Bone 161 (2022) 116404

Contents lists available at ScienceDirect

Bone

journal homepage: www.elsevier.com/locate/bone

Full Length Article

Bone geometry in older adults with subclinical hypothyroidism upon levothyroxine therapy: A nested study within a randomized placebo controlled trial

Annina Elisabeth Büchi [a,b], Martin Feller [a,b], Seraina Netzer [a,b], Manuel R. Blum [a,b], Elena Gonzalez Rodriguez [c], Tinh-Hai Collet [d], Cinzia Del Giovane [b], Diana van Heemst [e], Terry Quinn [f], Patricia M. Kearney [g], Rudi G.J. Westendorp [h], Jacobijn Gussekloo [f,i], Simon P. Mooijaart [e], Didier Hans [c], Douglas C. Bauer [j,k,l], Nicolas Rodondi [a,b], Daniel Aeberli [m,*]

[a] Department of General Internal Medicine, Inselspital, Bern University Hospital, University of Bern, Bern, Switzerland
[b] Institute of Primary Health Care (BIHAM), University of Bern, Bern, Switzerland
[c] Interdisciplinary Center of Bone Diseases, Rheumatology Unit, Bone and Joint Department, Lausanne University Hospital and University of Lausanne, Lausanne, Switzerland
[d] Service of Endocrinology, Diabetology, Nutrition and Therapeutic Education, Geneva University Hospitals, Geneva, Switzerland
[e] Department of Internal Medicine, section Gerontology and Geriatrics, Leiden University Medical Center, Leiden, the Netherlands
[f] Institute of Cardiovascular Medicine, University of Glasgow, Glasgow, Scotland, United Kingdom
[g] School of Public Health, University College Cork, Cork, Ireland
[h] Department of Public Health and Center for Healthy Aging, University of Copenhagen, Copenhagen, Denmark
[i] Department of Public Health and Primary Care, Leiden University Medical Center, Leiden, Netherlands
[j] Department of Medicine, University of California, San Francisco, USA
[k] Department of Epidemiology, University of California, San Francisco, USA
[l] Department of Biostatistics, University of California, San Francisco, USA
[m] Department of Rheumatology and Immunology, Inselspital, Bern University Hospital, Bern, Switzerland

ARTICLE INFO

Keywords:
Levothyroxine
Subclinical hypothyoroidism
Peripheral quantitative computational tomography
Bone geometry
Volumetric bone density

ABSTRACT

The effect of levothyroxine (LT4) therapy for subclinical hypothyroidism (SHypo) on appendicular bone geometry and volumetric density has so far not been studied. In a nested study within the randomized, placebo-controlled Thyroid Hormone Replacement for Subclinical Hypothyroidism (TRUST) trial, we assessed the effect of LT4 therapy on bone geometry as measured by peripheral quantitative computed tomography (pQCT). In the TRUST trial, community-dwelling adults aged ≥65 years with SHypo were randomized to LT4 with dose titration vs. placebo with mock titration. We analyzed data from participants enrolled at the TRUST site in Bern, Switzerland who had bone pQCT measured at baseline and at 1 to 2 years follow-up. The primary outcomes were the annual percentage changes of radius and tibia epi- and diaphysis bone geometry (total and cortical cross-sectional area (CSA) and cortical thickness), and of volumetric bone mineral density (bone mineral content (BMC) and total, trabecular and cortical volumetric bone mineral density (vBMD)). We performed linear regression of the annual percentage changes adjusted for sex, LT4 dose at randomization and muscle cross-sectional area. The 98 included participants had a mean age of 73.9 (±SD 5.4) years, 45.9% were women, and 12% had osteoporosis. They were randomized to placebo ($n = 48$) or LT4 ($n = 50$). Annual changes in BMC and vBMD were similar between placebo and LT4-treated groups, without significant difference in bone geometry or volumetric bone mineral density changes, neither at the diaphysis, nor at the epiphysis. For example, in the placebo group, epiphyseal BMC (radius) decreased by a mean 0.2% per year, with a similar decrease of 0.5% per year in the LT4 group (between-group difference in %ΔBMC 0.3, 95% CI –0.70 to 1.21, $p = 0.91$).

Abbreviations: SHypo, Subclinical hypothyroidism; TSH, Thyrotropin; FT4, Thyroxine; DXA, Dual energy X-ray absorptiometry; pQCT, Peripheral quantitative computed tomography; vBMD, Volumetric bone mineral density; TRUST, Thyroid hormone replacement for untreated older adults with subclinical hypothyroidism; BMC, Bone mineral content; CSA, Cross-sectional area; TBS, Trabecular bone score.

* Corresponding author at: Department of Rheumatology and Immunology, Inselspital, Freiburgstr. 15, Bern CH-3015, Switzerland.
E-mail address: daniel.aeberli@insel.ch (D. Aeberli).

https://doi.org/10.1016/j.bone.2022.116404
Received 27 January 2022; Received in revised form 16 March 2022; Accepted 31 March 2022
Available online 2 April 2022

Compared to placebo, LT4 therapy for an average 14 months had no significant effect on bone mass, bone geometry and volumetric density in older adults with subclinical hypothyroidism.
Trial registration: The trial was registered on ClinicalTrials.gov numbers NCT01660126 (TRUST Thyroid trial) and NCT02491008 (Skeletal outcomes).

1. Introduction

Subclinical hypothyroidism (SHypo) is defined as an elevated thyrotropin (TSH) level with free thyroxine (FT4) within the reference range [1]. Its prevalence increases with age, reaching >10% in men over 65 years and up to 21% in women over 75 years [1,2]. Even though levothyroxine (LT4) replacement therapy of SHypo with TSH <10.0 mIU/L is controversial particularly in older adults [3,4], many patients receive LT4, making it among the most prescribed drugs in the USA [5].

High thyroid hormone levels due to subclinical and overt hyperthyroidism (or overuse/overdosing of LT4) are associated with increased fracture risk [6–8], and the association may be stronger for FT4 than for TSH [6,9–11]. Thyroid hormones stimulate bone turnover by acting directly and indirectly on osteoclasts and osteoblasts, leading to bone mineral loss [12–15]. A direct effect of TSH itself has also been postulated [11,16]. In earlier small trials, either no effect, or a non-significant deleterious effect of LT4 on bone was observed [17–19]. TRUST (Thyroid Hormone Replacement for Untreated Older Adults with Subclinical Hypothyroidism) was a multicenter, international, double-blind parallel-group randomized controlled trial (RCT) of LT4 versus placebo [20]. In a recently published analysis of 196 TRUST patients, LT4 did not lead to lower areal bone mineral density measured by dual energy X-ray absorptiometry (DXA) [21]. However this analysis did not examine the individual bone compartments or the effects of weight-bearing on bone geometry and volumetric density.

Peripheral Quantitative Computed Tomography (pQCT) is a three-dimensional measuring technique that allows the assessment of cross-sectional bone geometry and volumetric bone mineral density (vBMD) independent of bone size. This is in contrast to two-dimensional methods like DXA [22]. Compared with DXA, assessment of bone geometry by pQCT allows accurate determination of the specific effect of LT4 on cortical and trabecular bone, the volumetric density of these compartments, and changes in internal and external bone circumference. So far, only little is known about the effect of TSH on bone geometry and vBMD: TSH-Suppressive LT4-Treatment in patients with thyroidectomy may leads to detrimental effects on appendicular bone [23], but long-term LT4-treatment within physiological limits seems to only minimally influence areal bone density [24]. High TSH levels have been shown to increase the risk for fractures [9] and to decrease cortical [10] rather than trabecular bone, and may impact bone quality. Data on vBMD and bone geometry are very limited, as most studies present either no information on bone density, or only use two-dimensional methods such as DXA. Of interest, a selective negative bone effect on radius but not tibia was stated by continuously elevated PTH with alteration of geometry, volumetric density, and both trabecular and cortical microarchitecture [25]. This deterioration of microarchitecture bone density and microarchitecture at peripheral sites was found to be a predictor of fracture risk in postmenopausal women [26]. To date, no such study has explored the impact of subclinical hypothyroidism and the effect of LT4 on appendicular bone geometry and volumetric density. Thus, our objectives were to examine the effects of LT4 in hypothyroid patients on radius and tibia and in particular on geometry and volumetric density, which might offer new insights on bone protective effect in architecture and resulting fracture risk.

2. Methods

2.1. Study population

This nested study focused on the skeletal outcomes of TRUST participants (clinicaltrials.gov NCT02491008, NCT01660126) who had pQCT measurements [20]. Community-dwelling individuals aged ≥65 years with persistent SHypo were included, as detailed before [20]. In short, persistent SHypo was diagnosed by elevated TSH levels (≥4.6 and ≤ 19.9 mIU/L) at two measurements at least 3 months apart, and FT4 levels within the assay reference range. Exclusion criteria were prior use of LT4, antithyroid, amiodarone or lithium treatment within 12 months before enrollment, thyroid surgery or radio-iodine, severe acute comorbidities, dementia, terminal illness or galactose intolerance [20]. For this study, we included participants from Bern, Switzerland, of the TRUST trial who had pQCT measurements at baseline and follow-up at 1 or 2 years. The pQCT measurements were started later after funding of this nested study (Fig. 1).

The trial was approved by the local ethics committee, and written informed consent was obtained from all participants. The trial was conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice guidelines.

2.2. Intervention

As previously described [20], participants were randomized 1:1 to placebo or LT4, with stratification according to country, sex and starting dose of LT4 using randomly permuted blocks. Treatment started with 50 μg daily (25 μg in individuals <50 kg body weight or with known coronary heart disease) of LT4 or the corresponding placebo. The dose was titrated according to target TSH levels ≥0.4 and <4.6 mIU/L in the LT4 arm, and a computerized mock titration was used in the placebo arm to ensure blinding. The intervention resulted in a significant reduction in TSH levels after 6–8 weeks and after 12 months ($p < 0.001$), resulting in normalization in the levothyroxine group (mean 3.63 mIU/l, SD 2.11 mIU/l) compared to placebo (5.48 mIU/l, SD 2.48 mIU/l) at 12 months. The average dose of levothyroxine at 1 year was 50 μg.

2.3. Clinical data

Baseline age, sex, current smoking and alcohol consumption, history of osteoporosis and diabetes mellitus, and all ongoing treatments were recorded. Bone-affecting treatments were classified in two categories [21]. Beneficial treatments included anti-osteoporotics (raloxifene, bisphosphonates, denosumab or teriparatide), hormonal replacement therapy, and hydrochlorothiazide. Treatments with known deleterious bone effects included systemic or topical glucocorticoids, proton pump inhibitors, aromatase inhibitors, serotonin recapture inhibitors, and antiepileptic treatments.

2.4. pQCT measurements

pQCT measurements were performed using a Stratec XCT 3000 scanner following the manufacturer's instructions and software (XCT

6.00 B, Stratec Medizintechnik, Pforzheim, Germany) [22]. This was calibrated with respect to water and the manufacturer's phantom, which is calibrated to the European Forearm Phantom (Erlangen, Germany). The measurements of the radius were performed on the non-dominant side, the measurements on the tibia on the contralateral leg. For radial measurements, the ulnar length was measured to the nearest 5 mm with a measuring tape from the olecranon to the ulnar styloid; the radial length was set equal to the measured ulnar length. Tibial length was measured from the medial knee joint cleft to the end of the medial malleolus. A scout view was acquired by the pQCT machine and the automated detection algorithm was used to place the reference line at the distal bone end. According to the manufacturer's recommendations, measurements were performed at 4% (epiphyseal) and 66% (diaphyseal) of the bone's total length measured from the reference line. Slice thickness was 2.2 mm, voxel size was set as 0.5 mm with a scanning speed of 20 mm/s.

2.4.1. Epiphyseal scans

A contour algorithm using a threshold of 180 mg/cm^3 (contour mode 1 and peel mode 1) traced the periosteal surface of the epiphysis of each bone. Bone mineral content (BMC) per cm slice thickness, total cross-sectional area (CSA) and total volumetric bone mineral density (vBMD) were determined. Concentric pixel layers where then digitally peeled off until a central area covering 45% of the total bone CSA was left, from this central area, trabecular BMD was determined.

2.4.2. Diaphyseal scans

The threshold was set to 280 mg/cm^3, and BMC and total CSA were calculated as described above. For cortical bone, the threshold was 710 mg/cm^3 (contour and peel mode 1), so that cortical CSA and BMD could be calculated. Cortical thickness was calculated on the assumption that the bone shaft is cylindrical from total CSA (including bone marrow and cortical CSA). Muscle CSA was determined by selecting an area with upper and lower thresholds of 280 mg/cm^3 and 40 mg/cm^3 respectively with contour mode 3 and peel mode 1. For subtracting the bone area, contour mode 1 and peel mode 2 were used.

2.5. Outcomes

Defined outcomes in bone parameters at the radius and tibia (epi- and diaphysis) were yearly changes (in percentage) of bone mineral content (BMC), total and cortical cross-sectional area (CSA), volumetric bone mineral density (vBMD) (total, trabecular and cortical) and cortical thickness.

2.6. Statistical analyses

For categorical variables, we calculated the number of participants and percentage, and for continuous variables we calculated the mean and standard deviation (SD). We assumed the baseline values of bone geometry (i.e. CSA) to be 100%. We then expressed the follow-up values

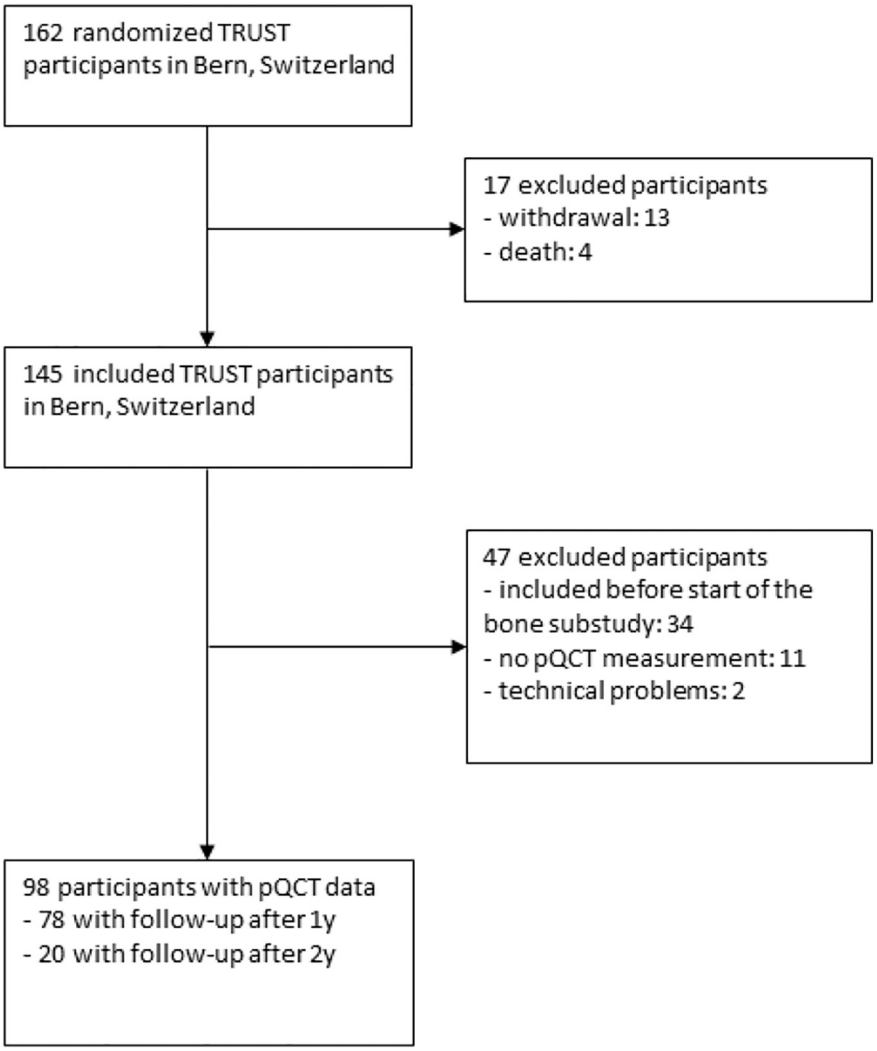

Fig. 1. Study flow chart.

as percentage of baseline for each participant, and calculated the absolute difference in % (follow-up – baseline). We then divided this value by the follow-up duration expressed in years (as 78 participants had follow-up pQCT assessment after 1 year, 20 after 2 years). This resulted in yearly changes expressed as percentage of baseline (i.e. %ΔBMC, %Δ totalCSA, %ΔtotalBMD, %ΔtrabBMD). Between-group comparisons (placebo – LT4) on the change between follow-up and baseline values and significance (*p*-values) were calculated using linear regression, adjusted for sex, starting dose of LT4 and muscle CSA at site of measurement (muscle mass at site of measurement influences bone density). In addition, we further adjusted the linear regression model for BMI, age and deleterious medications (i.e., proton pump inhibitors, systemic or topic glucocorticoids, aromatase inhibitors, serotonin recapture inhibitors, antiepileptic treatments) in a secondary analysis.

47 participants per group were needed to obtain 80% power, at a least significant difference (LSD) for the tibia of 0.7–1.7% (coefficient of variability) and 0.8–3.7% for the radius. Statistical significance was considered for p-values <0.05. All analyses were performed using Stata version 16 (StataCorp, College Station, TX).

3. Results

In total 98 participants had baseline and follow-up pQCT measurements (Fig. 1); 78 participants had pQCT after 1 year, 20 participants after 2 years of follow-up, due to the early closure of the study. The mean age at inclusion was 74.0 (SD 5.4) years, 45.9% were women (Table 1). 48 patients were included in the placebo group, 50 in the treatment group. There were no significant differences between groups except for sex and lower leg CSA. There were 19 (39.6%) women in the placebo, and 26 (52%) women in the LT4 group; the mean lower leg CSA was 7189 mm^2 in the placebo and 6555 mm^2 in the LT4 group. The pQCT measurements were similar between the placebo and LT4 groups at baseline (Table 2).

Table 1
Baseline characteristics of included participants, by treatment group.

	Placebo	Levothyroxine
Sample size	48	50
Female	19 (39.6)	26 (52.0)
Age (years)	74.0 ± 5.8	74.1 ± 4.9
Weight (kg)	76.5 ± 15.8	75.9 ± 15.2
Height (cm)	166.8 ± 8.1	166.4 ± 9.7
BMI (kg/m^2)	27.4 ± 4.8	27.4 ± 5.0
Current smoking	6 (12.5)	2 (4.0)
Excess alcohol consumption	2 (4.2)	2 (4.0)
TSH (mlU/L)		
Baseline	6.1 ± 1.5	6.3 ± 2.0
Median (IQR)	5.5 (5.2–6.8)	5.7 (5.1–6.8)
Range	4.6–11.8	4.6–16.8
Free T4 (pmol/L)	14.3 ± 1.7	14.0 ± 2.1
Osteoporosis history	6 (12.0)	6 (12.0)
Diabetes history	5 (10.0)	6 (12.0)
Calcium supplemented	1 (2.0)	2 (4.0)
Vitamin D treatment	6 (12.0)	11 (22.0)
Bone affecting medication		
Anti-osteoporotic or HRT	1 (2.0)	0 (0.0)
HCTZ	0 (0.0)	0 (0.0)
Systemic GC	0 (0.0)	0 (0.0)
Potentially deleterious	5 (10.0)	1 (2.0)
Forearm muscle CSA (cm^2)	3325 ± 934	2982 ± 841
Lower leg muscle CSA (cm^2)	7189 ± 1480	6555 ± 1322

Results are expressed as mean ± standard deviation for continuous variables and as number of participants (percentage) for categorical variables. LT4: Levothyroxine. BMI: body mass index. Excess alcohol consumption: more than 2 units per day. Anti-osteoporotic treatments: raloxifen, bisphosphonates, denosumab or teriparatide. HRT: hormonal replacement therapy. HCTZ: hydrochlorothiazide. GC: glucocorticoids. Deleterious: proton pump inhibitors, systemic or topic glucocorticoids, aromatase inhibitors, serotonin recapture inhibitors, antiepileptic treatments. CSA: cross-sectional area.

Specific pQCT changes between baseline and follow-up are shown in Table 2. There was a trend to significance in cortical BMD ($p = 0.06$), but no significant differences could be shown between placebo and LT4 groups in any measurement. Unadjusted analyses were similar (data not shown). Further adjustments for age, BMI and deleterious medication showed no significant differences between groups (Appendix Table 1).

4. Discussion

Our RCT did not show any effect of LT4 on bone geometry or vBMD, as assessed by pQCT in community-dwelling adults aged ≥65 with SHypo. Our study is the largest randomized controlled trial for SHypo treatment to date that included serial pQCT measurements. No other study has analyzed SHypo treatment effect on epi- and diaphyseal bone geometry of radius and tibia.

Our results are concordant with previous studies by DXA measurements where no effect on bone density or trabecular bone score (TBS) was found [17,18,21]. Meta-analyses on the association between SHypo and fracture risk [6,27] or BMD [27,28] showed no difference in observed results, but did not analyze outcomes specifically for treated Shypo. Only the double-blinded randomized controlled trial by Meier et al. reported a statistically but not clinically significant excess BMD loss of 1.3% (95% CI -2.9 to 0.5) [19] at the lumbar spine with treatment compared to placebo in 66 women.

For the assessment of bone geometry using pQCT, a negative change (placebo – LT4) for %ΔBMD, %Δ cortical thickness, %Δ cortical CSA and %ΔBMC would indicate protection by LT4, a negative change of %Δ total CSA total would indicate a deleterious effect of LT4 on bone. Increased total CSA due to periosteal apposition is regarded as compensation of cortical thinning, since cortical thinning reduces bone strength and leads to lower bending and torsional strength, and thus lower bone stability [22]. Data on vBMD and bone geometry changed by thyroid hormone status are scarce. One study found no effect of LT4 in thyroidectomy patients on bone density and geometry [29], another study found no effect of suppressive LT4 in young patients with thyroid carcinoma [30]. On the other hand, one study found an association of higher levels of thyroid hormones and less favourable volumetric bone density and geometry in healthy males [31], while Tournis et al. found adverse effects on trabecular and cortical bone [32], especially in non-weight bearing sites (such as the radius) in postmenopausal women in TSH-suppressive LT4 therapy in patients with thyroid carcinoma. Nicolaisen et al. found compromised microarchitecture of radius and tibia in hyperthyroid women [33], which was improved after restoration of euthyroidism, and similar improvements in bone geometry were observed for hypothyroid women when restored to euthyroidism [34].

As those results are conflicting and some studies only analyzed a very small number of patients, our results add valuable information. They are in line with the majority of studies that found no effect on bone density by LT4 in SHypo [17,18,21].

Besides its double-blind RCT design, a strength of our study is the adequate sample size to detect even small relevant changes in bone geometry, as indicated by narrow CI, which exclude clinically meaningful differences. As we calculated yearly changes for each patient, we were able to include a sufficient number of participants in each group as specified in the power calculation, achieving 48 patients (placebo) and

Table 2
Baseline, follow-up and yearly change in pQCT measurements by groups.

	Placebo Mean (SD) (n = 48)	LT4 Mean (SD) (n = 50)	Adjusted between-group difference^ (95% CI)	p-Value
Radius at 4% (epiphysis)				
BMC				
Baseline (g/cm)	1.4 (0.4)	1.2 (0.4)		
Follow-up (g/cm)	1.4 (0.4)	1.2 (0.4)		
Yearly change (%)	−0.2 (2.3)	−0.5 (2.5)	0.2 (−4.3; 3.8)	0.91
Total CSA				
Baseline (g/cm)	400.2 (80.9)	378.9 (80.2)		
Follow-up (g/cm)	403.5 (78.9)	380.1 (80.2)		
Yearly change (%)	0.5 (4.2)	0.3 (4.8)	2.3 (−5.4; 10.0)	0.56
Total BMD				
Baseline (g/cm)	341.4 (61.5)	324.9 (69.7)		
Follow-up (g/cm)	339.4 (66.1)	322.5 (69.3)		
Yearly change (%)	−0.6 (3.2)	−0.7 (3.8)	2.5 (−8.4; 3.5)	0.41
Trabecular BMD				
Baseline (g/cm)	201.7 (43.2)	189.1 (55.8)		
Follow-up (g/cm)	202.6 (42.6)	189.1 (55.5)		
Yearly change (%)	0.4 (2.7)	−0.1 (3.1)	2.7 (−2.2; 7.6)	0.28
Radius at 66% (diaphysis)				
BMC				
Baseline (mm^2)	1.0 (0.3)	1.0 (0.3)		
Follow-up (mm^2)	1.1 (0.3)	1.0 (0.3)		
Yearly change (%)	−0.6 (1.6)	−0.9 (2.1)	0.1 (−3.1; 3.2)	0.97
Total CSA				
Baseline (mm^2)	160.3 (36.0)	159.2 (41.1)		
Follow-up (mm^2)	160.2 (35.8)	159.6 (41.8)		
Yearly change (%)	−0.0 (2.4)	0.2 (3.2)	−2.1 (−1.7; 5.9)	0.27
Cortical CSA				
Baseline (mg/cm^3)	82.6 (22.1)	76.5 (23.2)		
Follow-up (mg/cm^3)	81.9 (22.1)	75.8 (24.0)		
Yearly change (%)	−1.0 (2.4)	−1.2 (3.8)	2.5 (−6.6; 1.7)	0.24
Cortical BMD				
Baseline (mg/cm^3)	1102.4 (56.0)	1097.5 (60.8)		
Follow-up (mg/cm^3)	1102.8 (55.5)	1093.6 (59.9)		
Yearly change (%)	0.0 (0.7)	−0.3 (1.1)	1.4 (−0.1; 2.9)	0.06
Cortical thickness				
Baseline (mm)	3.8 (0.7)	3.8 (0.7)		
Follow-up (mm)	2.2 (0.5)	2.0 (0.6)		
Yearly change (%)	−1.2 (2.5)	−1.3 (3.7)	4.2 (−9.4; 1.1)	0.12
Tibia at 4% (epiphysis)				
BMC				
Baseline (g/cm)	3.5 (0.8)	3.4 (0.9)		
Follow-up (g/cm)	3.5 (0.8)	3.3 (0.9)		
Yearly change (%)	−0.2 (1.7)	−0.9 (1.9)	0.0 (−3.1; 3.0)	0.99
Total CSA				
Baseline (g/cm)	1210.5 (221.6)	1204.3 (170.5)		
Follow-up (g/cm)	1201.9 (175.6)	1203.9 (222.3)		
Yearly change (%)	−0.4 (2.8)	−0.5 (2.2)	1.1 (−3.2; 5.4)	0.62
Total BMD				
Baseline (g/cm)	292.0 (53.8)	276.7 (49.2)		
Follow-up (g/cm)	293.0 (55.0)	275.7 (49.9)		
Yearly change (%)	0.3 (2.1)	−0.3 (1.6)	1.1 (−4.3; 2.0)	0.47
Trabecular BMD				
Baseline (g/cm)	221.7 (41.6)	213.0 (44.2)		
Follow-up (g/cm)	221.8 (40.7)	211.8 (45.3)		
Yearly change (%)	0.0 (2.1)	−0.6 (1.8)	0.6 (−2.7; 3.9)	0.74
Tibia at 66% (diaphysis)				
BMC				
Baseline (mm^2)	4.0 (0.8)	3.9 (0.8)		
Follow-up (mm^2)	4.0 (0.8)	3.9 (0.8)		
Yearly change (%)	−0.3 (0.8)	−0.6 (0.9)	0.2 (−1.2; 1.6)	0.79
Total CSA				
Baseline (mm^2)	672.0 (140.5)	659.9 (125.2)		
Follow-up (mm^2)	671.2 (140.8)	660.7 (127.7)		
Yearly change (%)	−0.1 (1.6)	0.0 (1.1)	−0.3 (−2.0; 2.6)	0.80
Cortical CSA				
Baseline (mg/cm^3)	300.8 (57.8)	296.4 (62.2)		
Follow-up (mg/cm^3)	299.2 (58.8)	293.4 (62.6)		
Yearly change (%)	−0.5 (1.3)	−0.9 (1.6)	1.3 (−3.8; 1.1)	0.29
Cortical BMD				
Baseline (mg/cm^3)	1088.0 (37.1)	1094.2 (36.2)		
Follow-up (mg/cm^3)	1087.8 (38.4)	1092.7 (36.0)		
Yearly change (%)	−0.0 (0.6)	−0.1 (0.5)	0.1 (−0.8; 1.0)	0.82

(*continued on next page*)

Table 2 (*continued*)

	Placebo Mean (SD) (n = 48)	LT4 Mean (SD) (n = 50)	Adjusted between-group difference^ (95% CI)	p-Value
Cortical thickness				
Baseline (mm)	3.8 (0.7)	3.8 (0.7)		
Follow-up (mm)	3.8 (0.7)	3.7 (0.7)		
Yearly change (%)	−0.5 (2.2)	−1.1 (2.1)	1.7 (−5.3; 1.8)	0.34

LT4: Levothyroxine. 4%: Epiphyseal pQCT scans. 66%: Diaphyseal pQCT scans. BMC: Bone mineral content per cm slice thickness. CSA: Cross-sectional area. BMD: volumetric bone mineral density. For baseline and follow-ups mean absolute values with standard deviation (SD) are presented. Mean yearly changes were calculated per patient and are presented as mean of change in % (SD) by assuming baseline is 100%, difference between groups expressed as absolute difference in % per year (95% CI) to make different lengths of follow-up comparable.

^ Calculation for the differences was Placebo minus LT4- derived by a linear regression model adjusted by sex, LT4 starting dosage and muscle CSA at location. Negative values of the difference indicate a larger change in LT4-, and positive values indicate a larger change in the placebo group.

50 patients (levothyroxine), thus reaching the 47 participants per group required by the sample size calculation. Our study has limitations. First, we might have been limited by the short follow-up period, although it was the longest blinded trial to date. Second, the sample size was not sufficient for subgroup analyses regarding TSH levels. In our study, TSH target values were <4.6 mU/L as proposed for older persons [35] and by study design [20], while there are different expert opinions [36] we cannot exclude that attaining lower TSH values might have a deleterious effect on bone health. Third, we have not done statistical adjustment of significance levels regarding the multiple outcomes, but we did not find any significant outcomes with $p < 0.05$. Fourth, there was a baseline difference between groups regarding lower leg CSA might be explained by the slightly different proportion of female participants in the two groups and was accounted for by inclusion of sex as a covariate in the linear regression.

For clinical implications, our results are reassuring in the context of the large number of individuals with SHypo treated with LT4. Only a large long-term, placebo-controlled trial would definitively determine if treatment of SHypo adversely affects bone health. Until long-term safety is ascertained, we suggest that physicians who wish to treat SHypo in their older patients prescribe the lowest LT4 dose required to achieve a clinical response and keep TSH within the reference range.

Funding

This project has been funded by grants from the Swiss National Science Foundation (SNSF 320030-150025, 320030-172676 and 32003B_200606 to Dr. Rodondi). The TRUST Thyroid trial was supported by research grant (278148) from the European Union FP7-HEALTH-2011 program and by grants from the Swiss National Science Foundation (SNSF 320030-150025 and 320030-172676 to Dr. Rodondi), the Swiss Heart Foundation, and Velux Stiftung (grant 974a to Dr. Rodondi). Dr. Collet's research is supported by grants from the Swiss National Science Foundation (PZ00P3-167826), the Swiss Society of Endocrinology and Diabetes, the Leenaards Foundation and the Vontobel Foundation. The research of Dr. van Heemst is supported by the European Commission project THYRAGE (Horizon 2020 Research And Innovation Program, 666869). Dr. Mooijaart was supported by research grant (627001001) from ZonMw under the ZonMw programme Evidence-based Medicine in Old age.

Data availability statement

The data that support the findings of this study are available upon reasonable request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

CRediT authorship contribution statement

Annina Elisabeth Büchi: Methodology, Formal analysis, Writing – original draft. **Martin Feller:** Methodology, Formal analysis, Writing – original draft, Writing – review & editing. **Seraina Netzer:** Formal analysis, Writing – review & editing. **Manuel R. Blum:** Writing – review & editing. **Elena Gonzalez Rodriguez:** Methodology, Writing – review & editing. **Tinh-Hai Collet:** Writing – review & editing. **Cinzia Del Giovane:** Methodology, Writing – review & editing. **Diana van Heemst:** Writing – review & editing. **Terry Quinn:** Writing – review & editing. **Patricia M. Kearney:** Writing – review & editing. **Rudi G.J. Westendorp:** Writing – review & editing. **Jacobijn Gussekloo:** Writing – review & editing. **Simon Mooijaart:** Writing – review & editing. **Didier Hans:** Writing – review & editing. **Douglas C. Bauer:** Conceptualization, Writing – review & editing, Supervision. **Nicolas Rodondi:** Conceptualization, Resources, Writing – review & editing, Supervision, Funding. **Daniel Aeberli:** Conceptualization, Resources, Writing – original draft, Writing – review & editing, Supervision.

Declaration of competing interest

AEB, MF, SN, EGR, THC, CDG, DvH, TQ, SM, PMK, RGJW, JG, DCB, NR, DA, MB have nothing to declare. DH holds stock in Medimaps Group, the makers of the Trabecular Bone Score software. The study medication (levothyroxine and matching placebo) was supplied by the healthcare business of Merck KGaA, Darmstadt, Germany at no cost. Merck KGaA, Darmstadt, Germany reviewed the manuscript only for medical accuracy before journal submission, but played no role in designing, analyzing, or reporting the trial. The authors are fully responsible for the content of this manuscript, and the views and opinions described in the publication reflect solely those of the authors.

Appendix

Appendix Table 1
Sensitivity analysis for differences between groups adjusted for potential confounding factors.

Adjusted for	Difference between groups (95% CI) of yearly changes^		
	Age	BMI	Deleterious medication
Radius at 4% (epiphysis)			
BMC	−0.1 (−4.1; 3.9)	−0.2 (−4.3; 3.9)	−0.2 (−4.3; 3.9)
Total CSA	2.4 (−5.2; 10.0)	2.3 (−5.4; 10.0)	2.1 (−5.6; 9.8)
Total BMD	−2.6 (−8.5; 3.4)	−2.5 (−8.5; 3.5)	−2.3 (−8.2; 3.6)
Trabecular BMD	2.7 (−2.2; 7.6)	2.7 (−2.2; 7.6)	2.7 (−2.2; 7.6)
Radius at 66% (diaphysis)			
BMC	0.1 (−3.1; 3.2)	0.0 (−3.1; 3.2)	−0.1 (−3.2; 3.1)
Total CSA	2.0 (−1.7; 1.4)	2.1 (−1.7; 5.9)	2.0 (−1.5; 1.5)
Cortical CSA	−2.4 (−6.4; 1.7)	−2.5 (−6.7; 1.6)	−2.4 (−6.6; 1.7)
Cortical BMD	1.4 (−0.1; 2.9)	1.4 (−0.1; 2.9)	1.4 (−0.1; 2.9)
Cortical thickness	−4.1 (−9.2; 1.1)	−4.2 (−9.5; 1.1)	−4.0 (−9.3; 1.2)
Tibia at 4% (epiphysis)			
BMC	−0.1 (−3.1; 3.0)	0.1 (−2.9; 3.1)	0.0 (−3.1; 3.1)
Total CSA	0.2 (−1.6; 2.0)	1.2 (−3.1; 5.5)	1.2 (−3.2; 5.5)
Total BMD	−1.2 (−4.3; 2.0)	−1.1 (−4.3; 2.0)	−1.2 (−4.3; 1.9)
Trabecular BMD	0.5 (−2.8; 3.8)	0.6 (−2.7; 3.9)	0.5 (−2.8; 3.8)
Tibia at 66% (diaphysis)			
BMC	0.2 (−1.2; 1.6)	0.2 (−1.2; 1.6)	0.2 (−1.3; 1.6)
Total CSA	0.3 (−2.2; 2.6)	0.3 (−2.0; 2.6)	0.2 (−2.1; 2.5)
Cortical CSA	−1.3 (−3.8; 1.2)	−1.3 (−3.8; 1.2)	−1.2 (−3.7; 1.2)
Cortical BMD	0.1 (−0.8; 1.0)	0.1 (−0.8; 1.0)	0.1 (−0.8; 0.9)
Cortical Thickness	−1.7 (−5.3; 1.8)	−1.7 (−5.2; 1.9)	−1.6 (−5.1; 1.9)

LT4: Levothyroxine. 4%: Epiphyseal pQCT scans. 66%: Diaphyseal pQCT scans. BMC: Bone mineral content per cm slice thickness. CSA: Cross-sectional area. BMD: volumetric bone mineral density.

^ Change after treatment presented as change in % with 95% CI. There were no significant p-values (not shown). We displayed the mean absolute difference in % per year (taking into account the varying follow-up durations). Calculation for the differences was placebo minus LT4 derived by a linear regression model adjusted by sex, LT4 starting dosage and muscle CSA at location and the additional adjustment mentioned in the header. Negative values of the difference indicate a larger change in LT4, and positive values indicate a larger change in the Placebo group. An increase for BMC, BMD (Total, cortical, trabecular) and cortical thickness is considered beneficial, an increase CSA (total and cortical) is considered deleterious.

References

[1] J.G. Hollowell, N.W. Staehling, W.D. Flanders, 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. 87 (2) (Feb 2002) 489–499.

[2] G.J. Canaris, N.R. Manowitz, G. Mayor, E.C. Ridgway, The Colorado thyroid disease prevalence study, Arch. Intern. Med. 160 (4) (2000) 526–534.

[3] G.E. Bekkering, T. Agoritsas, L. Lytvyn, et al., Thyroid hormones treatment for subclinical hypothyroidism: a clinical practice guideline, BMJ 365 (May 14 2019), l2006.

[4] P.W. Rosario, M.R. Calsolari, How selective are the new guidelines for treatment of subclinical hypothyroidism for patients with thyrotropin levels at or below 10 mIU/L? Thyroid 23 (5) (May 2013) 562–565.

[5] R. Rodriguez-Gutierrez, S. Maraka, N.S. Ospina, V.M. Montori, J.P. Brito, Levothyroxine overuse: time for an about face? Lancet Diabetes Endocrinol. 5 (4) (Apr 2017) 246–248.

[6] M.R. Blum, D.C. Bauer, T.H. Collet, et al., Subclinical thyroid dysfunction and fracture risk: a meta-analysis, JAMA 313 (20) (2015) 2055–2065.

[7] B. Abrahamsen, H.L. Jorgensen, A.S. Laulund, M. Nybo, T.H. Brix, L. Hegedus, Low serum thyrotropin level and duration of suppression as a predictor of major osteoporotic fractures-the OPENTHYRO register cohort, J.BoneMiner.Res. 29 (9) (Sep 2014) 2040–2050.

[8] P. Vestergaard, L. Mosekilde, Hyperthyroidism, bone mineral, and fracture risk–a meta-analysis, Thyroid 13 (6) (Jun 2003) 585–593.

[9] C.E. Aubert, C. Floriani, D.C. Bauer, et al., Thyroid function tests in the reference range and fracture: individual participant analysis of prospective cohorts, J. Clin. Endocrinol. Metab. 102 (8) (2017) 2719–2728.

[10] S.L. Greenspan, F.S. Greenspan, The effect of thyroid hormone on skeletal integrity, Ann. Intern. Med. 130 (9) (1999) 750–758.

[11] Y. Hwangbo, J.H. Kim, S.W. Kim, et al., High-normal free thyroxine levels are associated with low trabecular bone scores in euthyroid postmenopausal women, Osteoporos.Int. 27 (2) (Feb 2016) 457–462.

[12] C.D. Wirth, M.R. Blum, B.R. da Costa, et al., Subclinical thyroid dysfunction and the risk for fractures: a systematic review and meta-analysis, Ann. Intern. Med. 161 (3) (2014) 189–199.

[13] J.H. Bassett, G.R. Williams, Role of thyroid hormones in skeletal development and bone maintenance, Endocr. Rev. 37 (2) (Apr 2016) 135–187.

[14] P. Vestergaard, N.R. Jorgensen, P. Schwarz, L. Mosekilde, Effects of treatment with fluoride on bone mineral density and fracture risk–a meta-analysis, Osteoporos.Int. 19 (3) (Mar 2008) 257–268.

[15] P. Vestergaard, Discrepancies in bone mineral density and fracture risk in patients with type 1 and type 2 diabetes–a meta-analysis, Osteoporos.Int. 18 (4) (Apr 2007) 427–444.

[16] G. Mazziotti, F. Sorvillo, M. Piscopo, et al., Recombinant human TSH modulates in vivo C-telopeptides of type-1 collagen and bone alkaline phosphatase, but not osteoprotegerin production in postmenopausal women monitored for differentiated thyroid carcinoma, J.BoneMiner.Res. 20 (3) (Mar 2005) 480–486.

[17] R. Jaeschke, G. Guyatt, H. Gerstein, et al., Does treatment with L-thyroxine influence health status in middle-aged and older adults with subclinical hypothyroidism? J. Gen. Intern. Med. 11 (12) (Dec 1996) 744–749.

[18] D.S. Ross, 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. 95 (4) (Oct 1993) 385–388.

[19] C. Meier, M. Beat, M. Guglielmetti, M. Christ-Crain, J.J. Staub, M. Kraenzlin, Restoration of euthyroidism accelerates bone turnover in patients with subclinical hypothyroidism: a randomized controlled trial, Osteoporos.Int. 15 (3) (Mar 2004) 209–216.

[20] D.J. Stott, N. Rodondi, P.M. Kearney, et al., Thyroid hormone therapy for older adults with subclinical hypothyroidism, N. Engl. J. Med. 376 (26) (2017) 2534–2544.

[21] E. Gonzalez Rodriguez, M. Stuber, C. Del Giovane, et al., Skeletal effects of levothyroxine for subclinical hypothyroidism in older adults: a TRUST randomized trial nested study, J. Clin. Endocrinol. Metab. 105 (1) (Jan 1 2020).

[22] D. Aeberli, P. Eser, H. Bonel, et al., Reduced trabecular bone mineral density and cortical thickness accompanied by increased outer bone circumference in metacarpal bone of rheumatoid arthritis patients: a cross-sectional study, Arthritis Res.Ther. 12 (3) (2010) R119.

[23] J. Lehmke, U. Bogner, D. Felsenberg, H. Peters, H. Schleusener, Determination of bone mineral density by quantitative computed tomography and single photon absorptiometry in subclinical hyperthyroidism: a risk of early osteopaenia in post-menopausal women, Clin. Endocrinol. 36 (5) (May 1992) 511–517.

[24] S.L. Greenspan, F.S. Greenspan, N.M. Resnick, J.E. Block, A.L. Friedlander, H. K. Genant, Skeletal integrity in premenopausal and postmenopausal women receiving long-term L-thyroxine therapy, Am. J. Med. 91 (1) (Jul 1991) 5–14.

[25] S. Hansen, J.E. Beck Jensen, L. Rasmussen, E.M. Hauge, K. Brixen, Effects on bone geometry, density, and microarchitecture in the distal radius but not the tibia in women with primary hyperparathyroidism: a case-control study using HR-pQCT, J. BoneMiner.Res. 25 (9) (Sep 2010) 1941–1947.

[26] P. Dhiman, S. Andersen, P. Vestergaard, T. Masud, N. Qureshi, Does bone mineral density improve the predictive accuracy of fracture risk assessment? A prospective cohort study in Northern Denmark, BMJ Open 8 (4) (Apr 12 2018), e018898.

[27] R. Yang, L. Yao, Y. Fang, et al., The relationship between subclinical thyroid dysfunction and the risk of fracture or low bone mineral density: a systematic review and meta-analysis of cohort studies, J. Bone Miner. Metab. 36 (2) (Mar 2018) 209–220.
[28] D. Segna, D.C. Bauer, M. Feller, et al., Association between subclinical thyroid dysfunction and change in bone mineral density in prospective cohorts, J. Intern. Med. 283 (1) (Jan 2018) 56–72.
[29] E. Moser, T. Sikjaer, L. Mosekilde, L. Rejnmark, Bone indices in thyroidectomized patients on long-term substitution therapy with levothyroxine assessed by DXA and HR-pQCT, J. Thyroid. Res. 2015 (2015), 796871.
[30] G. Mendonca Monteiro de Barros, M. Madeira, L. Vieira Neto, et al., Bone mineral density and bone microarchitecture after long-term suppressive levothyroxine treatment of differentiated thyroid carcinoma in young adult patients, J. Bone Miner. Metab. 34 (4) (Jul 2016) 417–421.
[31] G. Roef, B. Lapauw, S. Goemaere, et al., Thyroid hormone status within the physiological range affects bone mass and density in healthy men at the age of peak bone mass, Eur. J. Endocrinol. 164 (6) (Jun 2011) 1027–1034.
[32] S. Tournis, J.D. Antoniou, C.G. Liakou, et al., Volumetric bone mineral density and bone geometry assessed by peripheral quantitative computed tomography in women with differentiated thyroid cancer under TSH suppression, Clin. Endocrinol. 82 (2) (Feb 2015) 197–204.
[33] P. Nicolaisen, M.L. Obling, K.H. Winther, et al., Consequences of hyperthyroidism and its treatment for bone microarchitecture assessed by high-resolution peripheral quantitative computed tomography, Thyroid 31 (2) (Feb 2021) 208–216.
[34] M.L. Obling, P. Nicolaisen, T.H. Brix, et al., Restoration of euthyroidism in women with Hashimoto's thyroiditis changes bone microarchitecture but not estimated bone strength, Endocrine 71 (2) (Feb 2021) 397–406.
[35] Z. Javed, T. Sathyapalan, Levothyroxine treatment of mild subclinical hypothyroidism: a review of potential risks and benefits, Ther.Adv.Endocrinol. Metab. 7 (1) (Feb 2016) 12–23.
[36] S.H. Pearce, G. Brabant, L.H. Duntas, et al., 2013 ETA guideline: management of subclinical hypothyroidism, Eur.Thyroid J. 2 (4) (Dec 2013) 215–228.