CLINICAL STUDY

Thyroid hormone status within the physiological range affects bone mass and density in healthy men at the age of peak bone mass

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Abstract

Context: The hormonal factors involved in the regulation of peak bone mass (PBM) in men have not been fully investigated. Apart from gonadal steroids and somatotrophic hormones, thyroid hormones are known to affect bone maturation and homeostasis and are additional candidate determinants of adult bone mass.

Objective: We aimed to investigate between-subject physiological variation in free and total thyroid hormone concentrations, TSH, and thyroid binding globulin (TBG) in relation to parameters of bone mass, geometry, and mineral density in healthy men at the age of PBM.

Design and setting: We recruited 677 healthy male siblings aged 25–45 years in a cross-sectional, population-based study. Areal and volumetric bone parameters were determined using dual-energy x-ray absorptiometry (DXA) and peripheral quantitative computed tomography (pQCT). Total and free thyroid hormones, TBG, and TSH were determined using immunoassays.

Results: Free and total thyroid hormone concentrations were inversely associated with bone mineral density (BMD) and bone mineral content (BMC) at the hip and total body (free triiodothyronine (FT₃), total T₃ (TT₃), and total T₄ (TT₄)) and at the spine (FT₃). TBG was negatively associated with BMC and areal BMD at all sites. At the radius, cortical bone area was inversely associated with TT₃, TT₄, and TBG, and trabecular bone density was inversely associated with free thyroxine, TT₄, and TBG. We observed inverse associations between cortical bone area at the mid-tibia and FT₃, TT₃, TT₄, and TBG. No associations between TSH and DXA or pQCT measurements were found.

Conclusion: In healthy men at the age of PBM, between-subject variation in thyroid hormone concentrations affects bone density, with higher levels of FT₃, TT₃, TT₄, and TBG being associated with less favorable bone density and content.

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Introduction

Peak bone mass (PBM) in young adults is a major determinant of bone mass later in life (1). Besides environmental factors such as exercise, smoking, and nutrition, genetic factors play a major role. These genetic and environmental influences are mediated in part by hormonal regulation of bone accrual during growth and maturation (1–3). The major and most extensively studied hormonal systems implicated in this regulation are the somatotrophic and the gonadal axes (3, 4). Another candidate hormonal determinant of PBM is thyroid hormone, known to have potentially marked effects on bone maturation and metabolism.

Thyroid hormone is essential for normal growth and bone development. Thyroid hormone deficiency results in delayed skeletal development, delayed bone age, and

growth arrest accompanied by epiphyseal dysgenesis (5). Hyperthyroidism in childhood, in contrast, induces not only accelerated skeletal development and growth with advanced bone age but also early premature fusion of the epiphyseal growth plates and cessation of growth (6).

In adults with hypothyroidism, bone turnover is reduced with a prolonged bone formation phase that leads to an increased mineralization phase and an apparent increase of bone mineral density (BMD). Hyperthyroidism in adulthood, on the other hand, is associated with increased bone turnover and a reduction in BMD at various skeletal sites due to increased cortical porosity and accelerated bone loss (7–11). Interestingly, population studies have shown that both hypo- and hyper-thyroidism in adults may be associated with an increased fracture risk (12).

However, little is known about the influence of substantial variation in thyroid parameters within the physiological euthyroid range on bone density and geometry. The effects of variation across the normal range of thyroid status on areal BMD (aBMD) and fracture susceptibility in middle-aged and older subjects were described in two recent studies. The first study reported the effects in postmenopausal women (13) and the second study in men and women above the age of 55 years (14). To our present knowledge, no studies have addressed the issue of influence of variation across the normal range of thyroid hormone status on PBM in male. We therefore studied the relationship between indices of thyroid hormone status within the physiological range and aBMD, volumetric BMD (vBMD) and bone geometry in healthy young male siblings at the age of PBM.

Materials and methods

Study design and population

A total of 767 young men were recruited from three semi-rural to suburban communities around Ghent, Belgium. Men aged 25–45 years were contacted by mail and asked whether they had a brother in the same age range also willing to participate. The study design and population characteristics have been previously described (3, 4, 15). The general aim of the study was to investigate the genetic and environmental determinants of PBM in men and therefore we sampled brothers together with their parents. For this publication, we only considered the brothers. After exclusions, 296 pairs of brothers were included in the study; 64 men were included as single participants, as their brothers could not participate in the study, 19 men were included as third brother in a family, and two as fourth brother. All brother analyses were done by family structure. The maximal age difference between brother pairs was arbitrarily set at 12 years. All participants were in good health and completed questionnaires about previous illness, lifestyle, physical activity, education, profession, nutrition, and smoking. Exclusion criteria were defined as illnesses or use of medication affecting body composition, hormone or bone metabolism: current or prolonged (> 3 months) use of glucocorticosteroids, anti-androgens, vitamin D supplements, insulin, thyroxine (T_4), and previous or current use of anti-epileptic drugs, hypogonadism, hyper- or hypothyroidism, cystic fibrosis, malabsorption or eating disorders, disorders of collagen metabolism or bone development, chronic renal failure, alcohol abuse, and autoimmune rheumatoid disease. The study protocol was approved by the ethical committee of the Ghent University Hospital, and informed consent was obtained from all participants. Smoking habits were registered as current or previous smoking.

Anthropometry and aBMD

Standing height was measured using a wall-mounted Harpenden stadiometer (Holtain Ltd, Crymych, UK). Body weight was measured in light indoor clothing without shoes. Anthropometric measurements were performed as described previously (4) according to the Anthropometric Standardization Reference Manual (16). aBMD at the lumbar spine and proximal femur (total hip region) of the nondominant limb was measured using dual-energy x-ray absorptiometry (DXA) with a Hologic QDR-4500A device (software version 11.2.1; Hologic, Bedford, MA, USA). The coefficient of variation (CV) was <1% as calculated from daily spine phantom measurements.

vBMD and bone geometry

A peripheral quantitative computed tomography (pQCT) device (XCT-2000, Stratec Medizintechnik, Pforzheim, Germany) was used to scan the dominant leg (tibia) and forearm (radius). The dominant side was selected to allow the assessment of the relationship between muscle area and bone parameters. The cortical volumetric BMD (vBMD; mg/cm^3), cortical cross-sectional area (mm^2), endosteal and periosteal circumferences, and cortical thickness (mm) were measured at the mid-radius (66% of bone length from the distal end) and mid-tibia (66%). Trabecular vBMD (mg/cm^3) was measured using a scan through the metaphysis (at 4% of bone length) at the nondominant radius. The CV for the calibration phantom was <1% as calculated from daily phantom measurements.

Biochemical determinations

Venous blood samples were obtained between 0800 and 1000 h after overnight fasting. All serum samples were stored at -80°C until batch analysis for parameters of thyroid function and bone metabolism.

Thyroid parameters included TSH, free T_4 (FT₄), free triiodothyronine (FT₃), total T_3 (TT₃), total T_4 (TT₄), thyroid binding globulin (TBG), as well as thyroperoxidase antibodies (TPOAb) and thyroglobulin antibodies (TgAb). Parameters of bone metabolism consisted of C-terminal telopeptide of type I collagen (CTX) and procollagen type 1 amino-terminal propeptide (P1NP). Commercial RIAs were used to determine serum levels of total T, sex hormone binding globulin (SHBG; Orion Diagnostica, Espoo, Finland), and estradiol (E_2 ; Clinical Assay; DiaSorin, Saluggia, Italy).

All thyroid parameter measurements with the exception of TT₃, TT₄, and TBG were performed using an immuno-electrochemiluminescence technique (Modular E 170, Roche Diagnostics GmbH). This assay has proven to give comparable results with other assays, according to the report from the IFCC WG for standardization of thyroid function tests (17).

Table 1 General characteristics and thyroid parameters of study participants ($n=677$) before exclusion of persons with positive thyroid autoimmunity. Variables are given as mean $\pm$ s.d. and when there was a non-Gaussian distribution, data were presented as median (first to third quartile).

Parameter	Values	Reference range
Age (years)	34.3 $\pm$ 5.5	
Height (cm)	179 $\pm$ 7	
Weight (kg)	81 $\pm$ 12	
BMI (kg/m ²)	25.2 $\pm$ 3.6	
Smokers (%)	22.9	
TSH (μ U/ml)	1.5 (1.14–2.14)	0.27–4.2
Free T ₃ (pg/dl)	391 $\pm$ 37	250–440
Free T ₄ (ng/dl)	1.44 $\pm$ 0.18	0.9–1.7
Total T ₃ (ng/dl)	147.4 (132.5–175.9)	87–180
Total T ₄ (μ g/dl)	10.2 (8.8–11.7)	5–12
TBG (mg/l)	18.3 $\pm$ 3.3	12–23
ATPO (U/l)	8.6 (6.8–10.9)	> 35 U/l: + auto-immunity
ATG (U/l)	12.3 (10–15.8)	> 115 U/l: + auto-immunity
Percentage autoimmunity	5%	

Conversion factor for FT₃ from pg/ml to pmol/l and for TT₃ from ng/dl to nmol/l is 0.0154; conversion factor for FT₄ from ng/dl to pmol/l and for TT₄ from μ g/dl to nmol/l is 12.87.

The intra- and interassay CV % were 1.0 and 6.1% for FT₄, 4.3 and 2.9% for FT₃, and 6.7 and 2.3% for TSH respectively. TT₃, TT₄, and TBG were measured using a RIA (DIAsource ImmunoAssays S.A., Nivelles, Belgium). The intra- and interassay CV were 4.7 and 3.7% for TT₃, 5.6 and 6.5% for TT₄, and 4 and 3.5% for TBG respectively.

Statistics

Descriptives are expressed as mean $\pm$ s.d. or median (first to third quartile) when criteria for normality were not fulfilled (Kolmogorov–Smirnov) and variables (bone parameters and hormone concentrations) were log transformed in subsequent linear models. Linear mixed-effects modeling was used to evaluate cross-sectional relationships in our study population, taking the interdependence of measurements within families into account. All analyses considering bone mass were adjusted for age, height, weight, and smoking status. Parameters of fixed effects were estimated via restricted maximum likelihood estimation and reported as standardized estimates of effect size (β) with their respective standard error. Associations were considered significant at P values <0.05 . Statistical analyses were performed using Spotfire S+ 8.1 (Insightful, Seattle, WA, USA) and Medcalc 11 (Mariakerke, Belgium). The formula for R^2 of Cox and Snell was used to calculate the proportion of variation explained by FT₃ and TBG.

Results

General characteristics and thyroid hormone status

The general characteristics, thyroid parameters, and pQCT measurements of the whole study population ($n=677$) are shown in Tables 1 and 2. Based on the exclusion criteria, subjects with thyroid disease were excluded a priori. We additionally excluded subjects with positive thyroid autoimmunity after determination of thyroid antibodies (TPOAb > 35 U/l or TgAb > 115 U/l), leaving 641 subjects. No differences in anthropometrics were observed between subjects with and without thyroid autoimmunity.

TT₄ was strongly ($\beta=0.51 \pm 0.03$, $P<0.0001$) and FT₄ was not associated with TBG ($\beta=-0.002$, $P=0.95$). TT₃ was also strongly associated with TBG ($\beta=0.56 \pm 0.03$, $P<0.0001$), as well as FT₃ ($\beta=0.17 \pm 0.03$, $P<0.0001$). Significant positive associations between free thyroid hormones and ratios of total hormones to TBG were observed ($\beta=0.24 \pm 0.04$, $P<0.0001$). No differences in anthropometrics were observed between subjects with and without thyroid autoimmunity.

Thyroid function in healthy young men in relation to age, body composition, and lifestyle factors

No effects of age, body height, or weight were observed on TSH, whereas free thyroid hormone concentrations decreased with age (FT₄: $\beta=-0.18 \pm 0.04$, $P<0.001$; FT₃: $\beta=-0.14 \pm 0.04$, $P<0.001$). TBG concentrations were positively associated with BMI ($\beta=0.13 \pm 0.04$, $P=0.001$) but not with age. FT₄, but not TT₄, concentrations were positively associated with body height ($\beta=0.09 \pm 0.04$, $P=0.03$), whereas no effect of body weight on FT₄ or TT₄ was observed. FT₃ and TT₃ were positively related to body weight ($\beta=0.09 \pm 0.04$, $P=0.03$ and $\beta=0.1 \pm 0.04$, $P=0.01$ respectively).

Current smokers displayed higher FT₄ (1.49 ± 0.20 vs 1.43 ± 0.18 ng/dl, $P=0.002$), higher FT₃ (4.04 ± 0.37 vs 3.87 ± 0.35 pg/ml, $P<0.001$), and lower TSH (1.4 (1.05–1.94) vs 1.53 (1.18–2.06) μ U/l, $P=0.04$) compared with nonsmokers or previous

Table 2 Descriptives of peripheral quantitative computed tomography bone parameters of study participants ($n=677$) before exclusion of persons with positive autoimmunity.

Radius	
Cortical bone area (mm ²)	101.3 $\pm$ 13.6
Cortical bone density (mg/cm ³)	1100 $\pm$ 35
Trabecular bone area (mm ²)	185.4 $\pm$ 26.1
Trabecular bone density (mg/cm ³)	228 $\pm$ 40
Tibia	
Cortical bone area (mm ²)	364 $\pm$ 47
Cortical bone density (mg/cm ³)	1112 $\pm$ 24

Figure 1 aBMD and BMC according to quartiles of FT₃.

smokers. All further analyses were adjusted for age, body height, weight, and smoking.

Thyroid hormone status in relation to areal bone mass (DXA)

We observed inverse associations of both FT₃ and TT₃ with bone mineral content (BMC) at the spine, the hip, as well as the whole body. In addition, areal BMD (aBMD) at both the level of the hip and the whole body was inversely associated with FT₃ and TT₃. At the level of the spine, aBMD was also inversely related to FT₃, but a similar trend for TT₃ was only borderline significant (Fig. 1, Table 3). FT₃ explained 0.52% of variation of hip BMD and 0.41% for BMD at the spine, when adjusted for age, length, weight, and smoking status.

Similarly, TT₄ was inversely associated with BMC at the three measurement sites, and with aBMD at the level of the hip and the total body (Table 3).

There was no significant relationship between either FT₄ or TSH and BMC or BMD as measured by DXA (Table 3).

We observed a strongly negative association between TBG and DXA parameters at all levels (Table 3). When adjusted for age, length, weight, and smoking status, TBG explained 2.6% of variation in total hip BMD, 0.66% of variation in BMD at the spine, and 1.6% of variation for the whole body. When introducing TBG in our statistical model as a covariate, only FT₃ remained significantly associated with aBMD at spine, hip, and whole body, although this association was weakened. The associations between total thyroid hormones and DXA measurements after adjustment for TBG were no longer significant (Data not shown).

Thyroid hormones in relation to bone geometry and volumetric bone density

The associations between thyroid hormones and pQCT-derived bone parameters are shown in Fig. 2. At the radius, cortical bone area was inversely associated with TT₃ ($\beta = -0.09 \pm 0.04$, $P = 0.01$), TT₄ ($\beta = -0.09 \pm 0.04$, $P = 0.01$), and TBG ($\beta = -0.12 \pm 0.04$, $P = 0.0008$) and trabecular bone density was inversely associated with FT₄ ($\beta = -0.08 \pm 0.04$, $P = 0.04$), TT₄ ($\beta = -0.1 \pm 0.04$, $P = 0.005$), and TBG ($\beta = -0.13 \pm 0.04$, $P = 0.001$). Inverse associations between cortical bone area at the mid-tibia and FT₃ ($\beta = -0.08 \pm 0.04$, $P = 0.02$), TT₃ ($\beta = -0.12 \pm 0.04$, $P = 0.0006$), TT₄ ($\beta = -0.10 \pm 0.03$, $P = 0.003$), and TBG ($\beta = -0.14 \pm 0.04$, $P = 0.0001$) were observed. However, the stronger described associations between total thyroid hormones and cortical bone area and trabecular bone density disappeared when TBG was added to the model as a covariate, similar to our observations with DXA measurements (data not shown).

Table 3 Relationship between TSH, thyroid hormone concentrations, thyroid binding globulin, and dual-energy x-ray absorptiometry bone parameters. Results from mixed effects models to account for family structure and adjusted for age, height, weight, and smoking, after exclusion of positive thyroid auto immunity. Data are presented as standardized estimates $\pm$ S.E.M.

	TSH (μ U/ml)	FT ₃ (pg/ml)	TT ₃ (ng/dl)	FT ₄ (ng/dl)	TT ₄ (μ g/dl)	TBG (mg/dl)
aBMD lumbar spine (g/cm ²)	$\beta = -0.003 \pm 0.04$ $P = 0.94$	$\beta = -0.10 \pm 0.04$ $P = 0.008$	$\beta = -0.08 \pm 0.04$ $P = 0.05$	$\beta = -0.03 \pm 0.04$ $P = 0.5$	$\beta = -0.05 \pm 0.04$ $P = 0.2$	$\beta = -0.12 \pm 0.04$ $P = 0.003$
aBMD total hip (g/cm ²)	$\beta = 0.03 \pm 0.03$ $P = 0.40$	$\beta = -0.08 \pm 0.03$ $P = 0.02$	$\beta = -0.14 \pm 0.04$ $P = 0.002$	$\beta = -0.05 \pm 0.04$ $P = 0.15$	$\beta = -0.11 \pm 0.04$ $P = 0.002$	$\beta = -0.17 \pm 0.04$ $P < 0.0001$
aBMD whole body (g/cm ²)	$\beta = 0.008 \pm 0.04$ $P = 0.83$	$\beta = -0.09 \pm 0.03$ $P = 0.007$	$\beta = -0.11 \pm 0.04$ $P = 0.01$	$\beta = -0.07 \pm 0.04$ $P = 0.053$	$\beta = -0.1 \pm 0.04$ $P = 0.006$	$\beta = -0.14 \pm 0.04$ $P = 0.0001$
BMC lumbar spine (g)	$\beta = -0.005 \pm 0.03$ $P = 0.90$	$\beta = -0.08 \pm 0.03$ $P = 0.02$	$\beta = -0.12 \pm 0.05$ $P = 0.009$	$\beta = -0.03 \pm 0.04$ $P = 0.5$	$\beta = -0.07 \pm 0.04$ $P = 0.004$	$\beta = -0.15 \pm 0.04$ $P < 0.0001$
BMC total hip (g)	$\beta = 0.04 \pm 0.03$ $P = 0.29$	$\beta = -0.08 \pm 0.03$ $P = 0.02$	$\beta = -0.15 \pm 0.05$ $P = 0.002$	$\beta = -0.05 \pm 0.04$ $P = 0.16$	$\beta = -0.1 \pm 0.03$ $P = 0.002$	$\beta = -0.16 \pm 0.03$ $P < 0.0001$
BMC whole body (g)	$\beta = 0.01 \pm 0.03$ $P = 0.64$	$\beta = -0.08 \pm 0.03$ $P = 0.006$	$\beta = -0.14 \pm 0.05$ $P = 0.004$	$\beta = -0.04 \pm 0.03$ $P = 0.20$	$\beta = -0.08 \pm 0.03$ $P = 0.01$	$\beta = -0.14 \pm 0.03$ $P < 0.0001$

Figure 2 Standardized estimates representing associations between TSH, FT₃, FT₄, and TBG and cortical and trabecular bone area and density, adjusted for age, height, weight, and current smoking at the radius and tibia (bar=95% confidence limit).

No associations between TSH and pQCT-derived bone parameters were observed (Fig. 2).

Thyroid hormones in relation to bone turnover markers

There was no relationship between biochemical markers of bone formation (P1NP) or resorption (CTX) and free or total thyroid hormones and TSH (e.g. FT₃ and P1NP: $P=0.6$). The higher described association between FT₃ and aBMD and BMC did not change when adding markers of bone turnover as a covariate (e.g. addition of P1NP: aBMD hip and FT₃: $P=0.005$, aBMD spine and FT₃: $P=0.004$, aBMD total body and FT₃: $P=0.007$).

TBG in relation to sex steroid hormones and SHBG

In order to explore possible mechanisms underlying the association between TBG and bone parameters, we investigated the relationship between sex steroids and TBG. No associations between estrogens (free and total) or testosterone (free and total) and TBG were observed. The associations between TBG and DXA and pQCT measurements remained when sex steroids were added to the model (data not shown). The negative associations between TBG and DXA and pQCT parameters remained intact when SHBG was added to the model as a covariate.

Discussion

This study demonstrates that between-subject variation in thyroid hormone status within the physiological range is related to aBMD and vBMD in healthy men at the age of PBM. Higher FT₃, TT₃, TT₄, and TBG are associated with lower aBMD and BMC at various

skeletal sites. However, after correction for TBG, only FT₃ remains significantly associated with aBMD. Measuring vBMD and bone geometry by pQCT, we observe inverse relationships between FT₄, TT₄, and TBG and trabecular bone density as well as inverse associations between FT₃, TT₃, TT₄, and TBG and cortical bone area.

The hormonal determinants of bone mass in men have not been fully investigated. Whereas our current understanding on the effects of thyroid hormone status on the skeleton in the adult is merely based on the studies in postmenopausal women or in situations characterized by thyroid dysfunction, this is the first study that considers young men at the age of PBM. Moreover, this report describes relationships between thyroid status and bone parameters determined not only by two-dimensional areal DXA estimations but also by three-dimensional geometric and volumetric pQCT measurements in men.

Since von Recklinghausen first described a case of thyrotoxic bone disease in 1891, hyperthyroidism is a well-known cause of osteopenia and osteoporosis (18). Studies on the relation between hyperthyroidism, aBMD, and fracture risk confirm a decrease of aBMD and an increase of fracture risk. This risk augments with advancing age and returns to normal upon treatment of the hyperthyroid state (9, 19, 20). Untreated subclinical hyperthyroidism in postmenopausal women has also been associated with decreased aBMD and a higher fracture risk compared with women who received treatment, although findings have not been unequivocal (21–24). Little data exists on the influence of subclinical hyperthyroidism on the skeleton in men (5).

Thyrotoxicosis results in increased global bone turnover as indicated by elevated values of the biochemical markers of bone formation and bone resorption (5), which in general correlate well with thyroid hormone concentrations, especially in cases of (subclinical) hyperthyroidism (3). Furthermore, histomorphometric data indicate that excessive thyroid hormone results in a shortening of the different phases of the remodeling cycle and an ~10% relative deficit of bone formation per cycle (8). Therefore, the decrease in BMD observed in thyrotoxicosis is explained both by an (reversible) expansion of the so-called remodeling space, with increased cortical porosity, and by accelerated bone loss that might primarily affect trabecular bone. Conversely, hypothyroidism is characterized by a lower global bone turnover and prolongation of the remodeling cycle with a small positive bone balance per cycle. When situations of excessive or deficient thyroid hormone are prolonged, the effects on bone remodeling can potentially result in a decreased or increased mineralization degree of bone tissue respectively (7, 8).

This study differentiates from most clinical data on the relationship between thyroid and skeletal status first in that we considered young men at the age of PBM and secondly in that we studied the influence of variations in

thyroid hormone status within the physiological range. Therefore, we excluded subjects with a history of thyroid disease or treatment with thyroid hormone or with positive levels of thyroid autoantibodies from our analyses. We observed an inverse relationship between FT_4 and log TSH in our cohort, but this association was no longer significant after exclusion of subjects with thyroid autoimmunity, compatible with the premise that we studied euthyroid subjects. Our study differs from the preceding studies because we also investigated associations between thyroid hormone levels and volumetric bone density and cross-sectional bone geometry. Our data show that thyroid status is associated not only with trabecular bone density but also with bone size (cortical bone area).

The novelty of our results is that between-subject physiological variation in apparent thyroid hormone exposure is associated with skeletal characteristics in men at the age of PBM and that higher levels of thyroid hormones, even within the normal range, have a negative influence on parameters of bone strength at this young adult age. The negative association between thyroid hormone levels and aBMD is seen for all assessed skeletal sites, although most consistently for FT_3 .

In line with our findings in young men, van der Deure *et al.* (14) reported in elderly men and women a negative association between FT_4 and aBMD and, when corrected for BMI, no significant association with TSH, although there was a trend (for a positive relationship between TSH and aBMD). However, FT_3 was not determined in their study. Murphy *et al.* (13) found that both higher FT_4 and FT_3 were associated with reduced aBMD in euthyroid postmenopausal women. We found associations only between FT_3 and BMD or BMC, but not with FT_4 , which is in agreement with the premise that most actions of thyroid hormone in the body are mediated by the active form of thyroid hormone, T_3 . More specifically in bone, remodeling is considered to be predominantly mediated by via $TR\alpha$ (25).

However, expression of deiodinases in osteoblasts has been demonstrated (26). Not only circulating serum T_3 but also local production of T_3 in bone can exert effects on bone. Nevertheless, other regulatory mechanisms can play a role and the local production of T_3 is dependent on varying concentrations of serum T_4 . Our data provides evidence for a negative effect of circulating T_3 on bone, though this does not exclude local effects of deiodinase activity in bone, apart from the described systemic effects.

Because of the observation of significant negative associations between both total thyroid hormones and TBG and bone parameters, the question raised was if TBG could be the main mediator for the associations observed with total thyroid hormones. Indeed, after correction for TBG, the associations between total thyroid hormones and DXA and pQCT parameters became insignificant. We hypothesized that the effects

of TBG on bone might be mediated by sex steroids, because administration of estrogens and androgens is known to be associated with an increase and decrease in the level of TBG respectively (27). However, in our study, TBG was not associated with the levels of free or total endogenous estrogen or testosterone. Furthermore, the stronger described associations between TBG and bone parameters remained significant when sex steroids or SHBG were introduced to our model as covariates. Moreover, the associations of TBG and bone parameters were independent of SHBG and appeared to be associated more strongly to bone than SHBG. Another argument in favor of an independent role for TBG apart from sex steroids is the consistently negative association between TBG and bone parameters, whereas associations between sex steroids (E_2) or SHBG and bone parameters are merely positive.

As TBG is negatively correlated with thyroid state (it decreases in hyperthyroidism and increases in hypothyroidism) (28, 29), one would theoretically expect that the observed relationship with bone would be different. Nevertheless, the associations between TBG and bone parameters are in the same direction as the thyroid hormones themselves (negatively), as is the case with SHBG and sex steroids (positively). In this regard, the binding proteins seem to potentiate the effect of their corresponding free hormones. Underlying these observations, a hypothetical transporter, facilitating hormone transfer across cellular membranes, could be active. However, no transporter has been described for TBG and the endocytic megalin-carrier pathway for SHBG remains unproven. TBG could also be a marker for nutritional status because we indeed observed a positive relation between TBG and BMI, but a positive relationship between TBG and bone parameters would be expected and malnutrition was not present in our population of healthy young men.

Further studies should be designed to elucidate whether TBG is a real determinant of bone mass or only a marker of another unknown determinant affecting bone.

This association study does not allow to establish a causal relationship or to unravel underlying pathophysiological mechanisms. In the context of our primary focus of interest, i.e. the determinants of PBM in men, one of the major questions raised by our findings is whether the recorded associations reflect actual effects of thyroid hormone on bone homeostasis in these adult young men and/or earlier effects of thyroid hormone that occurred during bone accrual. An argument in favor of the latter possibility might be provided by the fact that we could not observe an association between thyroid hormone levels and biochemical markers of bone turnover and no effect of these markers on the relation between thyroid hormones and aBMD. However, values for biochemical markers of bone turnover are rather high, with a broad range in men in their twenties before decreasing to a nadir toward the

age of fifties (30, 31). It is thus possible that an effect of thyroid hormone on these variables has been obscured by other stronger influences. Another argument in favor of effects of thyroid hormone action on bone acquisition and maturation is our observation of associations of thyroid hormone levels with pQCT-derived geometric bone parameters. Changes of the periosteal circumference by periosteal modeling occur essentially and rapidly during growth but are slow processes in young adulthood (32).

Recently, some authors have challenged the conventional view that skeletal responses to abnormal thyroid status result solely from altered T_3 action in the bone and have proposed TSH as a negative regulator of bone turnover (23, 33–37). For example, Morris *et al.* (35) and Kim *et al.* (23) observed associations between low-normal serum TSH levels and osteopenia and osteoporosis prevalence, together with a graded increase in aBMD with increasing TSH in postmenopausal women. van der Deure *et al.* (14) found in the Rotterdam study – a population of elderly Caucasians – that femoral neck aBMD as well as a DXA derived cortical thickness estimation increased with serum TSH, but this relationship became non-significant when corrected for BMI. Mazziotti *et al.* (37) observed that TSH values in the lower part of the normal range were associated with vertebral fractures in euthyroid postmenopausal women, independently of FT_4 , aBMD, and age. These findings are opposed by results from the Tromsø and the HUNT 2 study, where, as in our population, no relation of TSH with aBMD was observed within the normal range of serum TSH (34, 36).

Moreover, these epidemiological studies cannot differentiate between the effects of excess thyroid hormone or TSH deficiency because the hypothalamo-pituitary–thyroid axis with its feedback regulation remains intact (38). Despite the demonstration of TSH receptor expression in osteoblasts and osteoclasts (33), *in vitro* studies have not established a clear and consistent role for TSH in bone cells so far. In contrast, elegant studies in mice provide strong arguments against direct effects of TSH on bone. Bassett *et al.* (38) observed that deletion of $TR\alpha$ in mice resulted in decreased T_3 action in bone without affecting systemic thyroid status. Delayed ossification and osteosclerosis indicate that responses to reduced T_3 action in bone predominate when TSH levels are normal. Similarly, mutation or deletion of $TR\beta$ results in increased T_3 action in bone and increased levels of TSH. Accelerated ossification and osteoporosis confirms that the effects of T_3 excess predominate even when TSH levels are also increased, providing further evidence supporting that skeletal responses to hypo- and hyperthyroidism are mediated by T_3 acting via $TR\alpha$ and not by a direct effect of TSH on bone. In our cohort of young men, we could not find any relation between TSH and aBMD, BMC or volumetric trabecular and cortical density nor bone geometry, hereby supporting the conventional view.

We can conclude from this study that between-subject variation of thyroid hormones in the physiological range has an effect on bone mass, density, and geometry in healthy young men at the age of PBM, with higher levels of FT_3 , TT_3 , TT_4 , and TBG being associated with lower aBMD and BMC at various skeletal sites.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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