fast, patients had a full clinical examination, including, height (meters [m]) and weight (kilograms [kg]). Blood was collected and measured for TSH, FT4, triiodothyronine (T3), calcium, parathyroid hormone (PTH), 25-hydroxy-vitamin D (25(OH)vitD), bone specific alkaline phosphatase (BAP), C-crosslinking terminal telopeptide of type I collagen (CTx) and procollagen type 1 aminoterminal propeptide (P1NP). Second morning void urine was measured for excretion of N-telopeptide of collagen cross-links (NTx). We choose not to measure Cathepsin K or TRAP levels for various reasons, including age- and gender differences (28,29). Furthermore, CTx seems to reflect bone resorption better than TRAP, especially during hyperthyroidism (29). Plasma, serum and urine samples were handled immediately and stored at –80o C in Sarstedt tubes. BMD (expressed in grams per square centimetre) was measured at the femoral neck and the lumbar spine (vertebrae L2-L4) by dual energy x-ray absorptiometry (NHAMES adjusted, Hologic 4500, Hologic Inc., Bedford, MA, USA). Osteopenia was defined as a T-score between –1 and –2.5 according to the WHO criteria. Osteoporosis was defined as a T-score below –2.5 according to the World Health Organization (WHO) criteria or the presence of fractures at an X-ray of the thoracic and lumbar vertebral column. As fractures of the lumbar vertebrae can affect BMD measurements by increasing X-ray resorption and therefore lead to inappropriate BMD values. We therefore excluded patients with fractures of the lumbar vertebrae from the BMD analyses. The following data were recorded: smoking habits, alcohol use, physical activity, calcium intake, medication (including self-prescription drugs) or vitamin or mineral supplements and daily calcium intake and for females: date of first menstruation (menarche), date of last menstruation, cycle regularity and estrogen substitution.

Serum biochemistry
Serum FT4 and TSH were measured using a chemoluminescence immunoassay with a Modular Analytics E-170 system (intraassay CV of 1.6-2.2 % and 1.3-5.0 % respectively (Roche, Almere, The Netherlands). Serum T3 was measured with a fluorescence polarization immunoassay, CV 2.5-9.0 %, on an ImX system (Abbott, Abbott Park, IL, USA). Thyroglobulin was measured by Dynotest TG-s (Brahms Diagnostica GmbH, Berlin, Germany). Plasma PTH was measured using an immunoradiometric assay (Nichols Diagnostic Institutes, Wijchen, The Netherlands), Calcium by colorimetry and 25(OH)vitD by RIA (Incstar/DiaSorin, Stillwater, MN, USA). Serum BAP was measured by RIA (Hybritech Europe, Liege, Belgium), CTx and P1NP by chemoluminescence immunoassay with the Modular Analytics E-170 system (Roche Diagnostics, Almere, The Netherlands). NTx was measured by ELISA (Ostex International Inc., Seattle, WA, USA). NTx was expressed as the ratio of urine creatinine excretion (NTx/creatinine) to correct for differences in creatinine excretion.

Genotyping
DNA was isolated from peripheral leucocytes by the salting out procedure (30). Genotypes were determined using 5 ng genomic DNA by a 5' fluoregenic Taqman assay and reactions were performed in 384-wells format on ABI9700 2x384well PCR machines with endpoint reading on the ABI 7900HT TaqMan® machine (Applied Biosystems, Nieuwerkerk aan den Ijssel, The Netherlands). Primer and probe sequences were optimized using the single nucleotide polymorphism assay-by-design service of Applied Biosystems.
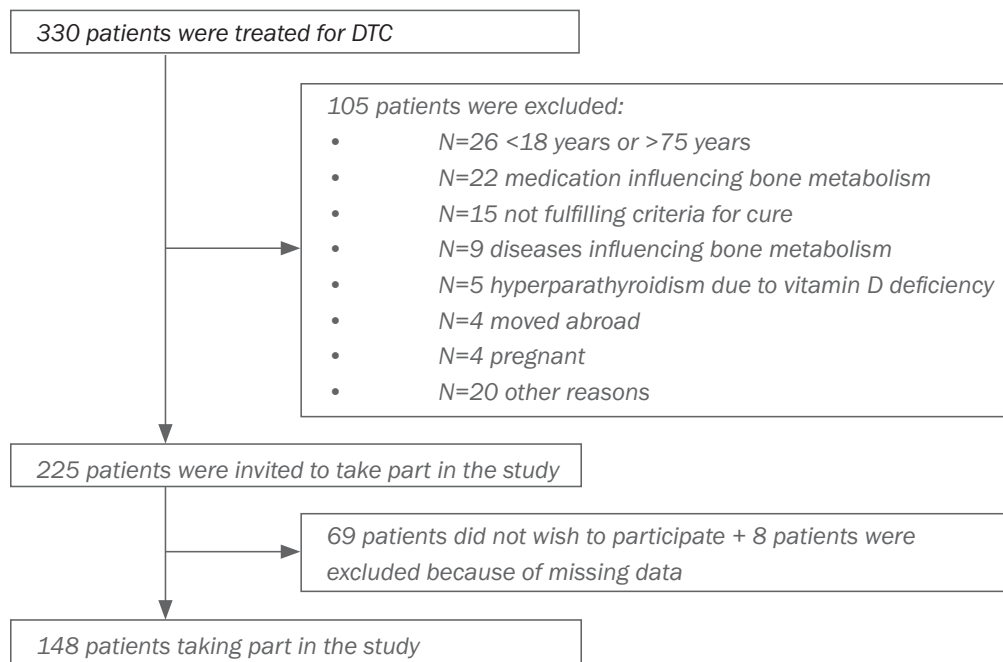
Statistical Analysis

Values are presented as mean ± standard deviation (SD), mean ± SEM, median (range) or as numbers or proportions of patients. Non-normally distributed data (TSH and PTH) were directly log transformed. Factors contributing to BMD and indicators of bone turnover were first identified using univariate regression analysis. During univariate regression analysis, corrected for age, gender and estrogen status (estrogen deplete/replete), the relationship between BMD and the following parameters were analyzed: BMI, smoking, alcohol use, physical activity, duration of follow-up after initial therapy for DTC, total dose of radioiodine received, serum levels of calcium (corrected for an albumin concentration of 42 g/L), 25(OH)vitD, lnPTH, FT4, T3, lnTSH and daily dose of thyroxin. The independent contributions of the TSHR-Asp727Glu polymorphism and serum TSH levels to BMD and indicators of bone turnover were studied by entering FT4, T3, age, gender, estrogen status and all significant covariates at univariate analysis in a multivariate model. Deviation from Hardy-Weinberg Equilibrium was analysed using a X^2-test. All calculations were performed using SPSS 12.0 for windows (SPSS, Inc., Chicago, IL). Differences were considered statistically significant at P<0.05

Results

Patient characteristics

Of a potential 330 patients with cured DTC, 105 were excluded for various reasons (Figure 1). Sixty-nine patients did not want or were not able to participate in the study for reasons of time or geographical distance. A total of 156 patients were thus included in the study. Eight patients were left out from analyses because of incomplete data. The basal characteristics of the 148 patients included in the study are shown in Table 1. Thirteen patients had post-surgical hypoparathyroidism for which they were adequately supplemented with active vitamin D metabolites and calcium as required. All patients were receiving L-thyroxin treatment at a mean dose of 182 ± 51 μg/day. The 25th, 50th and 75th percentiles of serum FT4 levels were 19.5, 22.1 and 24.9 pmol/L. A total of 4.3% of patients met the criteria of osteoporosis and 34% of the patients met the criteria for osteopenia (Table 2).

Figure 1 Flowchart of the Study

330 patients were treated for DTC

105 patients were excluded:
- N=26 <18 years or >75 years
- N=22 medication influencing bone metabolism
- N=15 not fulfilling criteria for cure
- N=9 diseases influencing bone metabolism
- N=5 hyperparathyroidism due to vitamin D deficiency
- N=4 moved abroad
- N=4 pregnant
- N=20 other reasons

225 patients were invited to take part in the study

69 patients did not wish to participate + 8 patients were excluded because of missing data

148 patients taking part in the study

Serum TSH levels, BMD and indicators of bone turnover
All thyroid related parameters as well as covariates that contributed significantly to BMD and indicators of bone turnover are shown in Table 3. No significant association was observed between serum TSH levels, serum thyroid hormone levels and BMD. However, using univariate analyses, the natural logarithm of serum TSH levels (and the unconverted values) appeared to be significantly inversely correlated to CTx, P1NP, BAP and NTx (Figure 2). The significance was sustained after correction for FT4, T3, gender, age, estrogen status and other significant determinants of bone metabolism using multivariate analysis. The relationship of serum TSH levels with indicators of bone turnover was independent from circulating thyroid hormone levels. Using multivariate analyses no significant or relevant relationship was found between FT4 or T3 and BMD or indicators of bone turnover.

Table 1. Characteristics of Patients

|	Total (n=148)
Age (years)	49.4 ± 12.7
Males	27 (18.2%)
Females, premenopausal / postmenopausal	76 (51.4%)/ 45 (30.4%)
Histology	
- Papillary Thyroid Carcinoma	122 (82.4%)
- Follicular Thyroid Carcinoma	26 (17.6%)
pTNM Stage	
- T1-3 N0 M0 / T1-3 N1 M0 / T4 or M1	90 (60.8%)/ 39 (26.4%)/ 19 (12.8%)
Relapse	18 (12.2%)
Follow-up Duration (years)	9.3 (1.2 –43.0)
FT4 (pmol/L)	22.3 ± 4.1
T3 (nmol/L)	1.47 ± 0.34
TSH (mU/L)	0.045 (0.003 – 6.830)
Thyroxin dose / weight (ug/kg)	2.15 ± 0.99
Age menarche women(years)	13.3 ± 1.4
Smoking	18 (12.2 %)
Regular physical activity	77 (52.0%)
BMI (kg/m2)	25.83 ± 4.50

Data are presented as mean ± SD, median (range) or number of patients (percentage).
FT4: Free Thyroxin, T3= Triiodothyronine, TSH= Thyrotropin, BMI: Body Mass Index

The TSHR Asp727Glu polymorphism, BMD and indicators of bone turnover
Genotype frequencies of the TSHR-Asp727Glu polymorphism (Asp/Asp = 131 (88.5%), Asp/Glu = 17 (11.5%) and Glu/Glu = 0 (0.0%)) did not deviate from Hardy Weinberg equilibrium proportions. The Glu727 allele had a frequency of 6.1 %, which is similar to previous studies in Caucasians (26). Patient groups were comparable with respect to age, gender, duration of follow-up, BMI and thyroid hormone parameters. Using univariate analysis, the TSHR-Asp727Glu polymorphism appeared to be a significant determinant of BMD. After correction for age, gender and estrogen status, carriers of the TSHR-Glu[727] allele had an 8.1% higher femoral neck BMD (p=0.023). When BMI was added as a covariate in the analysis of the relationship of the TSHR Asp727Glu polymorphism with femoral neck BMD, the significance level decreased to p=0.102, the standardized β decreasing from 0.172 to 0.127 (Table 3). There was no significant difference in BMD at the lumbar spine between groups.

Figure 2 Relationship between lnTSH and indicators of bone turnover
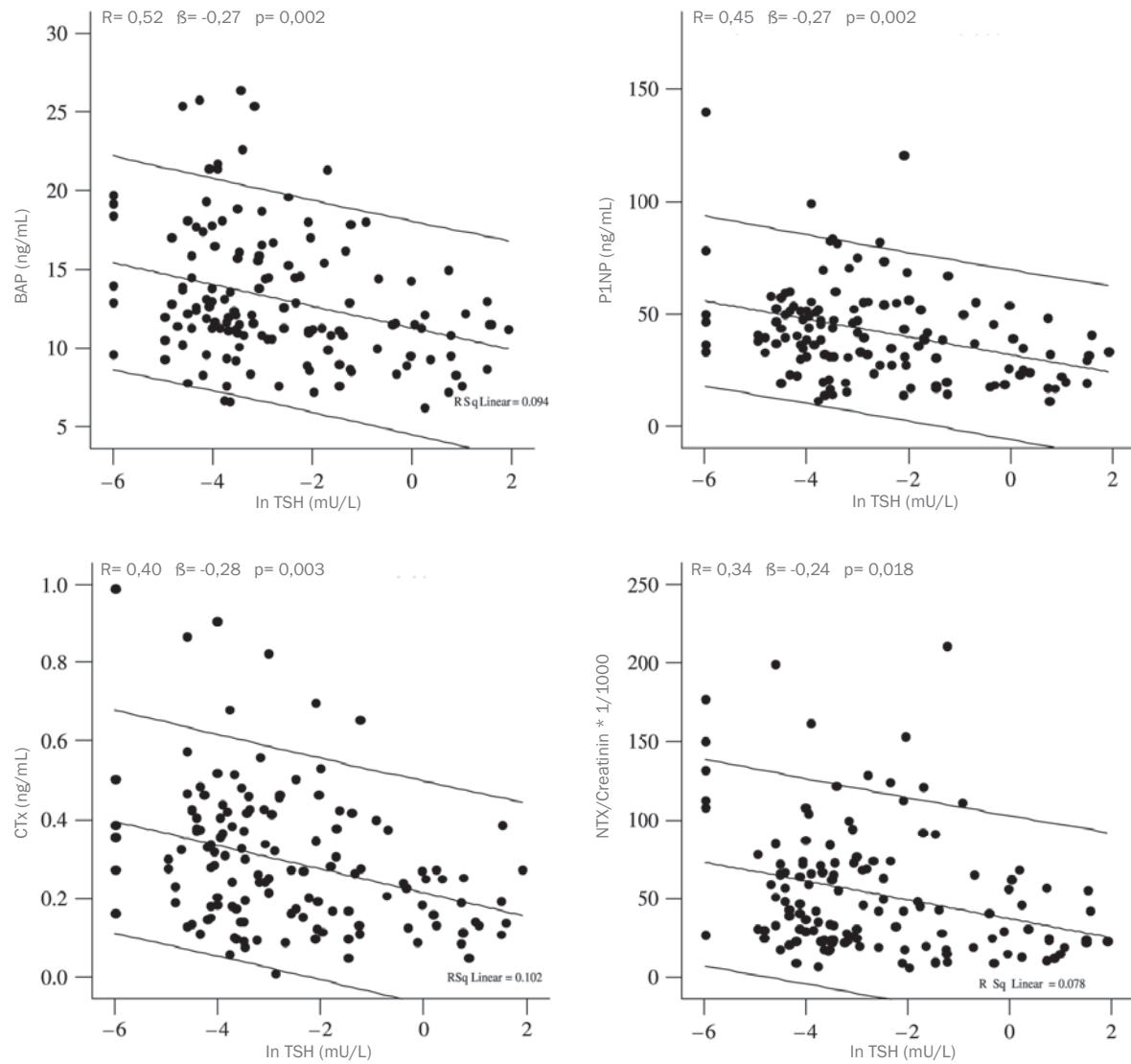
No significant differences were observed in indicators of bone turnover between groups (Table 2). The above mentioned relationship between TSH and parameters of bone metabolism was not influenced by the TSHR polymorphism.

Discussion

The purpose of the present study was to identify the relationship between TSH levels, BMD and indicators of bone turnover in 148 thyroidectomized patients. In support for a potential direct effect of TSH on bone, we observed an inverse relationship between serum TSH levels and indicators of bone formation (BAP and P1NP) and bone resorption (CTx and NTx), independent of serum thyroid hormone levels. These results are consistent with a suppressive effect of TSH on bone remodeling and are in keeping with the reported effects of TSH on bone metabolism in animal studies (14). It may well be that the lower range of TSH levels in our patient group, and the more uniform FT4 concentrations (25th and 75th percentiles being 19.5 and 24.9 pmol/L) have allowed to identify this relationship. We noticed no relationship between serum TSH levels and BMD, while we found an inverse relationship between serum TSH levels and indicators of bone turnover.

Table 2. Bone mineral Density and indicators of bone turnover

Bone Mineral Density	Men (n=27)	Women Premenopausal (n=76)	Women Postmenopausal (n=45)	Wild Type TSHR-Asp727glu (n=131)	Heterozygous TSHR-Asp727glu (n=17)
- Femoral neck (g/cm2)	0.90 (0.65-1.25)	0.88 (0.54-1.10)	0.79 (0.56-1.13)	0.86 (0.54-1.25)[1]	0.94 (0.68-1.25)[1]
- z-score	0.58 (-1.64-2.31)	0.51 (-1.95-2.32)	0.98 (-1.67-2.51)	0.55 (-1.95-4.14)[1]	1.13 (-1.18-3.52)[1]
- Lumbar vertebral column* (g/cm²)	1.14 (0.79-1.37)	1.07 (0.78-1.31)	1.06 (0.67-1.37)	1.06 (0.10-1.74)	1.10 (0.13-1.34)
- z-score	0.84 (-2.31-2.64)	0.68 (-2.07-2.76)	0.96 (-0.77-2.65)	1.25 (-2.31-6.61)	1.38 (-2.07-3.38)
Osteopenia # (%)	33.3	23.0	53.5	33.6	23.5
Osteoporosis# (%)	8.3	1.4	7.0	4.6	0
Calcium (mmol/L)	2.36 ± 0.02	2.40 ± 0.02	2.40 ± 0.02	2.39 ± 0.01 (n=130)	2.35 ± 0.02
25(OH)vitD (nmol/L)	66.04 ± 4.80	63.50 ± 3.57	63.5 ± 3.57	63.92 ± 2.32	65.13 ± 6.68 (n=16)
PTH (pmol/L)	4.36 ± 0.74	5.15 ± 0.46	5.15 ± 0.46	5.38 ± 0.31 (n=129)	3.62 ± 0.52 (n=16)
BAP (ng/mL)	12.96 ± 0.70	14.30 ± 0.78	14.30 ± 0.78	13.33 ± 0.41 (n=116)	12.05 ± 0.64 (n=14)
P1NP (ng/mL)	36.15 ± 3.65	49.88 ± 5.27	49.87 ± 5.27	42.76 ±2.27 (n=116)	40.91 ± 4.46 (n=14)
CTx (mg/mL)	0.25 ± 0.03	0.36 ± 0.04	0.36 ± 0.04	0.29 ± 0.02 (n=116)	0.32 ± 0.04 (n=14)
NTx /creatinine * 1/1000	29.96 ± 7.22	66.44 ± 8.61	66.44 ± 8.61	53.71 ± 3.86 (n=114)	49.86 ± 11.13 (n=14)

Data are presented as median (range), mean ± SEM or percentage.

* Patients with fractures of lumbar vertebrae excluded from BMD measurements of the lumbar spine.

 # According to WHO criteria.

1 Univariate analysis, p<0.05 Corrected for age, gender, estrogen status

PTH: Parathyroid hormone, BAP: Bone Specific Alkaline Phosphatase; P1NP: Procollagen type 1 Aminoterminal Propeptide; CTx: C-crosslinking Terminal Telopeptide of Type I collagen; NTx/Creatinin: Ratio of Urinary N-Telopeptide of Collagen Cross-links and Creatinin Concentration

Table 3. Association between serum TSH levels, BMD and indicators of bone turnover

Dependent	Thyroid related Variables			Covariables		
	Variable	Standardized Beta	p	Co-variable	Standardized Beta	P
BMD-Femoral Neck	FT4	0.063	0.413	BMI [#]	0.298	<0.001
	T3	-0.039	0.621			
	lnTSH	0.020	0.793			
	TSHR727Glu	0.172 (0.127)[&]	0.023 (0.102)[&]			
BMD-Lumbar Spine	FT4	0.038	0.635	BMI [#]	0.160	0.045
	T3	-0.014	0.867			
	lnTSH	-0.026	0.740			
	TSHR727Glu	-0.028	0.725			
BAP	FT4	0.148	0.103	BMI	0.177	0.050
	T3	0.251 (0.117)[&]	0.005 (0.165)[&]	Ca [#]	0.351	<0.001
	lnTSH [#]	-0.303 (-0.273)[&]	0.001 (0.002)[&]	lnPTH	0.208	0.021
	TSHR727Glu	-0.061	0.509			
P1NP	FT4	0.140	0.112	Ca	0.237	0.006
	T3	0.132	0.131			
	lnTSH [#]	-0.282 (-0.268)[&]	0.001 (0.002)[&]			
	TSHR727Glu	0.008	0.926			
CTx	FT4	0.157	0.078			
	T3	0.168 (0.062)[&]	0.058 (0.481)[&]			
	lnTSH [#]	-0.302 (-0.276)[&]	0.001 (0.003)[&]			
	TSHR727Glu	0.110	0.220			
NTx/Creatinine	FT4	0.234 (0.122)[&]	0.010 (0.200)[&]	lnPTH	0.197	0.030
	T3	0.108	0.240			
	lnTSH [#]	-0.286 (-0.238)[&]	0.002 (0.018)[&]			
	TSHR727Glu	0.021	0.820			

Univariate regression analysis, all corrected for gender, age and estrogen status. Due to a non-normal distribution, TSH and PTH were transformed by the natural logarithm.

[&] Values obtained with a multivariate regression model in which covariables that were significant using univariate analysis were included. [#] Variable with sustained significant association at multivariate analysis.

FT4: Free Thyroxin, T3= Triiodothyronine, TSH= Thyrotropin , BMD: Bone Mineral Density; BAP: Bone Specific Alkaline Phosphatase; P1NP: Procollagen type 1 Aminoterminal Propeptide; CTx: C-crosslinking Terminal Telopeptide of Type I collagen; NTx/Creatinine: Ratio of Urinary N-Telopeptide of Collagen Cross-links and Creatinine Concentration.

This could be due to the fact that the TSH levels Were measured at one occasion. BMD is acquired by a lifelong process, whereas indicators of bone turnover reflect short term biochemical effects. Kim *et al.* and Morris *et al.* found a relationship between not only below normal TSH and BMD, but also between low-normal TSH and BMD (21,31). An explanation for this difference could be that Kim *et al.* and Morris *et al.* excluded patients with thyroid disease whereas we studied DTC patients. It may be objected that TSH measured at one occasion may not reflect the overall suppression of TSH over time. To address this shortcoming, we collected all TSH measurements after initial therapy of the patients who participated in the study, with the exception of stimulated TSH levels. We calculated for each patient the slope of TSH levels, to verify changes over time. An average of 15 TSH measurements per patient were obtained and we calculated a slope of TSH values of -0.0001 (range -0.004-0) mU/L/year, thus indicating stable TSH levels over time.

We also hypothesized that the TSHR-Asp727Glu polymorphism, which is associated with serum TSH, but not FT4, may influence BMD and bone metabolism in humans. Although we found that carriers of the TSHR-Asp727Glu polymorphism had a higher BMD as compared with 131 non-carriers, the relationship in our study was no longer significant after correction for BMI. This may imply that the effect of the polymorphism is explained by BMI, which is also in agreement with the study of van der Deure *et al* (23). An explanation for the difference in outcome of our study and the study of van der Deure *et al.* could be that the number of patients in our study was too small to detect a significant correlation between the polymorphism and BMD. We did not find an association of serum TSH levels with lumbar spine BMD. This might be due to the fact that the BMD measurements of the lumbar spine are influenced by osteoarthritis and therefore cannot be accurately assessed (32).

The mechanisms of TSH effects on bone metabolism have not been fully elucidated. In a provocative study, Abe *et al.* suggested that TSH inhibits osteoclast formation and survival by attenuating JNK/c-jun and NFκB signaling in response to RANK-L and inhibits osteoblast differentiation and type 1 collagen expression as well by downregulating Wnt and VEGF signalling (14). The same group found that TSH directly inhibits Tumour Necrosis Factor-α (TNF-α) production and that TNF-α is the critical cytokine mediating the downstream antiresorptive effects of TSH on the skeleton (33). Other authors suggested that serum TSH activates the type 2 deiodinase in osteoblasts, thereby linking TSH and increased local thyroid hormone availability (15). Our data are in agreement with the notion of a direct effect of TSH on bone, as we found a significantly inverse relationship between serum TSH levels and indicators of bone formation and degradation, which is consistent with an overall suppressive effect on bone turnover. However, 2 recent papers by Bassett *et al.* added to the controversy on the net contribution of TSH to BMD and bone metabolism (19,34). Bassett *et al.* studied mice with complete or haploinsufficiency of TR-α and -β. They found skeletal hypothyroidism and osteosclerosis accompanied by reduced osteoclastic bone resorption in adult mice lacking TR-α, whereas young mice had delayed endochondral ossification, in the presence of normal circulating thyroid hormone and TSH concentrations. Adult mice lacking the TR-β, leading to elevated TSH and thyroid hormone levels, had skeletal hyperthyroidism, with evidence of increased bone resorption. The authors concluded that TR-α regulates both skeletal development and adult bone maintenance, with euthyroid status during development being essential to establish normal adult bone structure and mineralization. In the study of van der Deure *et al.* (23) a stronger effect of FT4 than TSH on BMD was observed, which supports the importance of thyroid hormone effects on bone. In our study, which involved a different patient group in many respects, we did not find a relationship between serum levels of FT4, TSH and BMD, which may well be due to the fact that serum thyroid hormone and TSH levels have changed after the thyroidectomy. Indeed, despite the low serum TSH levels in most patients, overall BMD was within the normal age-corrected range, which is in line with a recent report in DTC patients (35).

It is obvious that the net contribution of TSH in bone development and bone metabolism has not been established yet. We belief that our study may add important human data to the determination of the role of TSH in bone metabolism, since we were able to study the independent relation between serum TSH and bone in thyroidectomized patients.

In summary, we found an independent inverse relationship between serum TSH levels and biochemical indicators of bone turnover, which may point to a functional role of the TSHR in bone in humans. This study documents a direct effect of TSH, independent of thyroid hormone levels on BMD and indicators of bone turnover in humans. Further research into the mechanisms of TSH in bone metabolism needs to be performed.

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