

# Thyroid function and autoimmunity are associated with the risk of vertebral fractures in postmenopausal women

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**Abstract** Overt or subclinical thyroid dysfunction may affect the risk of fragility fractures. The aim of the present study was to assess the association of thyroid function and autoimmunity with vertebral fractures (VF) in a large sample of Greek postmenopausal women. This cross-sectional study recruited 335 euthyroid postmenopausal women, aged 35–79 years. Euthyroidism was verified by serum thyroid-stimulating hormone (TSH) within the laboratory reference range (0.4–4.5  $\mu$ IU/mL). VFs were diagnosed by lumbar spine lateral radiographs, according to quantitative procedures. Serum free triiodothyronine (FT3), free thyroxine (FT4), TSH, as well as levels of anti-thyroglobulin (anti-TG) and thyroid peroxidase antibodies (anti-TPO) were compared according to the presence of VFs. Multivariate logistic regression showed that the presence of VFs was predicted independently by ln-TSH levels (OR = 0.290,  $p$  = 0.037) and positive anti-TG antibodies (OR = 3.308,  $p$  = 0.026) in models adjusted for age, menopausal age, and ln-HOMA-IR. Stepwise logistic regression analysis showed that the presence of VFs was predicted by menopausal age (OR = 1.120,  $p$  = 0.001), ln-TSH (OR = 0.312,  $p$  = 0.052), and thyroid autoimmunity

(anti-TG and anti-TPO positive: OR = 6.637,  $p$  = 0.007) in a model that also included age and ln-HOMA-IR. Women with lower circulating TSH had higher risk of having a VF, independently of age, menopausal age, and insulin resistance. The presence of positive anti-TG/anti-TPO antibodies also indicated an elevated risk of fracture. Levels of thyroid hormones had no apparent effect on the risk of fracture. Further studies are necessary to establish the significance of our findings.

**Keywords** Vertebral fractures · Thyroid stimulating hormone · Insulin · Postmenopausal women

## Introduction

Osteoporotic vertebral fractures (VFs) are associated with significant disability and mortality, as well as with health and social service expenditure [1]. The spine presents a common site for osteoporotic fractures, as the lifetime probability of a major fracture at the age of menopause has been estimated to be as high as 15.1 % [2]. Moreover, VFs have been related to a marked increase of further fragility fractures [3, 4]; up to 20 % of individuals sustaining an initial VF will experience a second fracture within the following year [5]. Fracture risk is attributed to low bone density, as well as to several non-skeletal factors, which should be thoroughly evaluated in order to establish effective prevention strategies [6, 7].

Thyroid function is closely related to skeletal homeostasis. Overt and subclinical hyperthyroidism have been associated with increased risk of fracture in the general population [8–10]. Existing data, however, cannot support a clear impact of subclinical hypothyroidism on fracture risk in the general population [9, 10]. However, both

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subclinical hypo- and hyperthyroidism are related to higher FRAX scores of hip fracture in middle-aged women [11, 12]. Finally, both excess and deficiency of free thyroid hormones have been related to adverse effects on bone metabolism [13].

Euthyroid status is necessary for the regulation of adult bone structure [8, 14]. Fluctuation of TSH levels within the normal range has an impact on bone fractures in postmenopausal women: low normal TSH levels may increase the risk of VFs [15], hip fractures [16], or forearm and humerus fractures [17]. On the other hand, high normal TSH levels have been related with non-VFs [18]. However, a limited number of studies investigated the impact of thyroid hormones on the development of fractures, especially considering the underlying status of thyroid antibodies [15, 18, 19].

We have recently described the prevalence of vertebral fractures among Greek postmenopausal women [20]. The aim of the present study was to identify the potential association between the presence of VFs and thyroid function, as well as autoimmunity, in a sample of Greek postmenopausal euthyroid women.

## Materials and methods

### Subjects

This cross-sectional study included a total of 335 postmenopausal women, recruited from the Menopause Clinic of the 2nd Department of Obstetrics and Gynecology, University of Athens, Aretaieion Hospital. This clinic, active since 1998, serves both symptomatic and asymptomatic middle-aged women, providing information about menopause and offering screening and risk assessment for major morbidities of mid-life and beyond, such as osteoporosis, cardiovascular disease, cervical and breast cancer. A detailed electronic file is built for each informed, consenting woman containing demographic, life-style, and anthropometric parameters, as well as biochemical and hormonal assessments according to individual needs. All women who presented for their first evaluation between August 2007 and July 2013 and had a complete electronic file were screened for inclusion in this study. The menopausal status was defined as follicle stimulating hormone >25 mIU/mL and estradiol <50 pg/mL, after 12 consecutive months without menses.

Before recruitment, patients underwent the routine first-visit evaluation of our clinic, which includes PAP smear and transvaginal sonography, breast examination and mammography, fasting blood glucose, thyroid–liver–renal function tests, lipid profile, and bone densitometry. Clinical neurological examination was also performed to

exclude the presence of neurologic disorders, which may have affected fall risk. Exclusion criteria were the presence of gynecological malignancy, neurological disorders, clinically overt CVD, thromboembolism, and a previous diagnosis of DM or thyroid dysfunction. Since hyperthyroidism is an established risk factor for osteoporotic fractures, we decided to exclude women with thyroid hormones beyond the laboratory reference range, in order to evaluate only euthyroid women. Furthermore, we excluded women with secondary causes of osteoporosis (e.g. Paget's bone disease, renal osteodystrophy, hyperparathyroidism), as well as pre- and perimenopausal women. Women with adherence and retention concerns (e.g., alcoholism) were not included in the study. Renal function was assessed using both serum levels of creatinine and the Cockcroft-Gault equation,  $GFR = [(140 - \text{age}) \times (\text{actual weight in kg}) \times 0.85 \text{ (if female)}] / (72 \times \text{serum creatinine})$  [21]. Institutional review board approval was obtained by the ethics committee of Aretaieion Hospital.

### Anthropometric measurements

Weight and height were measured in the morning and in light clothing in order to estimate the body mass index (BMI). Weight was measured on an electronic scale, and height was measured in a stadiometer in the upright position. BMI was calculated using the equation  $BMI = \text{body weight (kg)} / \text{height}^2 \text{ (m}^2\text{)}$ .

### Biochemical and hormone assays

Serum glucose was measured using glucose assay (Abbott) with coefficient of variation (CV)  $\leq 5\%$  and sensitivity 2.5 mg/dL. Serum levels of total calcium, phosphorus, magnesium, and hemoglobin A1c (HbA1c) were assessed enzymatically by an autoanalyzer (ARCHITECT ci4100 Integrated System, Abbott Diagnostics Laboratories, Abbott Park, IL, USA, Abbott 65205, Wiesbaden, Germany). Serum levels of insulin were measured on an Architect i1000 analyzer (Abbott Ireland, Diagnostics Division, Lisnamuck, Longford, Ireland). The total CV% ranged from 1.9 to 5.2, and the analytical sensitivity was 1  $\mu\text{U/mL}$ . Creatinine levels were determined by an enzymatic method. The total CV% ranged from 1.9 to 5.2, and the analytical sensitivity was 1  $\mu\text{IU/mL}$ . TSH 3rd gen levels, free triiodothyronine (FT3), and free thyroxine (FT4) were measured with the Abbott Architect i1000 analyzer. The total coefficient of variation (CV%) and analytical sensitivity (AS) were as follows: TSH, CV% 1.7–5.3 and AS 0.0025  $\mu\text{IU/mL}$ ; FT3, CV% 2.3–5.0 and AS 1.0 pg/mL; FT4, CV% 3.6–7.8 and AS 0.4 ng/dL. Anti-myeloperoxidase antibodies (anti-TPO) and antithyroglobulin antibodies (anti-TG) were evaluated using the microparticle

enzyme immunoassay kits “anti-TPO Abbott AxSYM” and “anti-TG Abbott AxSYM”, respectively, on an AxSYM analyzer (Abbott Laboratories). Reference ranges were: TSH 0.40–4.5  $\mu$ IU/ml; insulin <25 mIU/L; FT4 0.71–1.85 ng/dL; FT3 1.7–3.7 pg/mL; anti-TPO, below 5 IU/mL, antithyroglobulin below 5 IU/mL. Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as follows: fasting insulin (microunits per milliliter)  $\times$  fasting glucose (millimoles per liter)/22.5.

**VF assessment**

Lumbar spine lateral radiographs were obtained according to quantitative procedures [22]. A single operator, blinded to the identity of the patient, performed the morphometric analysis. The heights of each vertebra, namely the anterior (Ha), posterior (Hp), and middle (Hm), were determined by placement of six points using a cursor and backlit digitizing board. The presence of morphometric VFs was defined using ratios of vertebral height for each vertebral body from T4 to L4: the Ha/Hp (wedge) ratio, the Hm/Hp ratio, and the ratio of the above vertebrae Hp/Hp, as well as the ratio of the below vertebrae Hp/Hp. A height ratio decrease of  $\geq 20\%$  was the threshold for diagnosing a VF, according to the visual criteria defined by Genant [22]. A vertebral body is considered fractured when at least one of its ratios falls below 3 SDs from normative mean values.

**Statistical analysis**

Statistical analysis of the data was performed using Statistical Package for the Social Sciences version 15.0 (SPSS Inc, Chicago IL, USA) and Stata statistical software package version 9.2 (StataCorp LP, College Station, TX, USA). Ln-transformed values of original parameters were used whenever necessary to improve normality in distribution for skewed parameters. Student’s *t* test for independent samples was used for comparisons between mean (or mean log-transformed) values of quantitative parameters between patients with and without prevalent fractures; if normality in distribution was still considered problematic after logarithmic transformation, the non-parametric Wilcoxon–Mann–Whitney test for independent samples was used instead. Fisher’s exact test and  $\chi^2$ -analysis were used for comparisons of categorical parameters between women with and without prevalent fractures. Thyroid hormones were described as “low-normal” or “high-normal”, if the estimated levels were below or higher than the respective median value for each hormone; levels of thyroid antibodies were dichotomized as “positive” and “negative” if the value of the antibody was higher or lower than fivefold the upper normal limit, respectively [23]. On a secondary level, the impact of thyroid hormones and TSH on the risk of VFs

has been also evaluated in the subgroup of patients with negative anti-thyroid antibodies. Multiple logistic regression was applied to further investigate possibly significant associations of parameters with presence/absence of prevalent fractures, while each hormonal parameter was then added, separately. In addition, stepwise logistic regression was used to assess the combined effect of significant indices of thyroid function on the risk of VFs. All regression models were a priori adjusted for risk factors that met the threshold of confounding (i.e.  $p < 0.1$ ). A cut-off point of  $p < 0.05$  was marked as “statistically significant”.

**Results**

**Study sample demographics**

The main demographic and anthropometric characteristics of the 335 euthyroid peri- and postmenopausal patients are presented in Table 1. At least one prevalent VF was identified in 24 (7.2 %) women in our study. No significant differences were observed regarding smoking frequency, alcohol consumption, frequency of physical exercise, level of

**Table 1** Mean ( $\pm$ SD) or *N* (%) of demographics and lifestyle characteristics in the study sample of Greek peri- and postmenopausal women (*N* = 335)

| Parameter                | Mean (SD) or <i>N</i> (%) | Range     |
|--------------------------|---------------------------|-----------|
| Menarche (age in years)  | 12.9 (1.4)                | 9–17      |
| Parity (number)          | 1.6 (0.9)                 | 0–5       |
| $\geq 1$ abortion (%)    | 54.8 %                    | –         |
| BMI (kg/m <sup>2</sup> ) | 26.4 (3.9)                | 19.1–35.8 |
| Education                |                           |           |
| None/elementary          | 62 (18.5 %)               | –         |
| Secondary                | 153 (45.7 %)              | –         |
| Tertiary                 | 120 (35.8 %)              | –         |
| Exercise                 |                           |           |
| <1 h/week                | 78 (23.3 %)               | –         |
| 1–3 h/week               | 61 (18.2 %)               | –         |
| >3 h/week                | 196 (58.5 %)              | –         |
| Alcohol                  |                           |           |
| None                     | 173 (51.6 %)              | –         |
| Occasionally             | 142 (42.4 %)              | –         |
| Daily                    | 20 (6.0 %)                | –         |
| Smoking                  |                           |           |
| Never/in the past        | 303 (90.4 %)              | –         |
| Currently                | 32 (9.6 %)                | –         |
| Dietary Ca intake        |                           |           |
| Poor                     | 68 (20.3 %)               | –         |
| Medium                   | 178 (53.1 %)              | –         |
| Rich                     | 89 (26.6 %)               | –         |

*BMI* body mass index, *Ca* calcium

**Table 2** Mean ( $\pm$ SD) of biochemical and hormonal parameters for the whole sample and according to the presence of vertebral fractures

| Parameter                           | All patients ( $N = 335$ ) |            | No fracture ( $N = 311$ ) | At least one fracture ( $N = 24$ ) | $p$ value (“no” vs. “at least one” fracture) |
|-------------------------------------|----------------------------|------------|---------------------------|------------------------------------|--|
|                                     | Mean (SD) or $N$ (%)       | Range      | Mean (SD) or $N$ (%)      | Mean (SD) or $N$ (%)               |  |
| Age (years)                         | 56.7 (7.1)                 | 35–79      | <b>56.4 (6.9)</b>         | <b>61.2 (8.0)</b>                  | <b>0.001</b>                                 |
| Menopausal age (years)              | 9.1 (6.9)                  | 1–34       | <b>8.7 (6.5)</b>          | <b>13.8 (9.1)</b>                  | <b>&lt;0.001</b>                             |
| Ca (mg/dL)                          | 9.46 (0.45)                | 8.2–11.1   | 9.46 (0.44)               | 9.35 (0.49)                        | 0.244  |
| P (mg/dL)                           | 3.47 (0.48)                | 2.1–5.2    | 3.45 (0.48)               | 3.61 (0.38)                        | 0.143  |
| Mg (mg/dL)                          | 2.21 (0.26)                | 0.8–3.6    | 2.22 (0.27)               | 2.13 (0.22)                        | 0.197  |
| Creatinine (mg/dL)                  | 0.76 (0.13)                | 0.50–1.30  | 0.75 (0.13)               | 0.78 (0.13)                        | 0.409  |
| GFR                                 | 91.6 (22.2)                | 46.0–161.9 | 92.2 (22.1)               | 83.7 (23.4)                        | 0.085  |
| FPG (mg/dL)                         | 92.8 (8.97)                | 66–120     | 93.1 (9.4)                | 91.4 (8.2)                         | 0.378  |
| Insulin <sup>a</sup> ( $\mu$ IU/mL) | 6.42 (1.63)                | 2.20–31.7  | 6.52 (1.64)               | 5.20 (1.34)                        | 0.063  |
| HbA1c <sup>a</sup> (%)              | 5.25 (1.09)                | 4.00–6.42  | 5.26 (2.65)               | 5.35 (1.12)                        | 0.459  |
| HOMA-IR <sup>a</sup>                | 1.47 (1.69)                | 0.39–7.75  | <b>1.49 (1.71)</b>        | <b>1.16 (1.35)</b>                 | <b>0.049</b>                                 |
| TSH <sup>a</sup> ( $\mu$ IU/mL)     | 1.26 (1.71)                | 0.41–4.5   | 1.27 (1.72)               | 1.08 (1.52)                        | 0.085  |
| FT3 (pg/mL)                         | 2.57 (0.59)                | 1.24–5.47  | <b>2.60 (0.60)</b>        | <b>2.27 (0.48)</b>                 | <b>0.016</b>                                 |
| FT4 (ng/dL)                         | 1.05 (0.15)                | 0.63–1.63  | 1.05 (0.15)               | 1.06 (0.17)                        | 0.820  |
| Anti-TPO                            | 18.2 % (61/335)            |            | 17.4 % (54/311)           | 29.2 % (7/24)                      | 0.162  |
| Anti-TG                             | 29.2 % (98/335)            |            | 27.9 % (87/311)           | 46 % (11/24)                       | 0.104  |

GFR was calculated using the Cockcroft–Gault formula

Bold indicates statistical significance which was set at the level of  $p$  value  $<0.05$

Ca calcium, P phosphorus, Mg magnesium, GFR glomerular filtration rate, FPG fasting plasma glucose, HbA1c glycated hemoglobin, HOMA-IR homeostasis model assessment of insulin resistance, TSH thyroid stimulating hormone, FT3 free triiodothyronine, FT4 free thyroxine, anti-TPO anti-thyroid peroxidase antibodies, anti-TG anti-thyroglobulin antibodies

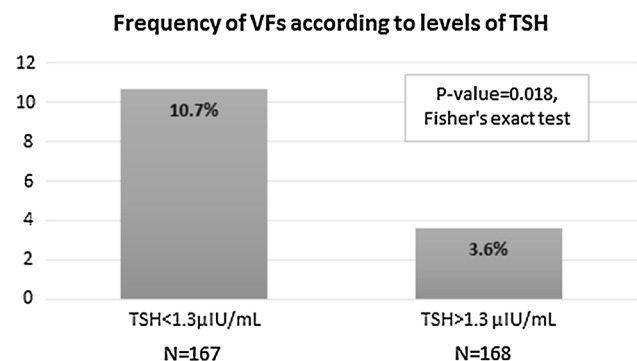
<sup>a</sup> Original mean values presented, but log-transformed values used for statistical analysis, due to skewness

dietary calcium intake, parity, and number of abortions or mean BMI values, comparing women with prevalent VFs to women without fracture (data not shown).

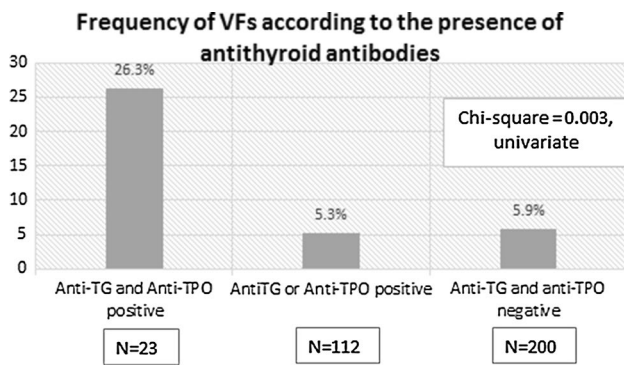
### Comparison between groups

Age and biochemical measurements in the whole study sample and according to the prevalence of lumbar fractures are summed up in Table 2. Women with prevalent VFs were older, with a higher menopausal age and lower levels of ln-HOMA-IR compared to women without fractures (age:  $61.2 \pm 8.0$  vs  $56.4 \pm 6.9$  years,  $p = 0.001$ ; menopausal age:  $13.8 \pm 9.1$  vs  $8.7 \pm 6.5$  years,  $p < 0.001$ ; ln-HOMA-IR:  $1.16 \pm 1.35$  vs  $1.49 \pm 1.71$ ,  $p = 0.049$ ). Women with prevalent VFs exhibited lower levels of FT3 compared to women without VFs ( $2.27 \pm 0.48$  vs  $2.60 \pm 0.60$  pg/mL,  $p = 0.016$ ,  $t$  test). Moreover, women with at least one VF had almost significantly lower levels of ln-insulin ( $5.20 \pm 1.34$  vs  $6.52 \pm 1.64$   $\mu$ IU/mL,  $p = 0.063$ ,  $t$  test) and ln-TSH ( $1.08 \pm 1.52$  vs  $1.27 \pm 1.72$   $\mu$ IU/mL,  $p = 0.085$ ,  $t$  test). In addition, the development of VFs associated with positive anti-thyroid antibodies (anti-TG: 46 vs 27.9 %,  $\chi^2 = 0.104$ ). Women with TSH levels  $<$  median value of 1.30  $\mu$ IU/mL had significantly higher prevalence of VFs compared to women

with TSH levels  $>1.30$   $\mu$ IU/mL ( $10.7$  vs  $3.6$  %,  $p = 0.018$ , Fisher’s exact test; Fig. 1). In addition, women with FT3 levels  $<$  median value of 2.51 pg/mL had significantly higher prevalence of VFs compared to women without VFs ( $11.4$  vs  $2.9$  %,  $p = 0.009$ , Fisher’s exact test). Women with positive anti-TPO and anti-TG antibodies presented the highest risk of VF compared to women with only one positive category of



**Fig. 1** Frequency of VFs according to levels of thyroid stimulating hormone (TSH) in this sample of 335 euthyroid postmenopausal women



**Fig. 2** Frequency of vertebral fractures according to the presence of anti-thyroid antibodies in this sample of postmenopausal women

antibodies or women without antibodies (Fig. 2). No major differences in any other biochemical measurement were noted between patients having and those not having prevalent lumbar fractures ( $p > 0.05$ ).

**Logistic regression**

The odds ratios of having a VF according to biochemical and hormonal parameters are presented in Table 3. After adjusting for age, years since menopause, and ln-HOMA-IR, as possible confounders, ln-transformed serum levels of TSH associated positively with the odds of having a fracture ( $p = 0.037$ ). In addition, levels of high-normal levels of TSH are associated with lower risk of VFs compared to low-normal levels of TSH (OR = 0.188,  $p = 0.015$ ). The impact of TSH on the development of VFs remains the same, even in the subgroup of patients with negative anti-thyroid antibodies (ln-TSH: OR = 0.201,  $p = 0.029$ ), adjusting for age, menopausal age, and insulin resistance. Moreover, the presence of anti-TG antibodies exhibited a positive association with the development of VFs, implying lack of protection. In addition, the simultaneous presence of both positive anti-TPO and anti-TG antibodies associated with the risk of VFs (OR = 6.328,  $p = 0.009$ ). Ln-transformed levels of insulin and HOMA-IR tended to affect the occurrence of VFs, after adjusting for the same confounders ( $p = 0.083$  and  $0.062$ , respectively). Levels of FT3 and FT4 presented no significant association with the risk of VFs in the total sample or in the subgroup of patients with negative anti-thyroid antibodies, in models of multivariate analysis adjusted for age, menopausal age, and insulin resistance (data not shown).

Stepwise logistic regression analysis showed that the risk of VFs was predicted by menopausal age and both levels of ln-TSH and positive anti-TG antibodies, in a stepwise multivariate logistic regression model that included also age and ln-HOMA-IR (model  $\chi^2 = 20.070$ ; ln-TSH: OR = 0.277,  $p = 0.026$ ; positive anti-TG: OR = 3.273,

**Table 3** Logistic regression analysis for the risk of having a vertebral fracture according to biochemical and hormonal parameters (each parameter examined separately)

| Parameter  | Adjusted for age, YSM, ln-HOMA-IR, and smoking <sup>a</sup> |              |                     |
|--|---|--------------|---------------------|
|  | OR  | p value      | 95 % CI             |
| Calcium (per 1 mg/dL)                                      | 0.361   | 0.104        | 0.106–1.234         |
| Phosphorus (per 1 mg/dL)                                   | 1.988   | 0.316        | 0.519–7.617         |
| Magnesium (per 1 mg/dL)                                    | 0.269   | 0.322        | 0.020–3.618         |
| FPG (per 1 mg/dL) <sup>b</sup>                             | 0.976   | 0.297        | 0.932–1.022         |
| Insulin <sup>c</sup> [per 1 ln( $\mu$ IU/mL)] <sup>b</sup> | 0.339   | 0.083        | 0.099–1.154         |
| HOMA-IR <sup>LN</sup> (per 1 ln unit) <sup>b</sup>         | 0.341   | 0.062        | 0.111–1.053         |
| TSH <sup>c</sup> [per 1 ln( $\mu$ IU/mL)]                  | <b>0.290</b>  | <b>0.037</b> | <b>0.091–0.928</b>  |
| TSH > 1.3 ( $\mu$ IU/mL) <sup>d</sup>                      | <b>0.188</b>  | <b>0.015</b> | <b>0.049–0.718</b>  |
| FT3 (pg/mL)  | 0.558   | 0.306        | 0.182–1.705         |
| FT3 > 2.51 pg/mL <sup>e</sup>                              | 0.444   | 0.183        | 0.134–1.467         |
| FT4 (ng/dL)  | 4.722   | 0.388        | 0.139–16.12         |
| FT4 > 1.04 ng/dL <sup>f</sup>                              | 1.559   | 0.408        | 0.545–4.459         |
| Positive anti-TPO  | 2.055   | 0.235        | 0.625–6.752         |
| Positive anti-TG   | <b>3.308</b>  | <b>0.026</b> | <b>1.150–9.515</b>  |
| Positive anti-TG and anti-TPO                              | <b>6.328</b>  | <b>0.009</b> | <b>1.589–15.197</b> |

Statistical significance was set at the level of  $p$  value  $< 0.05$

OR odds ratio, 95 % CI 95 % confidence interval, YSM years since menopause, FPG fasting plasma glucose, HOMA-IR homeostasis model assessment of insulin resistance, TSH thyroid stimulating hormone, FT3 free triiodothyronine, FT4 free thyroxine, anti-TPO anti-thyroid peroxidase antibodies, anti-TG anti-thyroglobulin antibodies

<sup>a</sup> Values correspond to model a priori including age, years since menopause, and ln-HOMA-IR; all other biochemical/hormonal parameters were included separately

<sup>b</sup> Regression model was not adjusted for ln-HOMA-IR values

<sup>c</sup> Log-transformed values used for statistical analysis, due to skewness

<sup>d</sup> Reference category: TSH lower than the median value of 1.3 IU/mL

<sup>e</sup> Reference category: FT3 lower than the median value of 2.51 pg/mL

<sup>f</sup> Reference category: FT4 lower than the median value of 1.04 ng/dL

$p = 0.030$ ). Moreover, the risk of VFs was predicted by the combined presence of anti-TG and anti-TPO (OR = 6.637,  $p = 0.007$ ) as well as menopausal age (OR = 1.12,  $p = 0.001$ ) and ln-TSH (OR = 0.312,  $p = 0.052$ ), in a stepwise multiple regression analysis model that included also age and ln-HOMA-IR as independent variables.

**Discussion**

The present study shows that higher TSH levels within the normal range are associated with lower risk of vertebral fracture in this sample of postmenopausal Greek women. Moreover, positive anti-thyroid antibodies further increased the risk of fracture. These associations occurred independently of confounders such as age, menopausal age, and BMI.



It is important to stress that lower TSH levels were associated with higher frequency of VFs within the euthyroid range, as we included women with normal thyroid function in the analyses. Elevated risk of hip fracture was observed in women with TSH levels within the low normal range (0.35–1.6  $\mu\text{IU/mL}$ ), according to a recent study of both genders that included 9421 women, aged  $\geq 65$  years [16]. In addition, the results of the OPENTHYRO cohort described an association between the risk of fractures and decreasing levels of TSH within the euthyroid range [17]. Concerning women, the same study showed that low-normal TSH was a significant predictor of hip, spine, forearm, and humerus fractures [17]. Similarly, the presence of VFs has been associated with lower levels of TSH within the normal range in a smaller cohort of 130 euthyroid postmenopausal women. In that study, women with low-normal TSH were almost three times more probable to have a vertebral fracture compared to the rest of the population [15].

On the contrary, a smaller number of studies found no association between thyroid function within the normal range and vertebral fractures. In the Osteoporosis and Ultrasound Study (OPUS), which included a large sample of 1278 euthyroid, elderly postmenopausal women, although they identified a 35 % reduction in incident non-vertebral fractures in women with TSH levels within the high-normal range, they observed no association between TSH levels and incident or prevalent VFs; however, they used a narrower reference range to define euthyroidism (0.15–3.64  $\mu\text{IU/mL}$ ) compared to our study [24]. Furthermore, no association between TSH levels within the normal range and bone fractures has been identified in a large mixed-gender sample of patients with chronic disabling diseases [25].

Hyperthyroidism is an established risk factor for osteoporotic fractures [26]. Controversy, however, exists whether milder subclinical conditions associated with low or low-normal TSH have an impact on bone health of older adults. Our results, along with other studies [11, 19, 20] indicate that these conditions might indeed pose a risk factor for osteoporotic fractures. Recent evidence indicates that TSH may have a direct protective role against osteoporosis. Results from histomorphometric analyses showed that TSH signaling slows bone resorption, by inhibiting osteoclasts differentiation *in vitro* [27, 28]. Similarly, Baliram et al. [29] indicated that TSH increases osteoblast differentiation and regulates osteoprotegerin production *in vitro*, with the latter possibly inhibiting osteoclastic resorption locally. The same group, trying to answer the question whether the increased thyroid hormones are the only pathogenetic factor leading to osteoporosis, compared two groups of hyperthyroid mice: (1) animals lacking the TSH receptor and (2) wild-type animals [30]. The researchers observed that net bone loss was more pronounced in mice lacking TSH-receptor versus wild type, indicating the protective role of TSH signaling in the regulation of bone turnover [30].

Positive anti-thyroid antibodies presented as an additional predictor of high fracture risk, especially in association with low-normal levels of TSH in this sample of postmenopausal women. According to our knowledge, the impact of anti-thyroid antibodies on the development of VFs in the euthyroid population has not been investigated yet. On the other hand, positive anti-TPO antibodies have been associated with FRAX score in postmenopausal women with subclinical hypothyroidism [12], but not in perimenopausal women with subclinical hyperthyroidism [11]. In addition, the presence of anti-TPO antibodies has been associated with worse quantitative calcaneus ultrasound parameters, in a large sample of Chinese men [31]. The combined assessment of antithyroid antibodies seems more rational, taking into account that anti-TPO and anti-TG antibodies fluctuate in parallel [32]. It has to be noted that positive anti-TG per se was associated with the presence of vertebral fractures, independently of age, menopausal age, and insulin resistance. However, the clinical implications of this finding cannot be elucidated, since anti-TPO represents a superior clinical marker of disease compared to anti-TG [32].

Levels of free thyroid hormones were not associated with the risk of VF, adjusting for age, menopausal age, and insulin resistance, according to our findings. Evidence on the impact of circulating free thyroid hormones on the risk of VFs is limited. No significant effect of serum FT4 levels on the risk of VFs has been identified in a cohort of 130 euthyroid postmenopausal women, in agreement with our findings [15]. A direct association between levels of free thyroid hormones and the risk of non-vertebral fractures has been observed in a population based sample of 2374 euthyroid postmenopausal women [18]. In addition, high levels of FT4 have been associated with lower quantitative calcaneus parameters in a large sample of Chinese men [31], as well as with lower lumbar spine BMD in perimenopausal women [33]. Finally, according to the results of a population-based cohort study, elevated levels of TSH ( $>4.0$   $\mu\text{IU/mL}$ ) have been associated with higher risk of hip fracture in women (HR = 1.75, 95 % 1.24–2.46) [19].

The present study has some limitations. First, the cross-sectional design does not permit the detection of causality. Women of our sample were recruited from an outpatient clinic and were thus more health concerned than the general population. Moreover, we did not evaluate bone turnover markers so that no definite conclusions can be drawn regarding the effect of thyroid function on bone metabolism. Finally, women with positive antithyroid antibodies cannot be defined as cases with euthyroid Hashimoto's, since the analysis has been performed retrospectively and data regarding sonographic assessment of the thyroid was sparse.

In conclusion, this cross-sectional study of Greek postmenopausal women, serum TSH within the euthyroid range had a significant inverse association with prevalent VFs.

Positive antithyroid antibodies emerged as another significant predictor of fracture risk. The risk of VF was even higher for women with positive antithyroid antibodies and low-normal TSH levels. These associations remained significant even after adjusting for traditional risk factors, such as age, menopausal age, and insulin resistance. Our findings, although indicative, may have implications on the evaluation of thyroid profile with respect to osteoporotic fracture risk.

#### Compliance with ethical standards

**Conflict of interest** The authors have nothing to disclose.

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