Treatment of subclinical hypothyroidism does not affect bone mass as determined by dual-energy X-ray absorptiometry, peripheral quantitative computed tomography and quantitative bone ultrasound in Spanish women

Juan D. Pedrera-Zamorano, Raul Roncero-Martin, Julian F. Calderon-Garcia, Mercedes Santos-Vivas, Vicente Vera, Mariana Martinez-Alvarez, Purificación Rey-Sanchez

Abstract

Introduction: The results of studies examining the influence of subclinical hypothyroidism (SCH) and levothyroxine (L-T$_4$) replacement therapy on bone have generated considerable interest but also controversy. The present research aims to evaluate the effects of L-T$_4$ treatment on different skeletal sites in women.

Material and methods: A group of 45 premenopausal (mean age: 43.62 ± 6.65 years) and 180 postmenopausal (mean age: 59.51 ± 7.90 years) women with SCH who were undergoing L-T$_4$ replacement therapy for at least 6 months were compared to 58 pre- and 180 postmenopausal women with SCH (untreated) matched for age. The mean doses of L-T$_4$ were 90.88 ± 42.59 µg/day in the premenopausal women and 86.35 ± 34.11 µg/day in the postmenopausal women. Bone measurements were obtained using quantitative bone ultrasound (QUS) for the phalanx, dual-energy X-ray absorptiometry (DXA) for the lumbar spine and hip, and peripheral quantitative computed tomography (pQCT) for the non-dominant distal forearm.

Results: No differences were observed between patients and untreated controls in these bone measurements except in the bone mineral density (BMD) of the spine ($p$ = 0.0214) in postmenopausal women, which was greater in treated women than in untreated controls.

Conclusions: Our results indicate that adequate metabolic control through replacement treatment with L-T$_4$ in pre- and postmenopausal women does not affect bone mass.

Key words: subclinical hypothyroidism, levothyroxine, bone mineral density, premenopausal, postmenopausal.

Introduction

Subclinical hypothyroidism (SCH) is a common problem, especially in middle-aged and older adults [1]. Its prevalence varies from 3% to 15%, depending on age, sex and the population under study and the diagnostic criteria used [2]. Subclinical hypothyroidism may progress to overt hypothyroidism in approximately 2–5% of cases annually [3]. The prev-
Treatment of subclinical hypothyroidism does not affect bone mass as determined by dual-energy X-ray absorptiometry, peripheral quantitative computed tomography and quantitative bone ultrasound in Spanish women

Physiological variation in thyroid status is related to bone mineral density (BMD) and fracture in healthy, euthyroid, postmenopausal women. Higher FT4 and free T3 (FT3) levels are associated with reduced BMD, and higher FT4 levels are associated with increased bone loss at the hip [9]. Abe et al. [10] found evidence for direct effects of TSH on components of skeletal remodeling, osteoblastic bone formation, and osteoclastic bone resorption, and these effects were mediated via the TSH receptor present on osteoblast and osteoclast precursors. Other studies suggested that TSH exerts a bone-protective action by negatively regulating osteoclastogenesis [11]. A recent study in postmenopausal women with normal TSH levels showed a favorable bone status compared to those with low TSH levels irrespective of the FT4 level [12]. This result is consistent with the view that TSH plays a role in the preservation of bone after menopause [13]. Other studies in postmenopausal women with SCH suggest that the elevation of serum TSH concentration affects not bone markers but bone structure as assessed by bone quantitative ultrasound (QUS) in the calcaneus [14].

Treatment with L-T4 has been shown to be effective in improving alterations produced in patients with SCH such as cognitive function [15]; however, the long-term effect of replacement treatment with levothyroxine (L-T4) on bones has produced controversial results. Some studies found that treatment with replacement doses of L-T4 resulted in a decrease in bone density [16, 17], while in others this treatment resulted in accelerated bone loss, although the absolute values were not in the range that is typical of osteoporosis [18]. Franklyn et al. [19] found that thyroxine alone does not have a significant effect on BMD.

The purpose of this study was to investigate the effect of chronic treatment with replacement L-T4 on bone in pre- and postmenopausal women with SCH and to compare the results obtained with women with untreated SCH. We evaluated bone status using quantitative bone ultrasound (QUS) for the phalanx, dual-energy X-ray absorptiometry (DXA) for the lumbar spine and hip, and quantitative bone ultrasound (pQCT) for the non-dominant distal radius. Using these three different techniques, our study enhances our understanding of the affected bone compartments and possible changes in bone quality in women.

**Material and methods**

We studied 225 women, including 45 premenopausal (mean age: 43.62 ±6.65 years) and 180 postmenopausal (mean age: 59.51 ±7.90 years) women, with SCH who were on L-T4 replacement therapy for at least 6 months at a mean dose of 90.88 ±42.59 µg/day for the premenopausal women and 86.35 ±34.11 µg/day for the postmenopausal women. Inclusion criteria were age over 18, being in treatment with thyroid hormone replacement for at least six months (for the treatment group and not receiving such treatment for the control group), and having TSH levels higher than 4.5 mIU/l and FT4 levels in the normal range (0.8–1.2 ng/dl) [1]. The exclusion criteria included clinical osteoporosis and routine medication that interfered with vitamin D or bone metabolism.

As controls, a group of 58 premenopausal women (mean age: 44.77 ±6.82 years) and 180 postmenopausal women (mean age: 58.75 ±7.92 years) with untreated SCH who were similar in race and geographical location were recruited by random digit dialing.

All the women were residing in the urban area of the health district of Cáceres, Spain. The postmenopausal women had primary or secondary studies and the premenopausal women secondary or university studies. The majority of them were married, had children, and their social status was average. None of the participants had dietary restrictions, neurological impairment, or physical disabilities, and their medical histories showed no presence of low-trauma fractures. All participating subjects gave written informed consent, and the research project was approved by the Ethical Review Committee at the Hospital “San Pedro de Alcántara” of Cáceres, and the Office for Protection from Research Risks at the University of Extremadura in accordance with the Helsinki Declaration of 1975.

We took a complete medical history and physically examined each subject before she was enrolled in the study. No women in the study were taking medications that would interfere with calcium metabolism (corticoids, oral anticoagulants, antipsychotics, etc.), with the exception of hormone replacement therapy with L-T4. All women led active lives but did not regularly practice sports. Alcohol intake was sporadic, not exceeding

Arch Med Sci 5, October / 2015 1009
Body composition was studied by means of bioelectrical impedance analysis (BIA) using a body composition analyzer (BC-418MA, TANITA, Tokyo, Japan). Food intake was quantified using dietetic scales, measuring cups, and spoons based on 7 days of diet record [20]. The intake of nutrients of all groups of women was consistent with the recommendations given by their government authorities (i.e., the recommended dietary allowances (RDA) given by European Union and Spanish authorities), with the exception of proteins, which were higher than the RDA, due to being in an area of high protein intake [21, 22].

Bone measurements

An ultrasound was performed on the 2nd to the 5th proximal phalanx of the nondominant hand using a DBM Sonic Bone Profiler (IGEA, Capri, Italy).

The femoral neck and L2-L4 spine BMDs were measured by DXA (Norland XR-800, Norland Inc., Fort Atkinson, USA) and are expressed as the amount of mineral (g) divided by the area scanned (cm²).

The pQCT measurements were performed on the nondominant distal forearm using a Stratec XCT-2000 device (Stratec Medizintechnik, Pforzheim, Germany).

Analytical studies

All subjects underwent biochemical measurements of blood glucose, transaminase, γ-glutamyl transferase (GGT), creatinine, calcium, phosphorus, total protein, bilirubin, alkaline phosphatase, and tartrate-resistant acid phosphatase (TRAP) levels, and coagulation study; and TSH and FT4 serum concentrations were measured by electrochemiluminescence immunoassay (ECLIA) using a commercial kit (Roche Diagnostics). For each subject, the calcium level was corrected for proteins, and normal calcium excretion and tubular phosphatase resorption were confirmed by conducting a biochemical study on a 24-hour urine sample.

Smoking; coffee, tea or alcohol intake; and exercise were not permitted during the 24 h before testing. We collected urine samples on the morning of testing after an overnight fast. Venous blood samples for hematological and biochemical studies were also obtained when the subjects were fasting (at 8:00 a.m.).

The blood samples were centrifuged, and the serum was stored at –20°C until analysis. We measured the concentrations of biochemical species in the serum using an Hitachi automated analyzer system 902 (Roche, Manheim, Germany) and the 24-hour urinary calcium excretion by atomic absorption spectroscopy using a Perkin Elmer model 5000 spectrophotometer (Perkin Elmer, Norfolk, CT, USA).

The baseline blood chemistry, amplitude-dependent speed of sound (Ad-SoS), DXA, and pQCT measurements were obtained in the same session and at an ambient temperature of 22°C.

Statistical analysis

All values are expressed as the mean ± SD. We confirmed the normal distribution of the data by calculating the skewness and kurtosis before applying standard tests. We compared the parameters (continuous variables) for each group (nominal variables) using the t test, an analysis of variance and covariance to determine the effects of the nominal variables. We also used single and multiple stepwise regressions and partial correlations (adjusted for age) to examine the relationships between continuous variables. A value of p < 0.05 was required for statistical significance. We processed the data using the StatView 5.01 statistical package (SAS Institute Inc., Cary, NC, USA).

Results

For the biological, anthropometric and biochemical variables (Table I), we found no significant differences between treated and untreated controls in premenopausal women. In postmenopausal women, the age of menarche was significantly lower in the treatment group than in the controls (p = 0.0083).

The results of the bone status analysis are shown in Table II. The only comparison that yielded significant differences was the comparison of the L2-L4 BMD between the postmenopausal treated patients and matched untreated controls (p = 0.0214), and BMD was higher in the treated women.

In the stepwise regression, we used the bone measurements as dependent variables and the other biological variables, anthropometric factors and thyroid function values as independent variables. In the group of premenopausal women on L-T₄ replacement therapy, the DXA measurements of the neck BMD and L₂-L₄ BMD were negatively correlated with age (β = −0.009, p = 0.0136;
Treatment of subclinical hypothyroidism does not affect bone mass as determined by dual-energy X-ray absorptiometry, peripheral quantitative computed tomography and quantitative bone ultrasound in Spanish women

**Discussion**

There are no significant differences in terms of the presence of osteoporosis and osteopenia between the total groups of women with treated SCH and untreated SCH; thus, in the group of women with SCH, 8% (n = 18) presented osteoporosis, 30.66% (n = 69) osteopenia, and 61.33% (n = 138) were normal according to densitometry; in the control group, 9.66% (n = 23) had osteoporosis, 35.71% (n = 85) osteopenia, and 54.62% (n = 130) were normal according to densitometry.

**Table I. Biological and anthropometric characteristics, thyroid function, and dose of levothyroxine in the groups of women studied**

| Parameter                  | Premenopausal | Postmenopausal | RDA           |
|----------------------------|---------------|----------------|---------------|
|                            | Treated SCH   | Untreated SCH  | Treated SCH   | Untreated SCH |
|                            | (n = 45)      | (n = 58)       | (n = 180)     | (n = 180)     |
| Age [years]                | 43.62 ±6.65   | 44.77 ±6.82    | 59.51 ±7.90   | 58.75 ±7.92   |
| Age of menarche [years]    | 12.46 ±1.32   | 12.74 ±1.54    | 12.61 ±1.50   | 13.04 ±1.55   |
| Years since menopause      | 10.91 ±7.91   | 9.87 ±8.13     |               |               |
| BMI [kg/m²]                | 27.02 ±6.39   | 26.58 ±4.39    | 28.53 ±4.55   | 27.75 ±4.27   |
| Trunk lean mass [kg]       | 24.42 ±2.56   | 23.93 ±2.03    | 23.75 ±2.09   | 23.39 ±2.00   |
| Trunk fat mass [kg]        | 11.23 ±5.48   | 11.82 ±4.22    | 13.09 ±1.10   | 12.19 ±3.93   |
| Trunk fat %                | 30.14 ±8.64   | 31.93 ±7.07    | 34.69 ±6.63   | 33.48 ±6.54   |
| T4 dose/day [µg]           | 90.88 ±42.59  | 86.35 ±34.11   |               |               |
| TSH [mU/l]                 | 1.38 ±0.29    | 6.72 ±1.42*    | 1.19 ±0.17    | 8.8 ±1.7*     |
| FT₄ [ng/dl]                | 1.09 ±1.10    | 0.98 ±0.74     | 1.18 ±0.54    | 1.19 ±0.21    |
| Calories/day               | 2245.84 ±643.28 | 2076.56 ±410.82 | 2100.48 ±616.66 | 2172.07 ±580.24 | 2265 |
| Carbohydrates [g]          | 271.56 ±104.77 | 273.33 ±73.97  | 260.89 ±94.13 | 265.73 ±80.86 | 330 |
| Fat [g]                    | 86.58 ±30.59* | 71.51 ±19.50   | 74.99 ±30.93  | 80.12 ±32.49  | 90 |
| Protein [g]                | 93.15 ±38.64  | 83.34 ±18.31   | 91.28 ±31.85  | 95.86 ±36.77  | 47 |
| Protein/weight [g/kg]      | 1.44 ±0.65    | 1.27 ±0.34     | 1.36 ±0.55    | 1.48 ±0.61    | 0.8 |
| Calcium [mg/day]           | 1045.27 ±453.92 | 1049.40 ±461.59 | 1061.71 ±540.89 | 1128.47 ±471.17 | 800 |
| Phosphorus [mg/day]        | 1407.66 ±504.49 | 1338.88 ±356.17 | 1387.53 ±508.05 | 1487.48 ±571.46 | 800 |

BMI – Body mass index, RDA – recommended dietary allowances, *p < 0.01, compared with the respective untreated group.

In the group of postmenopausal women on L-T₄ replacement therapy, the QUS parameter Ad-SoS was negatively correlated with age (β = −4.245, p = 0.0003). The neck BMD and L2–L4 BMD were negatively correlated with years since menopause (YSM) (β = −0.006, p = 0.0012; β = −0.006, p = 0.0105, respectively) and positively correlated with BMI (β = 0.008, p < 0.0001; β = 0.008, p = 0.0016, respectively). In the untreated group, the Ad-SoS was negatively correlated with age (β = −3.857, p = 0.0106), and the neck BMD and L2–L4 BMD were negatively correlated with YSM (β = −0.005, p = 0.0191; β = −0.010, p = 0.0009, respectively) and positively correlated with BMI (β = 0.007, p < 0.0001; β = 0.006, p = 0.0369, respectively). No variable was associated with any pQCT measure in the studied groups.

In the group of postmenopausal women on L-T₄ replacement therapy, the QUS parameter Ad-SoS was negatively correlated with age (β = −4.245, p = 0.0003). The neck BMD and L2–L4 BMD were negatively correlated with years since menopause (YSM) (β = −0.006, p = 0.0012; β = −0.006, p = 0.0105, respectively) and positively correlated with BMI (β = 0.008, p < 0.0001; β = 0.008, p = 0.0016, respectively). In the untreated group, the Ad-SoS was negatively correlated with age (β = −3.857, p = 0.0106), and the neck BMD and L2–L4 BMD were negatively correlated with YSM (β = −0.005, p = 0.0191; β = −0.010, p = 0.0009, respectively) and positively correlated with BMI (β = 0.007, p < 0.0001; β = 0.006, p = 0.0369, respectively). No variable was associated with any pQCT measure in the studied groups.

Our study evaluated the effect of at least 6 months of L-T₄ replacement therapy on bone mass in women with SCH compared to control women of the same age with SCH and without treatment. To this end, we used three techniques to determine bone status, QUS, DXA and pQCT, with the purpose of evaluating cortical and trabecular bone.

We found no significant differences in the bone parameters between premenopausal women on L-T₄ replacement therapy and the untreated controls. The effect of L-T₄ treatment on BMD in premenopausal women is uncertain. Saggese et al. [23], who studied a group of thirteen adoles-
cent SCH girls with a median age of 13.4 years who were on long-term L-T4 therapy, evaluated L2-L4 BMD by DXA and found no adverse effect on BMD or bone turnover. Moreover, the attainment of peak bone mass was not impaired. Larijani et al. [24] studied a group of 50 premenopausal women receiving suppressive therapy with L-T4 for 1 year and found no increased risk of osteoporosis using DXA. Greenspan et al. [25] observed minimal changes in bone density in premenopausal women with physiological doses of L-T4. However, other studies showed a reduction of BMD in premenopausal women receiving long-term L-T4 therapy. Kung and Pun [16] studied 26 premenopausal women with Hashimoto’s thyroiditis and reported that the BMD of the spine was unaffected, but the BMD of the femoral neck was reduced. A similar effect was observed in women taking L-T4 suppressive doses [26], in which excess exogenous thyroxine might predominantly deplete skeletal sites, and the BMD might be affected, particularly at the femoral neck, which is rich in cortical bone. A meta-analysis in 1996 [27] resulted in the conclusion that replacement therapy with L-T4 was associated with bone loss in the spine and hip in premenopausal women but not in postmenopausal women, and this effect was more marked in cortical bone than in trabecular bone.

In our stepwise regression in premenopausal patients, the DXA variables were negatively correlated with age. Our group has previously reported the negative effect of age on bone in premenopausal women via ultrasound on the phalanx [28]. We also found a negative relationship between the parameters of pQCT (total density and cortical + subcortical density) and the age of menarche in the patients of this study, indicating that cortical density is affected by this biological variable. One study in 2008 [29] found that in young adult women, an age of menarche that was late but within the normal range was associated with a deficit in cortical density. This result is consistent with our results, because those authors used DXA and pQCT to measure bone parameters and suggested that the estrogen exposure from the onset of sexual maturation to the end of growth influences the peak bone mass achieved.

In postmenopausal women, replacement treatment with L-T4 has been associated with a small but significant reduction in the BMD of the spine and hip. This negative effect on bones seems more pronounced in the cortical bone than in the trabecular bone [18]. Hadji et al. [18] studied a group of 156 women treated with replacement doses of L-T4 and indirectly evaluated bone mass using QUS at the heel; they observed a slight reduction in the ultrasound values. In our study of 180 postmenopausal women treated with L-T4,
we observed no differences in the QUS measurements of the phalanx between the treated patients and untreated controls. La Vignera et al. [30], in a study of 99 postmenopausal women between 50 and 56 years of age and treated with L-T4 for 1 year, observed a slight but significant reduction in the BMD of the lumbar vertebrae measured by DXA, which was more pronounced in patients on suppressive treatment than in those who were not on this treatment and was associated with increased serum alkaline phosphatase levels and increased urinary excretion of hydroxyproline. However, other authors found no reduction in BMD in postmenopausal women with SCH who were on L-T4 treatment [31, 32], which is in agreement with our results at the lumbar spine and hip.

The stepwise regression showed similar results in patients and controls. In QUS, Ad-SoS decreased with age in both patients and controls. The decrease in bone mass with age in postmenopausal women is widely documented [28, 33], but it seems that YSM is a more important predictor of bone loss than chronological age [34, 35]. This conclusion agrees with our results, because the DXA parameters correlated negatively with YSM in all women. The BMI also showed a positive relationship with the DXA parameters. Body mass index is often considered a positive correlate of BMD, but the link between BMI and BMD has not yet been clarified. The possible mechanistic explanations for the relationship between these physiological parameters include the actions of glucocorticoids, growth and sex hormones, leptin, and inflammatory adipokines [36].

With regard to the anthropometric parameters in patients with SCH, replacement therapy with L-T4 not only improves the lipid profile but also decreases the BMI [37]. Our study confirms this effect, because there were no significant differences in the BMI or in the lipid profile (data not shown) between treated and untreated women in the premenopausal or postmenopausal groups.

Few studies have used all three of these important techniques to assess bone mass, and none of them was related to treatment with L-T4. Moreover, research on the effects of long-term L-T4 treatment has been conducted mainly on patients taking suppressive doses of L-T4. We consider these facts and the large number of participants in the current study to be the main strengths of our work.

In conclusion, our results indicate the absence of adverse effects due to L-T4 replacement therapy in the QUS of the phalanges, BMD of either the spine or the hip, and the pQCT in the non-dominant distal radius in SCH-treated women.

**Conflict of interest**

The authors declare no conflict of interest.

**References**

1. Tarraga Lopez PJ, Lopez CE, de Mora FN, et al. Osteoporosis in patients with subclinical hypothyroidism treated with thyroid hormone. Clin Cases Miner Bone Metab 2011; 8: 44-8.
2. Prats JM. Effect of treatment with levothyroxine in the lipid profile of the patients with subclinical hypothyroidism. Endocrinol Nutr 2009; 56: 13-7.
3. Khandelwal D, Tandon N. Overt and subclinical hypothyroidism: who to treat and how. Drugs 2012; 72: 17-33.
4. Hollowell JG, Staehling NW, Flanders WD, et al. Serum TSH, T(4), and thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III). J Clin Endocrinol Metab 2002; 87: 489-99.
5. Surks MI, Ortiz E, Daniels GH, et al. Subclinical thyroid disease: scientific review and guidelines for diagnosis and management. JAMA 2004; 291: 228-38.
6. Bassett JH, Williams GR. The molecular actions of thyroid hormone in bone. Trends Endocrinol Metab 2003; 14: 356-64.
7. Kanatani M, Sugimoto T, Sowa H, Kobayashi T, Kanzawa M, Chihara K. Thyroid hormone stimulates osteoclast differentiation by a mechanism independent of RANKL-RANK interaction. J Cell Physiol 2004; 201: 17-25.
8. Britto JM, Fenton AI, Holloway WR, Nicholson GC. Osteoblasts mediate thyroid hormone stimulation of osteoclastic bone resorption. Endocrinology 1994; 134: 169-76.
9. Murphy E, Gluer CC, Reid DM, et al. Thyroid function within the upper normal range is associated with reduced bone mineral density and an increased risk of nonvertebral fractures in healthy euthyroid postmenopausal women. J Clin Endocrinol Metab 2010; 95: 3173-81.
10. Abe E, Marians RC, Yu W, et al. TSH is a negative regulator of skeletal remodeling. Cell 2003; 115: 151-62.
11. Ma R, Morshed S, Latif R, Zaidi M, Davies TF. The influence of thyroid-stimulating hormone and thyroid-stimulating hormone receptor antibodies on osteoelastogenesis. Thyroid 2011; 21: 897-906.
12. Baqi L, Payer J, Killinger Z, et al. The level of TSH appeared favourable in maintaining bone mineral density in postmenopausal women. Endocr Regul 2010; 44: 9-15.
13. Morris MS. The association between serum thyroid-stimulating hormone in its reference range and bone status in postmenopausal American women. Bone 2007; 40: 1128-34.
14. Nagata M, Suzuki A, Seguchi S, et al. Subclinical hypothyroidism is related to lower heel QUS in postmenopausal women. Endocr J 2007; 54: 625-30.
15. Aghili R, Khamseh ME, Malek M, et al. Changes of subtests of Wechsler Memory Scale and cognitive function in subjects with subclinical hypothyroidism following treatment with levothyroxine. Arch Med Sci 2012; 8: 1096-101.
16. Kung AW, Pun KK. Bone mineral density in premenopausal women receiving long-term physiological doses of levothyroxine. JAMA 1999; 285: 2688-91.
17. Schneider DL, Barrett-Connor EL, Morton DJ. Thyroid hormone use and bone mineral density in elderly women. Effects of estrogen. JAMA 1994; 271: 1245-9.
18. Hadji P, Hars O, Sturm G, Bauer T, Emons G, Schulz KD. The effect of long-term, non-suppressive levothyroxine treatment on quantitative ultrasonometry of bone in women. Eur J Endocrinol 2000; 142: 445-50.
19. Franklyn JA, Betteridge J, Daykin J, et al. Long-term thyroxine treatment and bone mineral density. Lancet 1992; 340: 9-13.
20. Pedrera-Zamorano JD, Lavado-García JM, Roncero-Martin R, Calderón-García JE, Rodríguez-Dominguez T, Canal-Macias ML. Effect of beer drinking on ultrasound bone mass in women. Nutrition 2009; 25: 1057-63.
21. Canal-Macias ML, Roncero-Martin R, Moran JM, Lavado-García JM, Costa-Fernandez MC, Pedrera-Zamorano JD. Increased bone mineral density is associated with breastfeeding history in premenopausal Spanish women. Arch Med Sci 2013; 30: 703-8.
22. Pedrera JD, Canal ML, Postigo S, Lavado J, Hernández ER, Rico H. Phalangeal bone ultrasound and its possible correlation with nutrient in an area of high protein intake. Ann Nutr Metab 2001; 45: 86-90.
23. Saggese G, Bertelloni S, Barocelli GI, Costa S, Ceccharelli C. Bone mineral density in adolescent females treated with L-thyroxine: a longitudinal study. Eur J Pediatr 1996; 155: 452-7.
24. Larijani B, Gharibdoost F, Pajouhi M, et al. Effects of levothyroxine suppressive therapy on bone mineral density in premenopausal women. J Clin Pharm Ther 2004; 29: 1-5.
25. Greenspan SL, Greenspan FS, Resnick NM, Block JE, Friedlander AL, Genant HK. Skeletal integrity in premenopausal and postmenopausal women receiving long-term L-thyroxine therapy. Am J Med 1991; 91: 5-14.
26. Garton M, Reid I, Loveridge N, et al. Bone mineral density and metabolism in premenopausal women taking L-thyroxine replacement therapy. Clin Endocrinol (Oxf) 1994; 41: 747-55.
27. Uzzan B, Campos J, Cucherat M, Nony P, Boissel JP, Perret GY. Effects on bone mass of long term treatment with thyroid hormones: a meta-analysis. J Clin Endocrinol Metab 1996; 81: 4278-89.
28. Pedrera Zamorano JD, Canal Macias ML, Lavado Garcia JM, Costa FC, Borrella DS, Rico LH. Reference curve of bone ultrasound measurements in proximal phalanges in normal Spanish women. J Clin Densitom 2003; 6: 373-80.
29. La Vignera S, Vicari E, Tumino S, et al. L-thyroxin treatment and post-menopausal osteoporosis: relevance of the risk profile present in clinical history. Minerva Ginecol 2008; 60: 475-84.
30. Ross DS. Bone density is not reduced during the short-term administration of levothyroxine to postmenopausal women with subclinical hypothyroidism: a randomized, prospective study. Am J Med 1993; 95: 385-8.
31. Ross DS. Hyperthyroidism, thyroid hormone therapy, and bone. Thyroid 1994; 4: 319-26.
32. Bączyk G, Opala T, Kleka P, Chuchracki M. Multifactorial analysis of risk factors for reduced bone mineral density among postmenopausal women. Arch Med Sci 2012; 8: 332-41.
33. Akdeniz N, Akpolat V, Kale A, Erdemoglu M, Kuyumcuglu U, Celik Y. Risk factors for postmenopausal osteoporosis: anthropometric measurements, age, age at menopause and the time elapsed after menopause onset. Gynecol Endocrinol 2009; 25: 125-9.