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

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Context: The relationship between thyroid function and bone mineral density (BMD) is controversial. Existing studies are conflicting and confounded by differences in study design, small patient numbers, and sparse prospective data.

Objective: We hypothesized that variation across the normal range of thyroid status in healthy postmenopausal women is associated with differences in BMD and fracture susceptibility.

Design: The Osteoporosis and Ultrasound Study (OPUS) is a 6-yr prospective study of fracture-related factors.

Setting: We studied a population-based cohort from five European cities.

Participants: A total of 2374 postmenopausal women participated. Subjects with thyroid disease and nonthyroidal illness and those receiving drugs affecting thyroid status or bone metabolism were excluded, leaving a study population of 1278 healthy euthyroid postmenopausal women.

Interventions: There were no interventions.

Main Outcome Measures: We measured free T_4 (fT4) (picomoles/liter), free T_3 (fT3) (picomoles/liter), TSH (milliunits/liter), bone turnover markers, BMD, and vertebral, hip, and nonvertebral fractures.

Results: Higher fT4 ($\beta = -0.091$; $P = 0.004$) and fT3 ($\beta = -0.087$; $P = 0.005$) were associated with lower BMD at the hip, and higher fT4 was associated with increasing bone loss at the hip ($\beta = -0.09$; $P = 0.015$). After adjustment for age, body mass index, and BMD, the risk of nonvertebral fracture was increased by 20% ($P = 0.002$) and 33% ($P = 0.006$) in women with higher fT4 or fT3, respectively, whereas higher TSH was protective and the risk was reduced by 35% ($P = 0.028$). There were independent associations between fT3 and pulse rate ($\beta = 0.080$; $P = 0.006$), increased grip strength ($\beta = 0.171$; $P < 0.001$), and better balance ($\beta = 0.099$; $P < 0.001$), indicating that the relationship between thyroid status and fracture risk is complex.

Conclusions: Physiological variation in normal thyroid status is related to BMD and nonvertebral fracture. (***J Clin Endocrinol Metab* 95: 0000–0000, 2010**)

Low bone mineral density (BMD), prior or parental history of fracture, low body mass index (BMI), use of glucocorticoids, smoking, excessive alcohol consumption, untreated thyrotoxicosis, and other factors increase susceptibility to osteoporosis. Even subclinical hyperthyroidism, defined by a suppressed TSH level in the presence of normal thyroid hormone concentrations, is associated with fracture (1, 2), and treatment with T_4 at doses that suppress TSH is associated with increased bone turnover and low BMD in postmenopausal women (3, 4). The prevalence of thyroid disease increases with age: 3% of women over 50 receive T_4, and more than 20% are overtreated (5). Subclinical hyperthyroidism affects a further 1.5% of women over 60, and its prevalence increases with age (6). Nevertheless, the role of thyroid hormones in the pathogenesis of osteoporosis remains uncertain (3, 7).

Longitudinal differences in free T_3 (fT3), free T_4 (fT4), and TSH in healthy individuals fluctuate by less than 50% of the population reference range (8). Each person has a unique hypothalamic-pituitary-thyroid (HPT) axis setpoint, implying that tissue thyroid hormone sensitivity varies between individuals. Data from the UK Adult Twin Registry estimate heritability for fT3 concentration at 23%, fT4 at 39%, and TSH at 65% (9), whereas estimates from Denmark were higher (10). A genome-wide screen identified eight quantitative trait loci linked to circulating fT3, fT4, and TSH levels, indicating that thyroid status is inherited as a complex trait (11), and genome-wide association studies demonstrate that many signaling pathways influence BMD and fracture susceptibility (12).

We hypothesized that variation across the normal range of thyroid status in healthy euthyroid postmenopausal women is associated with differences in BMD and fracture susceptibility.

Subjects and Methods

Osteoporosis and Ultrasound Study (OPUS)

OPUS is a prospective study of postmenopausal women recruited between April 1999 and April 2001 from five European centers (13). Investigations were approved at each institution according to the Declaration of Helsinki. Written consent was obtained from all subjects. Each center recruited approximately 100 women between 20 and 40 yr of age to calculate adjusted T-scores for BMD and approximately 500 postmenopausal women comprising 100 individuals in each 5-yr age band between 55 and 80. Ninety-nine percent of subjects were of white ethnicity. Individuals were excluded because of inability to undergo bone densitometry or perform specified investigations or because of cognitive limitations. A total of 566 premenopausal and 2374 postmenopausal women participated. Subjects completed a modified version of the European Vertebral Osteoporosis Study risk factor questionnaire (14, 15).

Biochemical measurements

Nonfasting samples were taken between 1200 and 1500 h, and serum was stored at −75 C. TSH, fT4, and fT3 were measured on a single automated analyzer using the ARCHITECT System (Abbott ARCHITECT i2000; Abbott Laboratories, Maidenhead, UK). This fT4 assay performs reliably compared with equilibrium dialysis, the gold standard method (16). All samples were analyzed within a 3-month period, and coefficients of variation were: TSH, <4.0%; fT3, <10.0%; and fT4, <10.4%.

Serum type I collagen C-terminal telopeptide (sβCTX), a bone resorption marker, and procollagen type I N-terminal propeptide (PINP) and osteocalcin (OC), bone formation markers, were measured on an Elecsys 2010 (Roche Diagnostics, Mannheim, Germany). Blood samples were collected at the same time of day to mitigate effects of diurnal variation. Urinary N-terminal telopeptide of type I collagen (uNTX), a bone resorption marker, and urinary creatinine were measured using dry slide methods (Ortho-Clinical Diagnostics, Rochester, NY). Coefficients of variation were <8.1% (sβCTX), <1.1% (PINP), <1.3% (OC), and <1.7% (uNTX). uNTX was expressed as a ratio to creatinine excretion (uNTX/Cr).

Physical measurements

Dual-energy x-ray absorptiometry scans were performed in posteroanterior projection (Hologic QDR-4500; Hologic Inc., Bedford, MA) in Kiel, Germany; Paris; and Sheffield, United Kingdom; or in anteroposterior projection (Lunar Expert devices; GE Lunar Corp., Madison, WI) in Aberdeen, United Kingdom, and Berlin (13, 15, 17, 18). Results were corrected for longitudinal changes (based on daily measurements of the European Spine Phantom) (19) and for differences among centers (20). Results of the same brand were adjusted according to cross-calibration phantom data, whereas results of different brands were standardized by expressing dual-energy x-ray absorptiometry results as standardized BMD (21, 22). For lumbar spine, the total BMD of vertebrae L2–L4 was evaluated, and measurements were performed only in women who had not sustained a vertebral fracture. Subjects in whom fewer than two vertebrae could be evaluated were excluded. For the hip, total BMD of the proximal femur was determined. Change in BMD was calculated as BMD at follow-up minus BMD at entry.

Lateral spine radiographs were obtained at study entry and after 6.1 ± 0.6 yr. Incident fractures were defined in vertebrae that sustained a deformity of greater than 20% in the anterior, middle, or posterior height ratio (13, 18). Hip fractures were ascertained by questionnaire after 6-yr follow-up and validated by analysis of medical records and imaging. Incident nonvertebral fractures were similarly recorded and validated. Grip strength was determined using a dynamometer (Takei Scientific, Tokyo, Japan). Two measurements were taken in each hand, and the mean was calculated. Subjects were asked to walk backward without deviating along a 5-m line, and the point of deviation was scored in 10-cm intervals to assess balance.

Study population

To determine reference intervals for normal euthyroid status, fT3, fT4, and TSH ranges were calculated for each decade of age (55–65, 65–75, >75) in 1754 healthy euthyroid postmenopausal women after exclusion of individuals with thyroid disease, chronic illness, other evidence of the sick euthyroid syn-
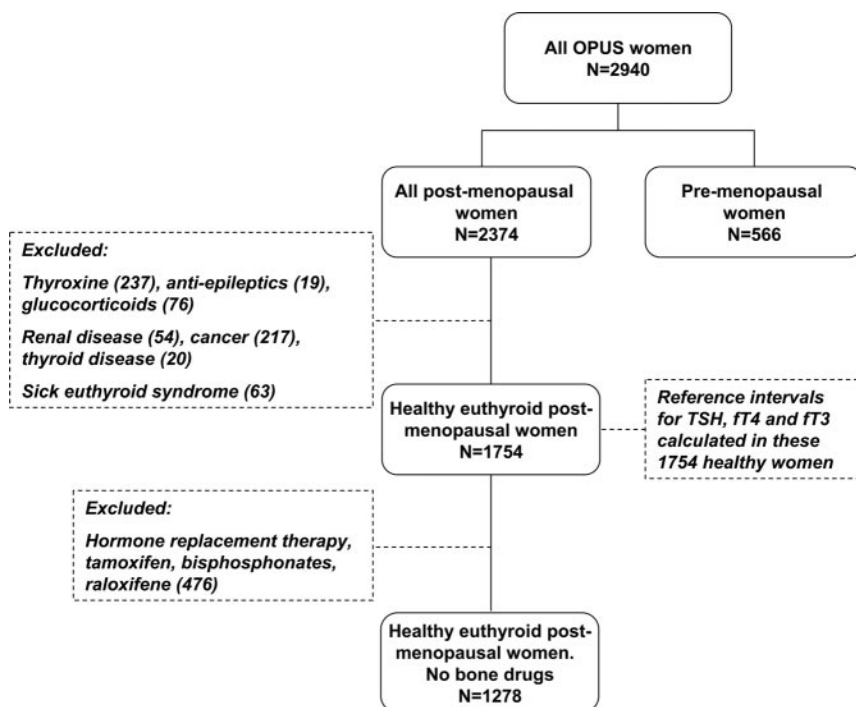
FIG. 1. Exclusion criteria to define populations of healthy euthyroid postmenopausal women and those women not receiving drugs affecting bone metabolism.

drome, or those receiving drugs affecting thyroid function. The 2.5th and 97.5th percentiles were calculated for fT4 and fT3. TSH values were ranked, and 2.5th and 97.5th percentiles were calculated. To analyze relationships between fT3, fT4, and TSH levels and bone parameters, a further 476 individuals receiving drugs that interfere with bone metabolism were excluded, resulting in a final analysis population of 1278. This rigorously defined group of healthy euthyroid postmenopausal women not receiving drugs affecting bone metabolism represents a normal healthy postmenopausal European Caucasian population.

Statistics

Analyses were performed using SPSS v.14 (SPSS Inc., Chicago, IL) or GraphPad Prism v.5 (GraphPad Software, Inc., San Diego, CA). Data were assessed for normal distribution using the Kolmogorov-Smirnov test, and nonparametric variables underwent logarithmic transformation, after which TSH, sβCTX, and uNTX/Cr datasets remained skewed.

fT4 and fT3 levels were grouped into quintiles and one-way between-groups ANOVA was conducted to explore relationships between fT4 or fT3 and BMD, bone formation markers, pulse rate, grip strength, and balance. Comparisons between highest and lowest quintiles were performed using Tukey's *post hoc* test. Comparisons with bone resorption markers were performed using Kruskal-Wallis (K-W) and Dunn's *post hoc* tests. TSH data were analyzed by Mann-Whitney U (M-WU) to com-

pare effects of TSH levels below 0.5 mU/liter with levels of at least 0.5 mU/liter.

Odds ratios (ORs) for vertebral fracture in relation to fT4, fT3, and TSH were determined using unadjusted and adjusted (for age, BMI, and BMD) logistic regression. Incident nonvertebral fracture (including hip fracture) risk was determined by Cox proportional-hazards modeling. The independent variable unit of analysis for OR and hazards ratio (HR) was 1 pmol/liter for fT4 and fT3 and 1 mU/liter for TSH. To conform to regression analyses, data were also presented per SD change in fT4, fT3, and TSH. Analysis of covariance was used to compare fT4 levels in women with an incident hip fracture to those without.

Stepwise adjusted regression analysis of relationships between thyroid status and BMD, bone turnover, pulse rate, grip strength, and balance was performed after adjustment for age or BMI, smoking, and age.

Results

Thyroid function in healthy postmenopausal women

To define a healthy euthyroid population, subjects receiving T_4 (n = 237), antiepileptics (n = 19), or glucocorticoids (n = 76); those with renal disease (n = 54), cancer (n = 217), or overt thyroid disease (TSH > 10 mU/liter and fT4 < 9 pmol/liter; or TSH > 10 mU/liter and fT3 < 2.5 pmol/liter; or TSH < 0.1 mU/liter and fT3 ≥ 6 pmol/liter) (n = 20); and individuals with sick euthyroid syndrome, defined as fT3 less than 2.5 pmol/liter plus one or more of malabsorption, rheumatoid arthritis, bone disease other than osteoarthritis, psoriasis, or asthma (n = 63), were excluded. Several subjects fulfilled two or more criteria, resulting in exclusion of 620 individuals to obtain a population of 1754 healthy euthyroid postmenopausal women (Fig. 1), in which reference intervals for normal thyroid function were defined (Table 1). There were no differences in smoking, alcohol consumption, prevalence of osteoarthritis, or family history of fracture between this healthy euthyroid group and the total population. There was a positive correlation between fT4 and fT3 (r = 0.404; $P < 0.01$), whereas fT4 and fT3 correlated negatively with TSH (rho = −0.183, $P < 0.001$; and rho = −0.050, $P < 0.05$, respectively). fT4 and fT3 increased

TABLE 1. Age-related reference ranges for thyroid function tests

Analyte	All women ≥ 55 yr old	55–65 yr	66–75 yr	>75 yr
TSH (mU/liter)	0.13–3.48	0.14–3.48	0.16–3.48	0.04–3.80
fT4 (pmol/liter)	9.15–16.99	8.87–16.89	9.24–16.91	9.61–17.12
fT3 (pmol/liter)	2.16–5.29	2.07–5.28	2.22–5.32	2.24–5.31

TABLE 2. Baseline characteristics

	Healthy euthyroid postmenopausal women, no bone-modifying drugs
n	1278
Age (yr)	68 ± 7
BMI (kg/m^2)	27.2 ± 4.7
Years since menopause	18.8 ± 9.1
TSH (mU/liter)	0.88 (range, 0.15–3.64)
fT4 (pmol/liter)	12.9 ± 2.0
fT3 (pmol/liter)	3.7 ± 0.8
Lumbar spine BMD (mg/cm^2)	1028 ± 180
Hip BMD (mg/cm^2)	839 ± 131
OC (ng/ml)	26.0 ± 10.6
PINP (ng/ml)	55.0 ± 20.0
sβCTX (ng/ml)	0.271 ± 0.248
uNTX/Cr (n$_M$/m$_M$)	56.6 ± 31.0
Grip strength (kg)	18 ± 5
Balance (m)	3.2 ± 2.0

with age (F = 21.26, $P <$ 0.0001; F = 3.482, $P <$ 0.05, respectively), whereas TSH remained constant (K-W, 0.0003; $P =$ 0.999).

Analysis population

To determine relationships between thyroid status and bone parameters, individuals receiving drugs affecting bone metabolism (n = 476) were excluded to define a final analysis group of 1278 healthy euthyroid postmenopausal women not receiving drugs affecting bone metabolism (Fig. 1 and Table 2).

In the analysis population, the following data were unavailable: lumbar spine BMD at entry into the study in six individuals, hip BMD at entry in 10, lumbar spine BMD after 6-yr follow-up in 489, and hip BMD at follow-up in 486. This resulted in a total of 788 women in which paired BMD data were available. TSH values were unavailable in 74 individuals, fT4 in 66, fT3 in 65, pulse rate in 34, grip strength in 193, balance in 38, OC in 61, PINP in 61, sβCTX in 61, and uNTX/Cr in 64. Taken together, a complete set of data were available in 593 women (Supplemental Table 1, published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org). Subjects in whom specific data were missing were excluded before statistical analysis.

Lower BMD in women with higher fT4 and fT3

Individuals with fT4 levels in the highest quintile had lower BMD than women with fT4 in the lowest quintile at both lumbar spine and hip at entry into the study (lumbar spine F = 2.29, $P =$ 0.050; hip F = 2.96, $P =$ 0.021) and at the hip after 6-yr follow-up (F = 4.32, $P =$ 0.041). Individuals with fT3 in the highest quintile had lower BMD at the hip after 6-yr follow-up (F = 2.68, $P =$ 0.045). The mean BMD values of women with fT4 and fT3 levels
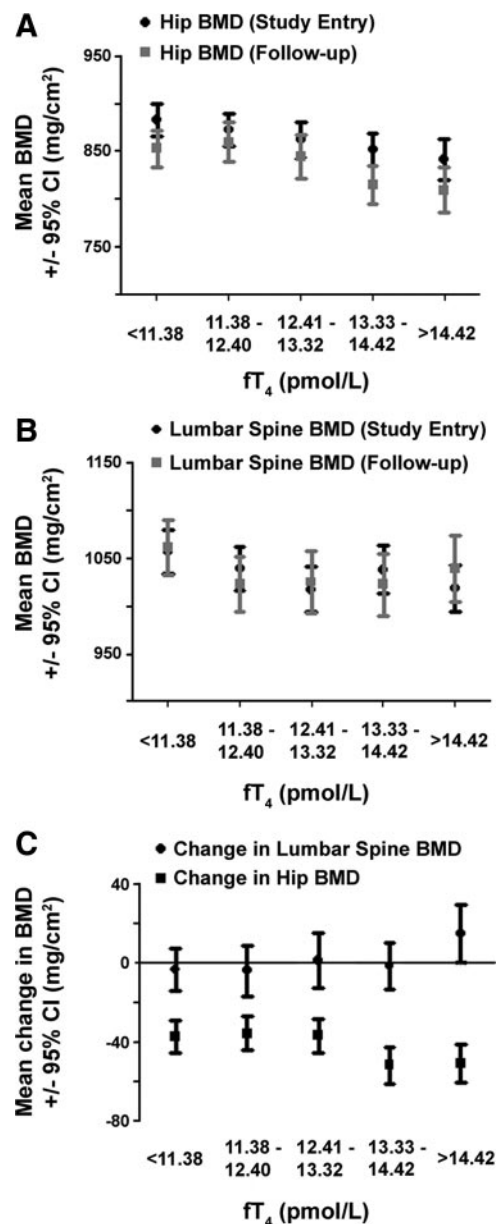

FIG. 2. Graphs showing hip (A) and lumbar spine (B) mean BMD ± 95% confidence interval (CI) at the time of entry into the study and after 6-yr follow-up in relation to quintiles of fT4 concentration. C, Mean change in BMD ± 95% CI in relation to fT4.

in the lowest and highest quintiles are included in Supplemental Table 2. Figure 2 shows quintile plots relating hip and lumbar spine BMD at study entry and after 6-yr follow-up and mean change in BMD to fT4 levels. These plots illustrate the inverse relationship between hip BMD and fT4 across the normal range of fT4 concentration. There was no relationship between TSH and BMD.

Increased risk of nonvertebral fracture in women with higher fT4 and fT3

There were 52 incident vertebral fractures in 44 individuals and 86 nonvertebral fractures, including seven hip fractures in seven individuals, among the 1278 healthy

TABLE 3. Relationship between thyroid function tests and fracture

	Logistic regression model			Cox proportional hazards model				
	OR (95% CI)			HR (95% CI)				
	Per unit change	Per SD change	*P*	Per unit change	Per SD change	*P*		
Prevalent vertebral fracture	1.08 (0.99–1.17)	1.15 (0.98–1.35)	0.087				fT4	Unadjusted
Incident vertebral fracture	0.91 (0.76–1.10)	0.84 (0.59–1.20)	0.336				fT4	Unadjusted
Incident nonvertebral fracture				**0.82 (0.72–0.93)**	**0.69 (0.54–0.87)**	**0.002**	fT4	Unadjusted
Prevalent vertebral fracture	0.92 (0.75–1.12)	0.94 (0.79–1.10)	0.407				fT3	Unadjusted
Incident vertebral fracture	1.06 (0.69–1.62)	1.05 (0.74–1.47)	0.794				fT3	Unadjusted
Incident nonvertebral fracture				**0.66 (0.51–0.86)**	**0.72 (0.58–0.86)**	**0.002**	fT3	Unadjusted
Prevalent vertebral fracture	1.04 (0.92–1.17)	1.03 (0.94–1.21)	0.569				TSH	Unadjusted
Incident vertebral fracture	1.20 (0.86–1.68)	1.24 (0.80–1.86)	0.286				TSH	Unadjusted
Incident nonvertebral fracture				1.18 (0.93–1.51)	1.22 (0.92–1.64)	0.167	TSH	Unadjusted
Prevalent vertebral fracture	1.03 (0.94–1.12)	1.06 (0.90–1.24)	0.581				fT4	Adjusted for age, BMI and lumbar spine BMD
Incident vertebral fracture	0.91 (0.75–1.10)	0.84 (0.58–1.20)	0.318				fT4	Adjusted for age, BMI and lumbar spine BMD
Incident nonvertebral fracture				**0.80 (0.70–0.92)**	**0.65 (0.51–0.85)**	**0.002**	fT4	Adjusted for age, BMI and lumbar spine BMD
Prevalent vertebral fracture	0.84 (0.68–1.03)	0.89 (0.73–1.02)	0.099				fT3	Adjusted for age, BMI and lumbar spine BMD
Incident vertebral fracture	1.10 (0.70–1.70)	1.08 (0.75–1.53)	0.685				fT3	Adjusted for age, BMI and lumbar spine BMD
Incident nonvertebral fracture				**0.67 (0.51–0.89)**	**0.73 (0.58–0.91)**	**0.006**	fT3	Adjusted for age, BMI and lumbar spine BMD
Prevalent vertebral fracture	1.02 (0.90–1.15)	1.02 (0.88–1.18)	0.775				TSH	Adjusted for age, BMI and lumbar spine BMD
Incident vertebral fracture	1.27 (0.91–1.77)	1.33 (0.89–1.98)	0.167				TSH	Adjusted for age, BMI and lumbar spine BMD
Incident nonvertebral fracture				**1.35 (1.03–1.76)**	**1.43 (1.04–1.97)**	**0.028**	TSH	Adjusted for age, BMI and lumbar spine BMD

Statistically significant results are shown in *bold*. Units of measure are: fT4, pmol/liter; fT3, pmol/liter; and TSH, mU/liter. CI, Confidence interval.

euthyroid postmenopausal women not receiving drugs affecting bone metabolism in whom data were available (Supplemental Table 1). Unadjusted and adjusted logistic regression indicated that fT4, fT3, and TSH levels were not related to vertebral fracture (Table 3).

Unadjusted Cox proportional hazards analysis revealed that the risk of incident nonvertebral fracture (including hip fracture) was increased by 18 and 34% in women with higher fT4 and fT3, respectively. After adjustment for age, BMI, and BMD, the risk of nonvertebral fracture was increased by 20 and 33% in women with higher fT4 or fT3, whereas in women with higher TSH, this risk was reduced by 35% (Table 3). Separate analysis of hip fractures by Cox proportional hazards was not pos-

sible because only seven were recorded. Nevertheless, women with an incident hip fracture had higher fT4 than women without (14.4 ± 2.2 *vs.* 12.7 ± 1.9 pmol/liter, analysis of covariance; F = 4.87; *P* = 0.03). After adjustment for BMI, this relationship did not change (F = 4.87; *P* = 0.03), but after adjustment for age the relationship was not significant (F = 3.25; *P* = 0.072).

Thyroid status is associated with pulse rate, grip strength, balance, and bone turnover

Pulse rate, grip strength, balance, and bone turnover markers were investigated as possible factors underlying associations between thyroid status and bone parameters. Individuals with fT3 in the highest quintile had increased

TABLE 4. Relationship between thyroid function tests, BMD, bone turnover, and extraskeletal parameters

	fT4 (pmol/liter)			fT3 (pmol/liter)			TSH (mU/liter)		
	Model R^2	β coefficient (95% CI) (lower, upper, per SD change)	P	Model R^2	β coefficient (95% CI) (lower, upper, per SD change)	P	Model R^2	β coefficient (95% CI) (lower, upper, per SD change)	P
BMD									
At study entry									
Lumbar spine BMD	0.126	−0.042 (−9.059, 1.407)	0.152	0.126	−0.043 (−21.127, 3.061)	0.143	0.127	−0.050 (−15.552, 1.040)	0.086
Hip BMD	**0.293**	**−0.051 (−7.299, −0.174)**	**0.040**	0.292	−0.042 (−15.779, 1.121)	0.089	0.290	−0.014 (−7.700, 4.144)	0.556
At 6 yr follow-up									
Lumbar spine BMD	0.128	−0.054 (−11.936, 1.870)	0.153	0.125	0.026 (−10.173, 21.269)	0.489	0.126	−0.040 (−24.494, 7.231)	0.286
Hip BMD	**0.304**	**−0.091 (−10.044, −1.976)**	**0.004**	**0.304**	**−0.087 (−23.439, −4.313)**	**0.005**	0.298	0.04 (−2.233, 11.222)	0.190
Change in lumbar spine BMD	0.113	0.021 (−2.038, 3.609)	0.585	**0.128**	**0.123 (4.227, 16.967)**	**0.001**	0.113	−0.004 (−6.833, 6.137)	0.916
Change in hip BMD	**0.027**	**−0.090 (−4.069, −0.439)**	**0.015**	0.023	−0.053 (−7.543, 1.082)	0.142	0.024	0.067 (−0.177, 5.852)	0.065
Bone turnover									
OC	0.032	−0.029 (−0.457, 0.151)	0.322	0.032	−0.009 (−0.831, 0.611)	0.764	0.035	−0.054 (−0.991, 0.017)	0.058
PINP	0.017	0.006 (−0.517, 0.639)	0.835	0.017	−0.008 (−1.570, 1.171)	0.776	0.018	−0.038 (−1.612, 0.307)	0.182
sβCTX	0.020	0.022 (−0.004, 0.010)	0.443	**0.023**	**−0.060 (−0.035, −0.001)**	**0.037**	0.021	−0.035 (−0.019, 0.005)	0.225
uNTX/Cr	0.017	0.033 (−0.399, 1.424)	0.270	0.018	0.049 (−0.330, 3.988)	0.097	0.016	−0.014 (−1.888, 1.140)	0.628
Extraskeletal parameters									
At study entry									
Pulse rate	0.022	0.034 (−0.121, 0.457)	0.253	**0.027**	**0.08 (0.285, 1.652)**	**0.006**	0.021	−0.020 (−0.652, 0.309)	0.483
Grip strength	**0.097**	**0.074 (0.040, 0.362)**	**0.014**	**0.121**	**0.171 (0.734, 1.488)**	**<0.001**	0.094	−0.042 (−0.458, 0.079)	0.165
Balance	0.109	0.052 (−0.003, 0.110)	0.064	**0.116**	**0.099 (0.111, 0.377)**	**<0.001**	0.106	−0.009 (−0.019, 0.079)	0.753

Statistically significant results are shown in *bold.* Data are adjusted for age, BMI, and smoking. CI, Confidence interval.

grip strength (F = 7.68; *P* < 0.001), better balance (F = 4.49; *P* = 0.023), and higher uNTX/Cr (K-W = 11.21; *P* < 0.01). Women with TSH less than 0.5 mU/liter had better balance (M-WU = 97,190; *P* = 0.036) and increased bone turnover (OC, M-WU = 97,840, *P* = 0.005; PINP, M-WU = 101,400, *P* = 0.039; sβCTX, M-WU = 100,700, *P* = 0.027; uNTX/Cr, M-WU = 87,880, *P* < 0.001) compared with women with TSH of at least 0.5 mU/liter. After adjusted regression analysis, however, TSH was not associated with bone turnover (see below and Table 4).

Adjusted regression

Stepwise regression was performed to investigate relationships between thyroid hormones and BMD, pulse rate, grip strength, balance, and markers of bone turnover.

After adjustment for age, higher fT4 was associated with lower hip BMD at study entry and after 6-yr follow-up, and with increased bone loss at the hip after 6-yr follow-up (Supplemental Table 3). Higher fT4 was also associated with increased grip strength. After adjustment for BMI, smoking, and age, higher fT4 was still associated with reduced BMD and bone loss at the hip and with increased grip strength (Table 4).

After adjustment for age, higher fT3 was associated with lower hip BMD after 6-yr follow-up. Higher fT3 was also associated with higher pulse rate, increased grip strength, and better balance, and with reduced bone loss

at the lumbar spine and lower levels of sβCTX (Supplemental Table 3). These associations remained after further adjustment for BMI and smoking (Table 4).

TSH was not associated with alterations in BMD, bone turnover markers, or other extraskeletal parameters.

Discussion

These studies demonstrate that physiological variation in thyroid status is related to BMD and fracture in healthy euthyroid postmenopausal women. Higher fT4 and fT3 were associated with reduced BMD, and higher fT4 was associated with increased bone loss at the hip. Cox proportional hazards analysis revealed an increased risk of nonvertebral fracture in women with higher fT4 and fT3 and protection from nonvertebral fracture with higher TSH. Thus, thyroid status within the upper normal range was associated with reduced BMD at the hip and an increased risk of nonvertebral fracture.

Although there was increased loss of hip BMD with increasing fT4 and fT3 levels, there was reduced loss of BMD at the lumbar spine with increasing fT3 and no association between vertebral BMD and fT4. These findings are consistent with the lack of relationship between fT4, fT3, and vertebral fracture. The reason for the discrepancy between hip and lumbar spine is not known, although

animal studies reveal that decreases in BMD after treatment with thyroid hormones occur preferentially at the hip (23).

After adjusted regression, relationships between fT4 or fT3 and BMD were not independent of TSH or each other but were consistent with the positive correlation between fT4 and fT3 and their inverse relationship with TSH. This interaction results from the physiological regulation of thyroid status mediated by the HPT axis, in which fT3, fT4, and TSH are dependent variables. Because of the correlation between fT4 and fT3, their interaction with TSH, and the results of regression analyses in which independent relationships between fT3, fT4, or TSH and bone parameters were lacking, effects of T_3, T_4, or TSH on the skeleton cannot be separated. Indeed, because skeletal responses to thyroid hormones and TSH are interdependent, it is impossible to resolve this issue in any study of healthy euthyroid individuals in whom the HPT axis maintains thyroid hormones and TSH in a physiological reciprocal relationship (24).

Nevertheless, independent associations between fT3 and pulse rate, grip strength, and balance were also identified. These findings reveal novel interactions between fT3 and the cardiovascular and neuromuscular systems that could modify the relationship between thyroid status and bone maintenance and complicate analysis of the mechanisms by which the HPT axis regulates BMD and fracture susceptibility (2).

As indicated, the current study identified the well-known inverse relationship between thyroid hormones and TSH as well as the positive association between fT4 and fT3. Interestingly, fT4 and fT3 levels increased with age, but TSH was unchanged. This appears surprising given that the Whickham study in the United Kingdom and the Framingham, National Health and Nutrition Examination Survey (NHANES) III, and Colorado studies in the United States indicated that the frequency of hypothyroidism increases with age (25–28). By contrast, Austrian and Danish studies have shown that hyperthyroidism is more common in the elderly. This discrepancy is thought to result from an increased prevalence of thyrotoxicosis in the elderly in countries with low iodine supply, whereas an increased prevalence of hypothyroidism occurs in iodine-sufficient countries (29–31). Importantly, large population studies are designed to characterize the extent of thyroid dysfunction in the population rather than to identify a healthy euthyroid population based on stringent exclusion criteria as in the current study. Indeed, there is little information about thyroid status in healthy euthyroid elderly individuals. Reduced synthesis and secretion of TRH and TSH appear to result in TSH levels in the low-normal range, and reduced thyroidal secretion of T_3 and T_4 is compensated by decreased clearance of thyroid hormones.

The most extensive reviews of the subject suggest that in healthy elderly subjects, fT4 levels are normal, whereas TSH levels are lower than in younger individuals. fT3 may be lower in the very elderly, but this finding has been disputed (31, 32). To our knowledge, no studies have examined healthy euthyroid postmenopausal women specifically. Thus, the small increases in fT4 and fT3 with age along with maintenance of stable TSH concentrations in the OPUS population may reflect the normal situation in healthy euthyroid postmenopausal women. It is important to note, however, that differences in local iodine sufficiency may complicate overall thyroid status in the OPUS population.

The current study is the largest, most rigorous investigation of the relationship between normal thyroid status and BMD and the only prospective analysis relating euthyroid status to incident fracture. Previous studies are confounded by differences in experimental design, and none included measurement of T_3. Two prospective studies investigated relationships between thyroid status, BMD, and fracture. Van der Deure *et al.* (33) followed 1151 men and women age 55 yr or older for 8.7 yr and included subjects with nonthyroidal illness and individuals receiving drugs affecting bone metabolism. BMD correlated inversely with fT4 and positively with TSH, although the relationship with fT4 was stronger. No associations between fT4 or TSH and fracture were identified (33). Bauer *et al.* (1, 34) measured TSH in 600 women more than 65 yr of age from a population of 9704 that included subjects with thyroid disease and nonthyroidal illness and individuals receiving drugs affecting bone metabolism. Women with suppressed TSH had an increased risk of hip and vertebral fracture during 3.7 yr of follow-up, although no relationship between TSH and BMD was observed in a sample of 458 women from the same population followed for 5.7 yr (1, 34). Two cross-sectional studies investigated relationships between euthyroid status and BMD. In the NHANES III study, which included postmenopausal women from diverse ethnic groups receiving T_4 or drugs affecting bone metabolism, BMD increased as TSH increased within the reference range and osteopenia or osteoporosis occurred more frequently in women with low-normal TSH. No relationships between total T_4 and BMD were identified (35). Kim *et al.* (36) studied 959 postmenopausal Korean women who were considerably younger than OPUS subjects. Women with TSH between 0.5 and 1.1 mU/liter had lower BMD compared with individuals with TSH between 2.8 and 5.0 mU/liter, but no relationship between fT4 and BMD was seen (36).

The inverse relationship between fT4 and BMD in the current study is consistent with Van der Deure *et al.* (33),

but differs from NHANES III and Kim *et al.* (35, 36) in which BMD correlated with TSH. In rodents, TSH has been proposed to inhibit bone turnover directly, and TSH deficiency was suggested to cause high bone turnover osteoporosis in hyperthyroidism (37). Although skeletal consequences of thyrotoxicosis could result from TSH deficiency or thyroid hormone excess, the current study and previous studies cannot determine their relative importance in skeletal homeostasis because the physiological reciprocal relationship between thyroid hormones and TSH remains intact in all subjects studied, as discussed earlier (24).

Nevertheless, all available studies are consistent with our finding that thyroid status at the "hyperthyroid" end of the normal range is associated with lower BMD and increased risk of nonvertebral fracture. Furthermore, studies of bone loss in postmenopausal women treated for subclinical hyperthyroidism (38) or receiving T_4 (4) also reveal that mild degrees of hyperthyroidism are associated with reduced BMD. These studies additionally suggest that thyroid status and BMD are related continuous variables because optimization of euthyroid status in postmenopausal women with mild or subclinical hyperthyroidism reversed bone loss (4, 38). In further agreement, Grimnes *et al.* (39) studied 993 postmenopausal women and 968 men from Tromso and showed that individuals with TSH below the 2.5th percentile had lower forearm BMD, whereas those with TSH above the 97.5th percentile had higher femoral neck BMD.

Although the current study was comprehensive, some limitations remain. Data were unavailable regarding prior history of thyroid disease. Blood samples were from nonfasting subjects. Although samples were taken at the same time of day to mitigate effects of diurnal variation, especially in sβCTX, it remains difficult to compare bone turnover results with other studies that used fasting early morning specimens. During 6-yr follow-up, there were only seven hip fractures, and so the association between higher fT4 and incident hip fracture should be considered with particular caution. Nevertheless, higher fT4 and fT3 were also associated with an increased risk of nonvertebral fracture, and higher TSH levels were protective. Overall, the fracture data support the hypothesis that thyroid status at the "hyperthyroid" end of the normal range is associated with an increased risk of nonvertebral fracture. Established criteria for detection of vertebral fractures were employed in this study (13, 18), and it is interesting that there was no relationship between thyroid status and vertebral fracture. This is consistent with a meta-analysis of thyrotoxic patients that revealed only an increased risk of hip fracture (40) and with animal studies demonstrating

differential sensitivity to thyroid hormones at different skeletal sites (23).

Overall, the current findings demonstrate that thyroid status within the upper normal range in healthy euthyroid postmenopausal women is associated with lower BMD and an increased risk of nonvertebral fracture. Thus, thyroid status may be a physiological determinant of bone maintenance and strength.

Acknowledgments

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Supplemental Table 1 Number of subjects in whom data was available for each variable

Test variable	Number
Lumbar spine BMD at study entry	1272
Hip BMD at study entry	1268
Lumbar spine BMD at 6 years follow-up	789
Hip BMD at 6 years follow-up	792
TSH	1204
FT4	1212
FT3	1213
Pulse rate	1244
Balance	1240
Grip strength	1085
Osteocalcin	1217
P1NP	1217
sβCTX	1217
uNTXCr	1214
Incident vertebral fracture	474
Incident hip fracture	365
Incident non-vertebral fracture	401

Supplemental Table 2 BMD in women with fT4 and fT3 in the lowest and highest quintiles

BMD (mg/cm^2)	fT4 (mean ± SD)		fT3 (mean ± SD)	
	Lowest quintile 10.3 ± 0.9 pmol/L	Highest quintile 15.6 ± 1.1 pmol/L	Lowest quintile 2.6 ± 0.3 pmol/L	Highest quintile 4.9 ± 0.4 pmol/L
Lumbar spine BMD (study entry)	1057±171	1018±164*	1047±161	1028±175
Hip BMD (follow-up)	882±138	841±152*	881±146	862±152
Lumbar spine BMD (study entry)	1062±177	1039±174	1025±160	1046±168
Hip BMD (follow-up)	853±129	809±126*	863±139	819±129*
Change in lumbar spine BMD	-3.0±64.4	14.9±73.4	-14.4±57.5	14.9±72.1*
Change in hip BMD	-38.0±50.8	-47.4±47.8	-41.2±50.6	-47.9±49.7

*P≤0.05 highest vs lowest quintile

Supplemental Table 3 Relationship between thyroid function, BMD, bone turnover and extra skeletal parameters

	fT4 (pmol/L)			fT3 (pmol/L)			TSH (mU/L)		
	Model R^2	Adjusted for age β coefficient 95% CI (lower, upper)	p	Mode l R^2	Adjusted for age β coefficient 95% CI (lower, upper)	p	Mode l R^2	Adjusted for age β coefficient 95% CI (lower, upper)	p
BMD:									
At study entry:									
Lumbar spine BMD	0.019	-0.044 (-9.456, 1.573)	0.161	0.018	-0.034 (-20.009, 5.492)	0.264	0.018	-0.035 (-13.740, 3.744)	0.262
Hip BMD	**0.136**	**-0.054 (-7.883, -0.021)**	**0.049**	0.135	-0.036 (-15.716, 2.935)	0.179	0.134	0.008 (-5.505, 7.528)	0.761
6 years follow-up:									
Lumbar spine BMD	0.004	-0.065 (-13.346, 1.369)	0.110	0.001	0.038 (-8.727, 24.752)	0.348	0.000	-0.011 (-19.267, 14.501)	0.782
Hip BMD	**0.126**	**-0.094 (-10.722, -1.699)**	**0.007**	**0.124**	**-0.081 (-23.655, -2.249)**	**0.018**	0.122	0.064 (-0.349, 14.641)	0.062
Change in lumbar spine BMD	0.101	0.018 (-2.169, 3.503)	0.644	**0.117**	**0.126 (4.493, 17.267)**	**0.001**	0.101	0.005 (-6.067, 6.924)	0.897
Change in hip BMD	**0.008**	**-0.087 (-4.002, -0.344)**	**0.020**	0.004	-0.053 (-7.534, 1.155)	0.150	0.005	0.058 (-0.598, 5.468)	0.115
Bone turnover									
Osteocalcin	0.009	-0.027 (-0.450, 0.164)	0.362	0.008	-0.010 (-0.855, 0.602)	0.733	**0.012**	**-0.063 (-1.079, -0.062)**	**0.028**
P1NP	0.000	0.007 (-0.508, 0.657)	0.802	0.000	-0.010 (-1.613, 1.147)	0.741	0.002	-0.046 (-1.745, 0.183)	0.112
sβCTX	0.010	0.024 (-0.004, 0.010)	0.411	**0.013**	**-0.060 (-0.035, -0.001)**	**0.037**	0.012	-0.041 (-0.021, 0.003)	0.152
uNTXCr	0.006	0.033 (-0.404, 1.426)	0.273	0.007	0.046 (-0.422, 3.914)	0.114	0.005	-0.019 (-2.015, 1.019)	0.520
Extra skeletal parameters:									
At study entry:									
Pulse rate	0.004	0.031 (-0.135, 0.448)	0.292	**0.010**	**0.08 (0.283, 1.660)**	**0.006**	0.003	-0.011 (-0.580, 0.386)	0.694
Grip strength	**0.095**	**0.075 (0.041, 0.363)**	**0.014**	0.119	**0.172 (0.741, 1.494)**	**<0.001**	0.092	-0.040 (-0.450, 0.085)	0.182
Balance	0.074	0.056 (0.000, 0.114)	0.053	**0.080**	**0.099 (0.107, 0.379)**	**<0.001**	0.071	-0.021 (-0.132, 0.059)	0.454

Statistically significant results in bold