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# Thyroid-stimulating hormone within low-normal range is related to imbalance of bone remodeling in euthyroid postmenopausal women with osteoporotic fractures

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## ABSTRACT

**Introduction.** Data relating to thyroid-stimulating hormone (TSH) and risk of fractures are limited, and the effect of TSH within the normal range on bone mineral density (BMD) and bone remodelling is controversial. We aimed to evaluate whether variations across TSH concentration within the normal range are associated with bone metabolism expressed by bone remodeling markers in euthyroid postmenopausal women with osteoporotic fractures.

**Material and methods.** The study group consisted of 60 elderly women admitted to the hospital due to nonvertebral osteoporotic fractures of which 57 were diagnosed as euthyroid. In all serum fT<sub>4</sub>, and TSH, P1NP (a bone formation marker) and CTX (a bone resorption marker) were measured.

**Results.** The majority of fractures occurred at the lower TSH tertile (0.14–2.19 mIU/L). Most of the patients (70%) in this tertile had TSH value below 1 mIU/L. There was a clear tendency towards lower P1NP in the first TSH tertile ( $p = 0.056$  and  $p = 0.057$ ) whereas most CTX values tended to be higher than the median concentration in the whole group. A significant positive correlation between TSH and P1NP was observed ( $r = 0.32$ ;  $p = 0.01$ ). TSH within the normal range and CTX explained 25% of the variability of P1NP in euthyroid women with osteoporotic fractures.

**Conclusions.** In elderly euthyroid women low-normal TSH level seems to be associated with the imbalance between bone resorption and formation. Diminished bone formation may predispose to increased risk of nonvertebral fractures. Bone marker testing in subjects with low-normal TSH may add new value in the assessment of fracture risk.

**Key words:** TSH concentration, osteoporotic fracture

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## Introduction

Thyroid hormones are involved in the regulation of bone metabolism, being essential for normal bone development. Hyperthyroidism in adults is related to increased bone turnover and bone loss and was shown to be associated with an increased lifetime risk for fractures [1]. In hypothyroidism bone metabolism is suppressed and bone mineral density (BMD) increased, but the fracture risk remains elevated. Data relating to thyroid-stimulating hormone (TSH) and risk of fractures are limited, and the effect of TSH concentrations

remaining within the normal range on BMD and bone turnover markers is a matter of controversy. TSH has been shown to be related with bone remodeling markers independently of thyroid hormone concentrations [2]. The results of in vitro experiments support the observations that TSH negatively regulates osteoclastogenesis and exerts a protective effect on bone [3]. According to recent reports, variations of serum TSH within the physiological range do not greatly influence BMD and there is no correlation between bone turnover markers and TSH [4, 5]. On the other hand, subclinical hyperthyroidism, and even thyroid function within the upper

normal range, have been shown to affect bone density and the risk of nonvertebral fractures in postmenopausal women [6, 7]. Maziotti et al. found that low-normal TSH is related to high prevalence of vertebral fractures in post-menopausal women with osteoporosis or osteopaenia, independently of thyroid hormones, age, and BMD [8]. Therefore, we aimed to evaluate whether variation across TSH concentration, within the reference range, is associated with bone metabolism expressed by changes in biochemical bone remodeling markers in euthyroid postmenopausal women with nonvertebral osteoporotic fractures.

## Materials and methods

### Study subjects

Our study group consisted of 60 women with mean age 77 (61–85) years admitted to the Department of Orthopaedics and Traumatology at the University Hospital in Bydgoszcz due to osteoporotic fracture. 76% of patients had femoral neck fracture and 24% had fractures of hip or knee joint. All women were postmenopausal and previously had not received any drugs affecting bone metabolism. Other inclusion criteria were as follows: no clinical history of recent significant trauma or prolonged immobilisation, absence of thyroid diseases, and no thyroid hormone replacement therapy. Thyroid function was defined by measurement of serum free-thyroxine (fT4), and serum TSH was assayed. Moreover, all patients were asked to fill-in the questionnaire on chronic diseases, personal history of fractures, taken drugs, and vitamin supplements. A total of 57 patients were euthyroid (fT4 0.7–1.48 ng/dL; TSH > 0.1– < 10 mIU/L) [7, 9], and three had subclinical hyperthyroidism (TSH < 0.1 mIU/L). We observed no relationship between TSH and fT4 in our study group, which supports the premise that we studied euthyroid patients ( $p = 0.29$ ) [5].

Written, informed consent was obtained from all subjects. The study was approved by the Bioethics Committee at Collegium Medicum, Nicolaus Copernicus University.

### Blood sampling and laboratory analyses

Non-fasting venous blood samples were collected from patients within 18 hours after fracture. Blood samples were collected at the same time of day (between 8:00 and 15:00 a.m.) to mitigate the effects of diurnal variation. There was no influence of the blood collection time on any parameters under study (Kruskal-Wallis test  $p > 0.09$ ). Serum was obtained within two hours to avoid proteolysis and stored deep-frozen at  $-70^{\circ}\text{C}$  in small aliquots until assayed. TSH and fT4 were measured on

an automated analyser using the ci8200 ARCHITECT System (Abbott Diagnostics; TSH detection limit of 0.01 mIU/L, which meets the requirements of a third generation TSH assay; fT4 detection limit of 0.4 ng/dL).

In the serum samples, directly after thawing, the following bone markers were measured: total propeptide of type 1 procollagen (P1NP), a bone formation marker (ECLIA Elecsys, ROCHE Diagnostics; detection limit 5 ng/mL; mid-95<sup>th</sup> percentile range for postmenopausal women 42.94–76.31 ng/mL), C-terminal telopeptide of type 1 collagen (CTX), a bone resorption marker (CTX ELISA, Immunodiagnostic Systems; detection limit 0.02 ng/mL; mean value for postmenopausal women 0.439 ng/mL; range 0.142–1.351 ng/mL).

### Statistical analysis

Statistical analysis was performed using Statistica 10.0 for Windows (Stat Soft) and statistical package PQStat ver. 1.4.2.324. Data were assessed for normal distribution using the Shapiro-Wilk test. All data are presented as median (Q1–Q3) because of non-Gaussian distribution. TSH levels were grouped into tertiles, and between tertiles Kruskal-Wallis one-way analysis of variance by ranks was performed. Spearman's rank correlation test and partial correlation between TSH and bone turnover markers, with the effect of age, were used. Multivariable regression analysis to assess the independent contributions of different variables to serum P1NP concentration was performed. Statistical significance was assumed when the  $p$ -value was equal to or less than 0.05, and a highly significant probability test was taken at the level of  $p < 0.01$ .

## Results

A group of euthyroid women with osteoporotic fracture was characterised by median TSH, and fT4, of 0.82 (0.57–1.55) mIU/L and 1.22 (1.11–1.32) ng/dL, respectively. Median concentrations of bone turnover markers CTX and P1NP were 0.535 (0.369–0.832) ng/mL and 47.3 (34.7–66.9) ng/mL, respectively.

The association between TSH concentration across tertiles and frequency of fractures is shown in Figure 1. The majority of fractures (84%) occurred in the first tertile, at the lowest TSH from 0.14 to 2.19 mIU/L, whereas only 5% of fractures occurred in women with TSH in the higher tertile (TSH 4.27–6.31 mIU/L).

The general characteristics of the study participants according to TSH tertiles is given in Table 1. The TSH concentration in the first tertile was significantly lower compared to that in other tertiles ( $p = 0.004$ ); in fact, 70% of cases in this tertile had a TSH value below 1 mIU/L. The lowest concentrations of bone forma-

tion marker P1NP was found in the lower TSH tertile ( $p = 0.056$  and  $p = 0.057$ , respectively). Low P1NP concentrations (below the median in the whole group) were found in 54% of patients with TSH 0.14–2.19 mIU/L, and this proportion was nearly two-fold higher compared to 2<sup>nd</sup> tertile. None of the patients in the high tertile had a P1NP below the median value. Concomitant low bone formation markers in this subgroup and higher CTX values (over the median) were found in 60% of women. At TSH over 2.20 mIU/L this proportion was slightly higher (70%).

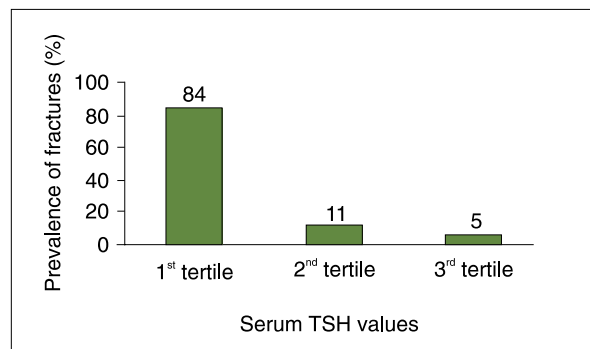
In euthyroid women with fractures a weak but highly significant positive correlation between TSH and P1NP was observed ( $r = 0.32$ ;  $p = 0.01$ ), which remained significant when adjusted for age. We observed no relationship either between TSH and other analysed

parameters or between thyroid hormones and analysed variables.

When rank correlations were analysed in TSH tertiles the relationship of P1NP with TSH remained positive and significant ( $p = 0.25$ ;  $p < 0.05$ ) in the lower tertile, and a weak tendency to inverse association of TSH and CTX could be noticed.

Moreover, CTX concentration was positively related with age ( $r = 0.25$ ;  $p < 0.05$ ) whereas CTX was significantly positively correlated with P1NP ( $r = 0.52$ ;  $p < 0.00002$ ) (Tab. 2).

Multivariable regression analysis was performed to assess the independent contributions of different variables to serum P1NP concentration in the whole study group. According to the results of bivariate correlation analysis, only TSH and CTX were taken into account (Tab. 3). Both TSH and CTX explained 25% of the variability of serum P1NP in euthyroid women with osteoporotic fractures.



**Figure 1.** Occurrence of fractures in euthyroid postmenopausal women according to TSH tertiles;  $p < 0.001$ . Tertile 1 vs. tertile 2 and tertile 3

## Discussion

This study shows that TSH variation within the physiological range is related to bone remodeling markers in postmenopausal euthyroid women who have sustained nonvertebral fragility fractures. The majority of fractures occurred at TSH concentrations in the lower tertile, and this was associated with the lowest concentrations of bone formation marker-P1NP. Interestingly, bone resorption in patients with low-normal TSH remained within the upper reference range. Our data support the concept of the imbalance between bone resorption

**Table 1.** General characteristics, thyroid parameters, and bone turnover markers of euthyroid postmenopausal women with osteoporotic fractures, according to TSH tertiles; Median (Q1–Q3)

	TSH		
	1 <sup>st</sup> tertile 0.14 to 2.19 mIU/L	2 <sup>nd</sup> tertile 2.20 to 4.26 mIU/L	3 <sup>rd</sup> tertile 4.27 to 6.31 mIU/L
N	50	7	3
Age (years)	77 (59–85)	82 (76–83)	83 (78–86)
TSH [mIU/L]	0.73 (0.52–1.12)	2.62(2.41–3.04)	5.24 (4.51–6.07)
fT4 [ng/dL]	1.22 (1.12–1.32)	1.15 (0.92–1.26)	1.29 (1.26–1.34)
P1NP [ng/mL]	44.2 (34.4–65.3)	63.1 (30.6–97.1)	64.4 (58.9–87.5)
CTX-I [ng/mL]	0.512 (0.366–0.817)	0.535 (0.409–0.651)	1.029 (0.410–1.640)

**Table 2.** Correlations between TSH, age, and bone turnover markers

	TSH	CTX
Age (years)	$r = 0.12$ $p < 0.3$	$r = 0.25$ $p < 0.05$
P1NP	$r = 0.32$ $p = 0.01$	$r = 0.52$ $p < 0.00002$

and bone formation in elderly euthyroid women with low-normal TSH levels and may suggest increased risk of nonvertebral (mostly hip) fractures.

The effect of TSH variations within the physiological range on BMD and bone turnover markers have been a matter of controversy. Mazioti et al. in a selected group of euthyroid postmenopausal women with low

**Table 3.** Multiple regression analysis on serum P1NP

	Independent variable	Adjusted R <sup>2</sup>	F	$\beta$	p
P1NP		0.25	12.4		< 0.0002
	TSH			0.19	< 0.07
	CTX			0.43	< 0.0009

bone mineral density showed that low-normal TSH levels are associated with high prevalence of vertebral fractures independently of thyroid hormones, age, and BMD [8]. In their study bone turnover markers were not measured, but the authors suggest additional measuring of TSH in postmenopausal women with osteopaenia and osteoporosis, even in the absence of thyroid disorders, because TSH levels below 1 mIU/L may predispose to vertebral fractures [8]. Another recent report by Murphy et al. showed that thyroid function within the upper-normal range is related to reduced BMD at the hip and increased risk of nonvertebral fractures in postmenopausal euthyroid women. However, in their study no relationship was found between TSH and BMD and bone turnover marker alterations [7]. Similarly, Roef et al., in a study performed in healthy men at the age of peak bone mass, observed that thyroid hormone status within the physiological range influences bone mass and density but has no relationship with bone formation marker P1NP and bone resorption marker CTX [5].

Our study was performed in women with sustained fragility fractures, and we found a positive significant correlation between serum TSH within the normal range and P1NP, a bone formation marker. Moreover, we showed that the majority of women with fractures in the lower TSH tertile had the lowest P1NP values and below the median for the whole group (< 47.3 ng/mL). In fact, the median P1NP concentration in our study group with fractures was lower than that reported by others for age-matched, postmenopausal women with osteoporosis [7, 10, 11]. On the other hand, median CTX in the lower TSH tertile, which remained within the reference range for age, and in the whole study group, was much higher than that observed by others [7, 10]. Zhao et al. found significantly higher levels of serum  $\beta$ -CTX in Chinese postmenopausal women with sustained osteoporotic fracture or vertebral fracture but no association of P1NP with vertebral fractures [10]. Our data suggest the possibility of uncoupling of the bone remodeling process in elderly women, particularly with low-normal TSH that may predispose to nonvertebral fractures. This seems to be in line with another important finding in the present study, supported by multivariable regression analysis, which revealed that P1NP variation may, to some extent, be explained by CTX and TSH concentrations in elderly euthyroid women with fragility fractures.

TSH has been reported to modulate bone remodeling independently of thyroid hormones, and a direct inhibitory effect on osteoclastic bone resorption has been shown [12]. TSH receptors in osteoclasts and osteoblasts were described, and reduced TSHR expression was found to be related to the development of osteoporosis [3]. We did not find any significant association of TSH with CTX because we studied a limited number of euthyroid women with TSH within the normal range. However, we did not observe significantly elevated CTX values across the TSH tertiles, as it was for P1NP. Therefore, we can only speculate that this could be an effect of a significant increase in TSH concentration.

Our study has some limitations. We included only women with fragility fractures, and because of technical reasons we did not measure bone mineral density by DEXA. However, we evaluated bone remodeling process with the most representative and recommended biochemical markers. The concentrations of biochemical bone markers were not always measured in the fasting state; however, as shown previously, concentrations of these markers in the serum, particularly P1NP, are virtually unaffected by food intake, and we excluded the influence of blood collection time.

Finally, we conclude that in elderly euthyroid women low-normal TSH level may be associated with the imbalance between bone resorption and bone formation. Diminished bone formation may predispose to increased risk of nonvertebral (mostly hip) fractures. Bone markers testing in subjects with low-normal TSH may add new value in the assessment of fracture risk.

Further studies should be designed to confirm the effect of TSH concentration within low-normal range on bone remodeling in a larger population.

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