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

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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-

alence of this condition increases with age and is greater in women than in men [4]. Subclinical hypothyroidism is defined as a serum concentration of thyroid-stimulating hormone (TSH) above the upper limit of the reference range when the concentration of serum-free T4 (FT4) is within the reference range [5].

Thyroid hormones are key regulators of bone formation and remodeling. Thyroid hormone T3 stimulates osteoblast activity both directly and indirectly through numerous growth factors and cytokines [6]. It also directly stimulates osteoclast differentiation [7], although it is unclear whether T3 can act on osteoblasts to indirectly stimulate osteoclastic bone resorption [8].

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-T$_4$ 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-T$_4$) on bones has produced controversial results. Some studies found that treatment with replacement doses of L-T$_4$ 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-T$_4$ 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-T$_4$ 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 mU/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 Caceres, 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-T$_4$. All women led active lives but did not regularly practice sports. Alcohol intake was sporadic, not exceeding

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100 ml/day in any case. Only 5% of the women smoked, and not more than 10 cigarettes per day. Height was measured using a Harpenden stadiometer with a mandible plane parallel to the floor, and weight was measured using a biomedical precision balance. Both measurements were determined with the subjects wearing only light clothing and no shoes. The body mass index (BMI) was calculated as weight in kilograms divided by the square of the height in meters (BMI (kg/m^2)).

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 (cm^2).

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-T$_4$ replacement therapy, the DXA measurements of the neck BMD and L2–L4 BMD were negatively correlated with age (β = –0.009, $p = 0.0136$;

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

Parameter	Premenopausal		Postmenopausal		RDA
	Treated SCH (*n* = 45)	Untreated SCH (*n* = 58)	Treated SCH (*n* = 180)	Untreated SCH (*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/m^2]	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*	
FT$_4$ [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 ±450.89	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.

β = –0.007, *p* = 0.0432, respectively). In the pQCT experiments, the total density and cortical + subcortical density were negatively correlated with age of menarche (β = –19.944, *p* = 0.0113; β = –32.165, *p* = 0.0116, respectively). No variable showed an association with bone measurements in the untreated SCH group. No variable showed an association with Ad-SoS in the studied groups.

In the group of postmenopausal women on L-T$_4$ 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.

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.

Discussion

Our study evaluated the effect of at least 6 months of L-T$_4$ 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-T$_4$ replacement therapy and the untreated controls. The effect of L-T$_4$ treatment on BMD in premenopausal women is uncertain. Saggese *et al.* [23], who studied a group of thirteen adoles-

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Table II. The results of the bone status analysis

Parameter	Premenopausal				Postmenopausal			
	Untreated SCH (n = 58)	Treated SCH (n = 45)	Value of p	Mean difference (confidence interval 95%)	Untreated SCH (n = 180)	Treated SCH (n = 180)	Value of p	Mean difference (confidence interval 95%)
Phalanx Ad-SoS [m/s]	2123.42 ±47.23	2117.52 ±62.05	0.602	5.90 (−16.47 to 28.28)	2039.21 ±73.41	2024.23 ±71.01	0.068	14.97 (−1.09 to 31.04)
Femoral neck BMD [g/cm²]	0.906 ±0.133	0.921 ±0.147	0.603	−0.01 (−0.069 to 0.404)	0.804 ±0.115	0.805 ±0.116	0.904	−0.001 (−0.254 to 0.2250)
L2–L4 BMD [g/cm²]	1.058 ±0.137	1.095 ±0.150	0.195	−0.037 (−0.094 to 0.019)	0.926 ±0.157	0.964 ±0.156	0.021*	−0.038 (−0.07 to −0.005)
Total radius density [mg/cm³]	360.16 ±49.63	381.76 ±59.63	0.101	−21.60 (−47.51 to 4.30)	345.18 ±42.27	332.70 ±48.22	0.138	12.47 (−4.06 to 29.01)
Trabecular radius density [mg/cm³]	187.47 ±34.05	191.46 ±31.84	0.621	−3.99 (−20.04 to 12.06)	187.21 ±37.38	175.92 ±32.95	0.09	11.29 (−1.78 to 24.37)
Cort + Sub radius density [mg/cm³]	501.13 ±71.66	537.50 ±90.48	0.063	−36.36 (−74.76 to 2.03)	474.07 ±58.09	460.63 ±71.43	0.262	13.43 (−10.18 to 37.06)

Ad-SoS – Amplitude-dependent speed of sound, BMD – bone mineral density, Cort + – Sub Cortical + subcortical, *p = 0.0214 compared with untreated postmenopausal group.

cent SCH girls with a median age of 13.4 years who were on long-term L-T$_4$ 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 reciving suppressive therapy with L-T$_4$ 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-T$_4$. However, other studies showed a reduction of BMD in premenopausal women receiving long-term L-T$_4$ 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-T$_4$ 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-T$_4$ 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-T$_4$ 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-T$_4$ 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-T$_4$,

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-T$_4$ 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-T$_4$ 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-T$_4$ 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-T$_4$. Moreover, research on the effects of long-term L-T$_4$ treatment has been conducted mainly on patients taking suppressive doses of L-T$_4$. 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-T$_4$ 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.

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