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Thyroid function tests in the reference range and fracture risk: individual participant analysis of prospective cohorts

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ABSTRACT

Context

Hyperthyroidism is associated with increased fracture risk, but it is not clear if lower TSH and higher free thyroxine (FT4) in euthyroid individuals are associated with fracture risk.

Objective

To evaluate the association of TSH and FT4 with incident fractures in euthyroid individuals.

Design

Individual participant data analysis.

Setting

Thirteen prospective cohort studies with inception between 1981 and 2002.

Participants

Adults with baseline TSH 0.45-4.49 mIU/L.

Main Outcome Measures

Primary outcome was incident hip fracture. Secondary outcomes were any, non-vertebral, and vertebral fractures. Hazard ratios (HR) with 95% confidence interval (CI) were adjusted for age and sex. For clinical relevance, we studied TSH according to five categories: 0.45-0.99mIU/L; 1.00-1.49mIU/L; 1.50-2.49mIU/L; 2.50-3.49mIU/L; 3.50-4.49mIU/L (reference). FT4 was assessed as study-specific standard deviation increase, because assays varied between cohorts.

Results

During 659,059 person-years, 2,565/56,835 participants had hip fracture (4.5%; 12 studies with available data). The pooled adjusted HR (95%CI) for hip fracture was 1.25 (1.05-1.49) for TSH 0.45-0.99 mIU/L, 1.19 (1.01-1.41) for TSH 1.00-1.49 mIU/L, 1.09 (0.93-1.28) for TSH 1.50-2.49 mIU/L, and 1.12 (0.94-1.33) for TSH 2.50-3.49 mIU/L (*P* for trend = 0.004). Hip fracture was also associated with FT4 (HR 1.22, 95%CI 1.11-1.35 per one standard deviation increase in FT4). FT4 only was associated with any and non-vertebral fracture. Results remained similar in sensitivity analyses.

Conclusions

Among euthyroid adults, lower TSH and higher FT4 are associated with an increased risk of hip fracture. These findings may help refine the optimal ranges of thyroid function tests.

Registration: The protocol was published on PROSPERO (registration number CRD42016039125).

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INTRODUCTION

Overt hyperthyroidism is a well-known risk factor for fracture and is associated with decreased bone mineral density (BMD) (1). We recently showed that subclinical hyperthyroidism was also associated with increased fracture incidence (2). Thyroid hormones stimulate bone turnover acting directly and indirectly on osteoclasts and osteoblasts (3). Anabolic action is net during growth, but in adults, catabolic action leads to greater bone loss and higher fracture risk (3). Thyroid hormones might also decrease muscular strength and coordination, and increase the risk of fall (4, 5). Administering TSH reduces bone resorption and increases bone formation in post-menopausal women monitored for thyroid cancer (6). Conversely, high TSH levels can degrade bone quality by increasing cortical, rather than trabecular bone.

The reference range for thyroid function tests – "euthyroidism" – is defined by the 2.5 to 97.5 percentiles in an apparently healthy population. However, the studies from which TSH reference range was derived did not exclude participants with occult or underlying disease, e.g. those with positive anti-thyroid antibodies, which might bias the reference range towards higher TSH values (7, 8). In medicine, reference ranges can be derived from normative data, as for thyroid function tests, or preferably determining levels associated with important risks or outcomes, as for lipids, or blood pressure.

TSH within the lower reference range has been associated with osteoporosis and fracture mostly in cross-sectional studies of healthy post-menopausal women, but prospective data are limited and conflicting (5, 9-13). If we can better understand the association between TSH and health outcomes, we could make more accurate estimates of fracture risk, which would help refine thyroxine treatment targets. We hypothesized that lower TSH and higher FT4 in euthyroid participants were associated with increased risk of fractures. We therefore aimed to assess the association between TSH within the reference range, free thyroxine (FT4), and fracture risk by analyzing individual participant data (IPD) of population-based prospective cohort studies participating to the international Thyroid Studies Collaboration (2, 14).

METHODS

Data source, searches and study selection

The study protocol was registered on PROSPERO prior to study conduct (available on http://www.crd.york.ac.uk/PROSPERO; registration number: CRD42016039125).

We updated our previous systematic literature search, which had identified in Ovid (MEDLINE) and EMBASE from inception to March 2015 all prospective cohorts of adults with baseline TSH and FT4 measurement and follow-up evaluation for incident fracture (2). Additionally, we searched for studies with participants with only euthyroidism, which may have been omitted in our initial search. Our Ovid (MEDLINE) and EMBASE search (until 05/19/2016) used following medical search terms: *euthyroid, euthyroidism* or *normal TSH* and *fractures* or *osteoporosis*. After retrieving studies according to titles and abstracts, two authors (C.E.A. and D.S.) independently reviewed full-texts to confirm study eligibility. Disagreements were resolved by consensus with a third author (N.R.). We also requested unpublished fracture data from all cohorts of the Thyroid Studies Collaboration (2, 14-17). Exclusion criteria were: 1) cohorts using first-generation TSH assays because these assays were not sensitive enough; 2) studies with only participants aged <18 years; 3) studies with only participants with thyroid medication (thyroxine or anti-thyroid drugs); 4) studies with only participants with TSH outside the reference range (<0.45mIU/L or >4.49mIU/L); and, 5) studies exclusively on participants after thyroid surgery. Agreement between reviewers was 100% (K =1.00). For the IPD analysis, we included all participants aged ≥18 years at enrollment with measured TSH at baseline evaluation, and fracture assessment, as defined below, at follow-up.

Data extraction and quality assessment

If the cohorts identified met our eligibility criteria, they were invited to provide IPD. Each study was approved by its local ethics committee. All participants gave informed consent for the original studies. We collected information on demographics, anthropometrics, medications, other risk factors for fracture, history of thyroid disorders, BMD and incident fracture.

Risk of bias and study quality were independently assessed by C.E.A and D.S., using the following Newcastle-Ottawa Quality Assessment Scale items (18): 1) cohorts selection; 2) cohorts representativeness; 3) ascertainment of exposure; 4) availability of relevant confounding factors for adjustment; 5) outcome assessment based on objective fracture assessment, with adjudication procedure for fractures other than hip; 6) length of follow-up; 7) adequacy of follow-up; 8) researchers/participants/physicians blinding to thyroid values; and, 9) publication status. In sensitivity analyses, we excluded cohorts that did not meet one or more item(s).

Data synthesis and analysis

Definition of thyroid function

All included studies used a third-generation TSH radioimmunoassay. Details on the assays used for TSH and FT4 measurement are described in **Supplemental Table 1**. To maximize comparability, we used uniform TSH thresholds based on previously established thresholds, as done in previous reports of the Thyroid Studies Collaboration (2, 14). We defined euthyroidism as TSH 0.45-4.49 mIU/L. For clinical relevance, we separated TSH values into five categories: 0.45-0.99mIU/L; 1.00-1.49mIU/L; 1.50-2.49mIU/L; 2.50-3.49mIU/L; 3.50-4.49mIU/L. The later was used as reference category because we hypothesized, based on our previous publication (2), that lower TSH might be associated with higher fracture risk. Because of different FT4 reference ranges across studies, we used standard deviation (SD) rather than specific cut-offs. FT4 was available for all but two studies in the euthyroid range (19, 20).

Definition of outcomes

Our primary outcome was incident hip fracture, including femoral neck, pertrochanteric, and subtrochanteric fractures, as previously defined (2). Briefly, we excluded pathologic (i.e. associated with metastasis or rare bone disease) and periprosthetic fractures. Any, non-vertebral and clinical vertebral incident fractures were secondary outcomes. We excluded 1) vertebral fractures diagnosed with only radiologic imaging to keep focus on clinical relevance; 2) cervical and sacral vertebral fractures because fractures at these locations are usually associated with trauma rather than osteoporosis. “Any fractures” included fractures at any location, except for skull, face, ankle, finger, or toe, since these are not related to osteoporosis. “Non-vertebral fractures” was the same as “any fractures" except it excluded vertebral fractures. For any and non-vertebral fractures, we excluded cohorts that collected fracture data on only part of the skeleton. **Supplemental Table 2** describes fracture definitions by study.

Statistical analyses

We used a shared frailty Cox regression model with random-effects at study level to conduct an IPD meta-analysis, which used data from all included cohorts to assess the relationship of incident fractures with TSH categories and FT4, respectively (21, 22). The random-effects accounted for the between-study variation caused by different definitions of TSH reference range across the studies, incorporating the extra uncertainty in the confidence intervals. We used Schoenfeld residuals to test the proportional-hazards assumption (23). Results were presented as hazard ratios (HR) compared to the reference category. Time-to-event was defined for each outcome from baseline TSH measurement to first fracture event. We adjusted primary analyses for age and sex, and then for other risk factors for fracture (body mass index [BMI], smoking, and history of diabetes), because they might mediate the association between thyroid function tests and fractures. We conducted following predefined sensitivity analyses: 1) excluding participants with thyroid medication (thyroxine or anti-thyroid medication) at baseline; 2) excluding participants with thyroid-altering medication at baseline (thyroid medication, oral corticosteroids, amiodarone, iodine); 3) excluding participants with anti-fracture medication at baseline (bisphosphonate, calcitonin, selective estrogen receptor modulator, parathyroid hormone); 4) including only studies with formal fracture adjudication; 5) including only studies that uniformly defined fractures (except for hip fracture, since it has a common definition and is rarely reported in error); 6) excluding cohorts with loss to follow-up rates >5%; 7) excluding participants who developed overt or subclinical thyroid dysfunction over time; 8) including only participants with TSH remaining within the reference range during follow-up; and 9) further adjusting for BMD, which reflects bone loss and may be a potential mediator between TSH or FT4 and incident fractures. In this last analysis, we used BMD as a continuous variable, and included only studies that used dual energy X-ray absorptiometry (DXA) devices with femoral neck BMD for hip fractures (available for six studies) (5, 10, 14, 24-26), lumbar spine BMD for vertebral fractures (available for one study) (10), and whole body BMD for any fractures (available for one study) (10) . We conducted predefined stratified analyses by sex, age (<75 versus ≥75 years), and duration of follow-up (<5 versus ≥5 years).

For the FT4 analysis, we used the whole range of FT4 values including only participants with TSH within the reference range. FT4 values were converted to ng/mL (12.87pmol/L = 1ng/mL). We used study-specific SD to assess fracture risk per one SD increase in FT4 because FT4 assays varied between cohorts (14). We performed the same sensitivity and stratified analyses as for TSH.

We used STATA release 13.1 for all analyses (StataCorp LP, College Station, Texas). All tests were two-sided, at a 0.05 level of significance.

RESULTS

Our updated literature search identified nine additional reports (**Supplemental Figure 1**) (2). Eight of them concerned studies already identified in our previous search (2). The newly identified study (Study of Osteoporotic Fractures) (19) agreed to participate. We excluded the Nagasaki Adult Health Study (27), because it used first-generation TSH assays, which have a low functional sensitivity (1mIU/L) (28). For the same reason, this study had been included in our previous work (2) only in the analysis on subclinical hypothyroidism, but not on subclinical hyperthyroidism (16). We included thirteen studies (5, 10, 14, 17, 19, 25, 26, 29-34) from the USA, Europe, and Australia with 61,959 participants, and a median duration of follow-up of 12.1 years (interquartile range [IQR] 8.5-12.9), totaling 659,059 person-years. Median age was 64 (range 18-102) with 60.5% women (**Table 1**). Median (IQR) TSH was 1.60mIU/L (1.10-2.30); 3.1% of participants used thyroid medication at baseline and 5.5% during follow-up; 17.7% had a TSH 0.45-0.99mIU/L, 24.8% 1.00-1.49mIU/L, 37.4% 1.50-2.49mIU/L, 14.2% 2.50-3.49mIU/L and 5.9% 3.50-4.49mIU/L. Hip fracture occurred in 2,565 participants (4.5%; 12 studies), any fracture in 2,333 (8.9%; 9 studies), non-vertebral fracture in 1,874 (8.5%; 9 studies), and vertebral fracture in 263 (1.3%; 7 studies). Overall quality was good (**Supplemental Table 3**): one study reported loss to follow-up >5% (5), four did not perform formal fracture adjudication (25, 29, 32, 33), and three had not published fracture data in a separate manuscript (17, 29, 33).

Tests of the proportional-hazards assumption on the basis of Schoenfeld residuals indicated that assumptions were met for all analyses ($P > 0.11$ for all).

Thyroid function and hip fractures

Compared with the reference group (TSH 3.50-4.49mIU/L), pooled age- and sex-adjusted HR (95% CI) for hip fracture was 1.25 (1.05-1.49) for TSH 0.45-0.99mIU/L, 1.19 (1.01-1.41) for TSH 1.00-1.49mIU/L, 1.09 (0.93-1.28) for TSH 1.50-2.49mIU/L, and 1.12 (0.94-1.33) for TSH 2.50-3.49mIU/L (*P* for trend 0.004, **Figure 1**). After adjusting for BMI, smoking status, and history of diabetes, HR (95% CI) was 1.24 (1.03-1.49) for TSH 0.45-0.99mIU/L compared with the reference group, while HR (95%CI) for TSH 1.00-1.49mIU/L was somewhat attenuated and no longer statistically significant (1.15 [0.97-1.38]). The risk of hip fracture in participants with TSH 0.45-0.99mIU/L remained significantly higher in all sensitivity analyses, and was even higher after adjusting for femoral neck BMD (**Table 2**). For TSH 1.00-1.49mIU/L, the risk of hip fractures remained significantly higher in all sensitivity analyses, except after adjusting for femoral neck BMD, or after excluding participants with thyroid-altering medication at baseline. This association remained not significant for TSH 1.50-2.49mIU/L, or TSH 2.50-3.49mIU/L. We found no significant interaction for sex, age, or duration of follow-up (**Supplemental Figure 2**), although confidence intervals were larger and point estimates smaller for age <75 years and follow-up <5 years. Conversely, there was significant interaction for publication status with a HR (95% CI) of 1.35 (1.13-1.61) for the ten studies that published risk of hip fracture associated with thyroid function tests in a separate manuscript, and 0.44 (0.21-0.90) for the two studies (17, 29) that did not previously publish hip fracture data associated with thyroid function tests in a separate article (*P* for interaction 0.0001, **Supplemental Table 4**).

The HR (95% CI) for hip fracture was 1.24 (1.12-1.37) per one SD increase in FT4 (**Figure 2**). We found no significant interaction with sex, age, duration of follow-up, or publication status of hip fracture data (**Figure 2, Supplemental Table 4**), although point estimate was smaller when follow-up was <5 years. All sensitivity analyses yielded similar results (**Table 2**). In the 25,760 participants of the five cohorts with available data on thyroid function tests during follow-up (25, 29, 31-33), 146 (0.6%) participants developed subclinical hyperthyroidism and 46 (0.2%) overt hyperthyroidism. When we included only endogenous forms of thyroid dysfunction (i.e., participants without thyroxine use at baseline, N=25,049), 102 (0.4%) and 25 (0.1%) participants developed subclinical and overt hyperthyroidism, respectively. The HR (95% CI) for hip fracture for TSH 0.45-0.99 mIU/L compared with the reference group was 1.70 (1.13-2.57) in the sensitivity analysis including only participants with TSH remaining within the reference range (four cohorts with data on hip fracture and thyroid function tests during follow-up) (25, 29, 31, 32).

Thyroid function and any, non-vertebral, and vertebral fractures

For all TSH categories when compared with the reference group, we found no significant association for any, non-vertebral, or vertebral fractures (**Supplemental Table 5**). The HR (95% CI) per one SD increase in FT4 was 1.08 (1.02-1.15) for any fracture, and 1.10 (1.03-1.18) for non-vertebral fracture. These associations remained significant in most sensitivity analyses (**Table 3**), except when adjusting for BMD. Association between FT4 and vertebral fracture was not statistically significant, possibly because of the lower number of data (**Table 3**). We found no significant interaction in the analyses stratified by sex, age, duration of follow-up, or publication status for any of these fracture outcomes (**Table 3**, **Supplemental Table 4**).

DISCUSSION

In this analysis of 61,959 euthyroid participants of thirteen prospective cohorts with 659,059 person-years of follow-up, lower TSH levels within the reference range were associated with increased risk of hip fracture, and higher FT4 levels with increased risk of hip, any, and non-vertebral fracture.

While overt and subclinical hyperthyroidism have been associated with increased fracture risk (1, 2), previous studies on the relationship between TSH within the reference range and fracture risk had conflicting results. The Clalit Health Services, a large historical cohort study, found a borderline increased incidence of hip fracture with TSH 0.35-1.6mIU/L when compared with TSH 1.7-2.9mIU/L, but in women only (odds ratio [95%CI] 1.28 [1.03-1.59]), while the association with other osteoporotic fractures was not statistically significant (11). A small cross-sectional study (N=129) found an association between low TSH and vertebral fracture (12). The Cardiovascular Health Study found no significant association between TSH within the reference range or FT4 assessed as continuous variables and hip fracture (13), but, consistent with our findings, curves bent with an increased fracture risk for TSH <1.5 mIU/L and for FT4 >1.4 ng/mL. Our thorough IPD analysis across multiple prospective cohorts confirms the association between low TSH and hip fractures, and an association between high FT4 and all but vertebral fractures in participants with TSH within the reference range, suggesting that even a modest increase in thyroid hormone levels among euthyroid adults is associated with higher fracture risk.

Our study was strengthened, first, by an IPD analysis that allowed us to standardize the definitions of predictors and outcomes, adjust for similar potential confounders, and avoid aggregation bias for subgroup analyses. This was the best way to perform time-to-event analysis. Second, our study is the largest to assess fracture risk in prospective cohorts with TSH within the reference range. Third, we included all international prospective cohorts available on this topic, since all the studies we identified agreed to participate.

Our study had several limitations. First, our population consisted mostly of Caucasians and included few young adults. Second, thyroid function tests were performed only at baseline in most cohorts, so we may have included adults who later developed subclinical or overt thyroid dysfunction. However, our sensitivity analysis including only participants with persistent TSH within the reference range yielded an even stronger association between low TSH and hip fracture. In addition, other participants may have had a non-thyroidal illness, a potential cause of suppressed TSH; however, the prevalence of non-thyroidal illness was likely low as we only studied community-dwelling adults. Third, fractures were adjudicated in nine of thirteen cohorts, and we could not uniformly define each fracture type across all cohorts. Nevertheless, sensitivity analyses limited to cohorts with the most uniform facture definitions or adjudicated fracture yielded similar results. Fourth, we did not know fracture mechanism, but we excluded pathological fractures and fracture locations typically not associated with osteoporosis to reduce bias related to traumatic fractures. Fifth, data on fractures other than hip location were available in a more limited number of studies, reducing the number of outcomes and the related power to identify associations. Sixth, we had no information on other factors that may have influenced bone integrity or accounted for variations in circulating TSH or FT4, such as nutrition or deiodinase activities. Finally, thyroid antibodies were not systematically measured and their potential impact on bone metabolism could not be assessed.

Our findings may have two important clinical implications. First, TSH reference range is still a matter of debate (35). TSH reference range was indeed defined in a population that included persons with occult or underlying thyroid disease (7, 8). TSH between 0.4 and 2.5mIU/L is associated with a lower incidence of thyroid dysfunction (36), but previous studies showed various adverse outcomes associated with subclinical thyroid dysfunction (14-16), and with TSH at both extremities of the reference range (e.g. higher risk of cardiovascular disease with high TSH/low FT4, and higher risk of fractures, osteoporosis, and dementia with low TSH/high FT4) (9, 13). There may be optimal values of thyroid function tests within the reference range.

Second, similar to previous studies showing stronger association of adverse outcomes with FT4 than TSH (37, 38), FT4 was associated with hip, any and non-vertebral fracture, while TSH was associated only with hip fracture. TSH and thyroid hormones may act differently on peripheral organs, including bones: TSH may act on osteoblasts and osteoclasts via specific receptors (3), while thyroid hormones may act on target tissues via nuclear receptors controlled locally by deiodinases (3, 39, 40). This may explain why TSH and FT4 are associated with different fracture types. FT4 may therefore help evaluate osteoporosis and fracture risk, which is now usually done with the World Health Organization FRAX score, but future studies should determine if adding FT4 improves clinical accuracy of this score. Of note, FT4 was not significantly associated with vertebral fracture. One explanation may be that FT4 acts differently on vertebral bone. It may however also be due to lack of power, as we could include about ten times fewer vertebral than other fractures (**Table 3**).

We may have expected a stronger association of fracture risk with TSH and FT4, respectively, after excluding participants with thyroid medication at baseline, but the risk was only slightly increased, probably because of the low number of participants with thyroid medication at baseline (N=1897, 3%).

In conclusion, analyzing individual data of 61,959 adults from thirteen large prospective cohorts, we found that TSH at the lower extremity of the reference range was associated with higher risk of hip fractures, and high FT4 with higher risk of hip, any, and non-vertebral fractures. Our findings may help refine the current definition of optimal thyroid function. Meanwhile, clinicians should be aware that lower TSH and higher FT4, even within the reference range, are associated with an increased risk of hip fracture.

AUTHORS CONTRIBUTIONS:

Carole Aubert and Prof Rodondi had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Aubert, Bauer, Rodondi

Literature search and review: Aubert, Segna

Acquisition, analysis, or interpretation of data: Åsvold, Aubert, Blum, Bremner, Cappola, Ceresini, den Elzen, Gussekloo, Kearney, Khaw, Peeters, Stott, Walsh, Westendorp, Rodondi.

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Table 1. Study population and baseline characteristics of the participants in the 13 included studies (n=61,959)

Study name, place	**Description of study population**	**Number of participants**	**Age, median (range)***	**Women, No. (%)**	**TSH, median, mIU/L**	**Thyroid medication at baseline, No. (%)†,‡**	**Thyroid medication during follow-up, No. (%)†,§**	**Start of follow-up, year**	**Duration of follow-up, median (IQR), years‖**	**Pe y**
Busselton Health Study, Australia (29)	Adults	1,907	51 (18-90)	919 (48.2)	1.42	10 (0.5)	15 (0.8)	1981	20.0 (17.6-20.0)	33
CHS, USA (4 communities) (25)	Adults with Medicare eligibility	2,853	71 (65-100)	1,694 (59.4)	2.03	145 (5.1)	299 (10.5)	1989-1990	12.9 (7.5-18.9)	36
EPIC-Norfolk Study, England (30)	Adults aged 45-79y	11,986	58 (40-78)	6,365 (53.1)	1.70	275 (2.3)	NA	1995-1998	12.4 (11.7-13.3)	14
Health ABC Study, USA (4 communities) (14)	Adults aged 70-79y with Medicare eligibility	2,347	74 (69-81)	1,165 (49.6)	1.99	177 (7.5)	383 (13.9)	1997	12.7 (8.1-13.2)	24
HUNT Study, Norway (31) ¶	Adults	31,388	57 (19-99)	21,186 (67.5)	1.60	999 (3.2)	NA	1995-1997	12.2 (11.6-12.8)	34
InCHIANTI Study, Italy (17)	Adults aged ≥65y	1,066	71 (21-102)	590 (55.3)	1.38	17 (1.6)	28 (2.6)	1998	9.1 (7.8-9.3)	8,
Leiden 85-Plus Study, The Netherlands (32)	Adults aged 85y	456	85 (85-85)	293 (64.3)	1.66	6 (1.3)	11 (2.4)	1997-1999	4.8 (2.2-8.1)	2,
MrOS, USA (6 clinical centers) (10)	Men aged ≥65y	1,410	73 (65-99)	All men	1.97	83 (5.9)	98 (6.9)	2000-2002	11.1 (8.1-11.8)	13
OPUS, Germany, France, UK (5)**	Women aged 20-80y	1,205	63 (20-80)	All women	0.96	0 (0.0)	NA	1999-2001	6.0 (5.8-6.3)	7,
PROSPER, Ireland, Scotland, The Netherlands (33)	Older adults at high cardiovascular risk	5,124	75 (69-83)	2,527 (49.3)	1.75	135 (2.6)	163 (3.2)	1997-1999	3.2 (3.0-3.5)	15
Rotterdam Study, The Netherlands (34)	Adults aged ≥55y	1,611	68 (55-93)	957 (59.4)	1.54	21 (1.3)	NA	1989-1992	15.2 (10.4-16.2)	21
Sheffield Study, England (26)	Women aged 50-85y	291	63 (50-86)	All women	2.00	2 (0.7)	9 (3.1)	1990-1991	10.0 (5.5-10.1)	2,
SOF, USA (4 clinical centers) (19)††	Women >65y	314	71 (65-88)	All women	1.50	15 (4.8)	NA	1986-1998	14.3 (9.8-19.8)	4,
Overall	**13 cohorts**	**61,959**	**64 (18-102)**	**37,506 (60.5)**	**1.60**	**1,885 (3.1)**	**831 (5.5)**	**1981-2002**	**12.1 (8.5-12.9)**	**65**

Abbreviations: CHS, Cardiovascular Health Study; EPIC, European Prospective Investigation of Cancer; Health ABC, Health, Aging and Body Composition; HUNT, Nord-Trø Health Study; InCHIANTI, Invecchiare in Chianti; IQR, interquartile range; MrOS, Osteoporotic Fractures in Men Study; No., number; OPUS, Osteoporosis and Ultrasound PROSPER, Prospective Study of Pravastatin in the Elderly at Risk; SOF, Study of Osteoporotic Fractures; TSH, thyroid-stimulating hormone; UK, United Kingdom; USA, United St America; y, years.

* We excluded participants younger than 18y.

† Thyroid medication was defined as thyroxine or anti-thyroid medication.

‡ Data on thyroid medication at baseline was missing for 255 participants in the HUNT Study, 59 participants in the MrOS, one participant in the Rotterdam Study, four participants SOF and seven participants in the Health ABC Study.

§ Data on thyroid mediation at follow-up was missing for 243 participants in the MrOS, 96 participants in the InCHIANTI Study, 45 participants in the Sheffield Study, and all parti in the HUNT Study, EPIC-Norfolk Study, Rotterdam Study, OPUS and SOF.

‖ Duration of follow-up was defined as the maximum duration of follow-up that was available, i.e. the time to the first hip (or any if unavailable) fracture or censor date/death.

¶ We included participants excluded from the original article of the HUNT Study (participants <40y, with previous fracture and/or with previous thyroid disease), which expla different number of the sample.

** We included only the thyroid hormone sub-study of the OPUS, which excluded participants on thyroid medication.

†† We included only a subsample of the SOF, i.e., the participants with TSH measurement at baseline.

‡‡ It was calculated as time to hip fracture; for the PROSPER, it was calculated as time to any fracture, since data on hip fracture was unavailable.

Table 2: Sensitivity analyses for the risk of hip fracture according to thyroid-stimulating hormone and free thyroxine

	Analysis by TSH category*		**Analysis by SD increase in FT4†**	
	No. of events/ participants	Hazard ratio (95% CI)‡	No. of events/ participants	Hazard ratio (95% CI)§
Main analysis	610/13,390	1.25 (1.05-1.49)	542/20,633	1.24 (1.12-1.37)
Medication use				
Excluding participants with thyroid medication at baseline[‖]	557/12,728	1.28 (1.06-1.53)	526/20,158	1.26 (1.13-1.40)
Excluding participants with thyroid-altering medication at baseline[¶]	542/12,396	1.28 (1.07-1.55)	506/19,679	1.26 (1.13-1.40)
Excluding participants with anti-fracture medication at baseline**	605/12,739	1.27 (1.07-1.52)	539/20,563	1.24 (1.12-1.38)
Definition of fracture				
Including only studies with formal fracture adjudication††	496/12,048	1.31 (1.06-1.60)	416/17,913	1.21 (1.07-1.36)
Other				
Excluding one study with loss to follow-up >5%‡‡	606/12,748	1.26 (1.05-1.50)	536/19,463	1.24 (1.11-1.37)
BMD				
Further adjusting for femoral neck BMD at baseline§§	94/2,020	1.68 (1.08-2.61)[‖‖]	142/4,147	1.22 (1.01-1.47)

Abbreviations: BMD, bone mineral density; CHS, Cardiovascular Health Study; CI, confidence interval; EPIC, European Prospective Investigation of Cancer; FT4, free thyroxine; Health ABC, Health, Aging and Body Composition; HUNT, Nord-Trøndelag Health Study; InCHIANTI, Invecchiare in Chianti; MrOS, Osteoporotic Fractures in Men Study; No., number; OPUS, Osteoporosis and Ultrasound Study; PROSPER, Prospective Study of Pravastatin in the Elderly at Risk; SD, standard deviation; SOF, Study of Osteoporotic Fractures; TSH, thyroid-stimulating hormone.

All analyses were adjusted for age (as a continuous variable) and sex. Data for hip fractures were available for 12 cohorts (all but PROSPER).

* We present a selected analysis for the TSH category 0.45-0.99mIU/L compared with the reference category (TSH 3.50-4.99mIU/L). No. are for participants in these both TSH categories only.

† FT4 was measured in all studies but the SOF and the Health ABC Study (FT4 not measured in participants with TSH within reference range).

‡ Hazard ratios are for TSH 0.45-0.99mIU/L, compared with the reference group 3.50-4.99mIU/L.

§ Hazard ratios are per one standard deviation increase in FT4.

[‖] Thyroid medication was defined as thyroxine or anti-thyroid medication.

[¶] Thyroid-altering medication included oral corticosteroid, amiodarone, iodine, thyroxine, or anti-thyroid medication.

** Anti-fracture medication was defined as bisphosphonate, calcitonin, selective estrogen receptor modulator, or parathyroid hormone.

†† EPIC-Norfolk Study, HUNT Study, InCHIANTI Study, MrOS, OPUS, Rotterdam Study, Sheffield Study, Health ABC Study, and SOF (Health ABC Study and SOF only in the TSH analysis).

‡‡ OPUS.

§§ Femoral neck BMD at baseline was available in following studies: CHS, MrOS, Rotterdam Study, Sheffield Study, OPUS, Health ABC Study.

[‖‖] Participants within the TSH category 3.50-4.49mIU/L had lower femoral neck BMD at baseline than participants within the TSH category 0.45-1.50mIU/L (mean [SD]: 0.77g/cm^2 [0.16] versus 0.79 g/cm^2 [0.15], respectively, $P = 0.002$), which explains the higher hazard ratio after adjusting for femoral neck BMD at baseline.

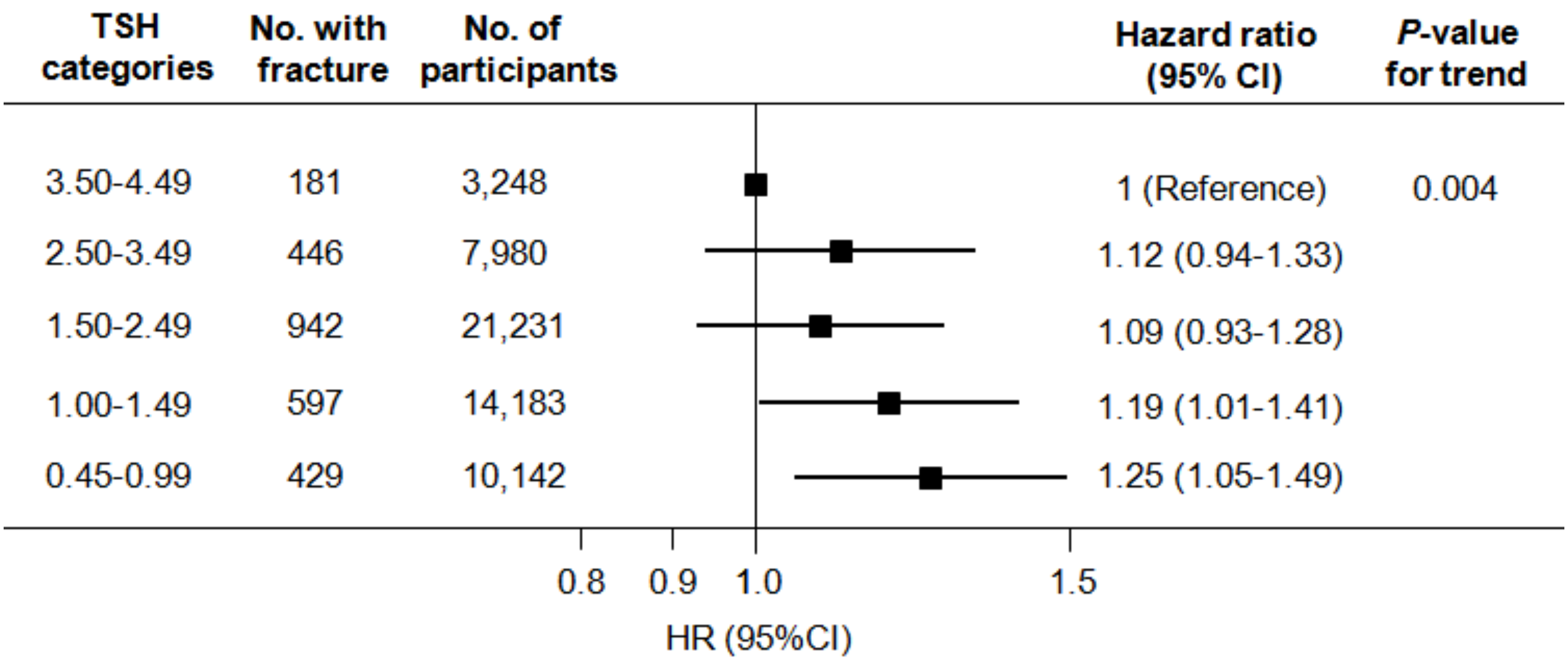
Table 3. Sensitivity and stratified analyses for the risk of any, non-vertebral, and vertebral fractures, per one standard deviation increase in free thyroxine

	Any fracture*		Non-vertebral fracture†		Vertebral fracture‡	
	No. of events/ participants	Hazard ratio (95% CI)	No. of events/ participants	Hazard ratio (95% CI)	No. of events/ participants	Hazard ratio (95% CI)
Main analysis	1,629/22,977	1.08 (1.02-1.15)	1,273/19,101	1.10 (1.03-1.18)	129/17,711	1.06 (0.86-1.3
SENSITIVITY ANALYSES						
Medication use						
Excluding participants with thyroid medication at baseline§	1,552/22,440	1.09 (1.02-1.16)	1,240/18,697	1.14 (1.06-1.23)	125/17,309	1.08 (0.86-1.3
Excluding participants with thyroid-altering medication at baseline‖	1,537/21,976	1.09 (1.02-1.15)	1,200/18,256	1.11 (1.03-1.19)	125/16,868	1.07 (0.86-1.3
Excluding participants with anti-fracture medication at baseline¶	1,622/22,927	1.08 (1.02-1.15)	1,263/19,038	1.10 (1.03-1.18)	127/17,666	1.05 (0.85-1.2
Definition of fracture						
Including only studies with formal fracture adjudication**	1,026/15,805	1.11 (1.02-1.19)	1,111/17,208	1.11 (1.03-1.19)	113/15,806	1.07 (0.86-1.3
Including only studies with most uniform definition of fracture††	1,155/19,728	1.06 (0.99-1.14)	685/14,461	1.08 (0.98-1.19)	65/14,462	1.10 (0.83-1.4
Other						
Further adjusting for BMI, smoking status, and diabetes mellitus	1,591/22,536	1.21 (1.00-1.46)	1,140/17,562	1.09 (1.01-1.18)	126/17,290	1.03 (0.83-1.2
Excluding studies with loss of follow-up rate >5%	NA	NA	1,174/17,981	1.13 (1.04-1.22)	NA	NA
BMD						
Further adjusting for lumbar spine BMD at baseline‡‡	NA	NA	NA	NA	39/1,399	0.96 (0.68-1.3
Further adjusting for whole body BMD at baseline§§	183/1,399	0.89 (0.75-1.04)	NA	NA	NA	NA
STRATIFIED ANALYSES						
Stratified for sex						
Women	1,013/11, 321	1.11 (1.03-1.19)	827/10,075	1.10 (1.01-1.20)	62/8,679	1.12 (0.83-1.5
Men	616/11,656	1.05 (0.95-1.15)	446/9,026	1.08 (0.96-1.22)	67/9,032	1.00 (0.75-1.3
P-value for interaction	NA	0.39	NA	0.79	NA	0.61
Stratified for age						
<75 years at baseline	1,041/18,367	1.10 (1.02-1.19)	955/17,144	1.10 (1.02-1.20)	87/15,917	0.96 (0.74-1.2
≥75 years at baseline	588/4610	1.06 (0.96-1.16)	318/1,957	1.10 (0.97-1.25)	42/1,794	1.25 (0.88-1.7
P-value for interaction	NA	0.47	NA	0.99	NA	0.25
Stratified for duration of follow-up						
<5 years	446/5,920	1.04 (0.93-1.15)	47/888	0.82 (0.59-1.14)	7/888	0.60 (0.26-1.3
≥5 years	1,183/17,057	1.09 (1.02-1.18)	1,226/18,213	1.10 (1.03-1.18)	122/16,823	1.07 (0.87-1.3
P-value for interaction	NA	0.39	NA	0.07	NA	0.18

Abbreviations: BMD, bone mineral density; BMI, body mass index; CI, confidence interval; EPIC, European Prospective Investigation of Cancer; FT4, free thyroxine; Health ABC, Health, Aging and Body Composition; InCHIANTI, Invecchiare in Chianti Study; MrOS, Osteoporotic Fractures in Men Study; NA, not appropriate; No., number; OPUS, Osteoporosis and Ultrasound Study; PROSPER, Prospective Study of Pravastatin in the Elderly at Risk; SD, standard deviation; SOF, Study of Osteoporotic Fractures; TSH, thyroid-stimulating hormone.
All analyses were adjusted for age (as a continuous variable) and sex; FT4 was measured in all studies but SOF and Health ABC Study (FT4 not measured in participants with TSH within the reference range).
Hazard ratios are per one standard deviation increase in FT4.

* Data on any fractures were available for 7 studies (MrOS, EPIC-Norfolk Study, InCHIANTI Study, Leiden 85-Plus Study, PROSPER, Rotterdam Study, Busselton Health Study).
† Data on non-vertebral fractures were available for 7 studies (MrOS, EPIC-Norfolk Study, InCHIANTI Study, Rotterdam Study, Busselton Health Study, Sheffield Study, OPUS).
‡ Data on vertebral fractures were available for 5 studies (MrOS, EPIC-Norfolk Study, InCHIANTI Study, Rotterdam Study, Busselton Health Study). Vertebral fracture was defined as a clinical symptomatic dorsal or lumbar fracture.
§ Thyroid medication was defined as thyroxine or anti-thyroid medication.
‖ Thyroid-altering medication included oral corticosteroid, amiodarone, iodine, thyroxine, or anti-thyroid drug.
¶ Anti-fracture medication was defined as bisphosphonate, calcitonin, selective estrogen receptor modulator, or parathyroid hormone.
** EPIC-Norfolk Study, InCHIANTI Study, MrOS, OPUS, Rotterdam Study, Sheffield Study.
†† EPIC-Norfolk Study, InCHIANTI Study, Leiden 85-Plus Study, MrOS, PROSPER.
‡‡ Lumbar spine BMD was available in MrOS only.
§§ Whole body BMD was available in MrOS only.

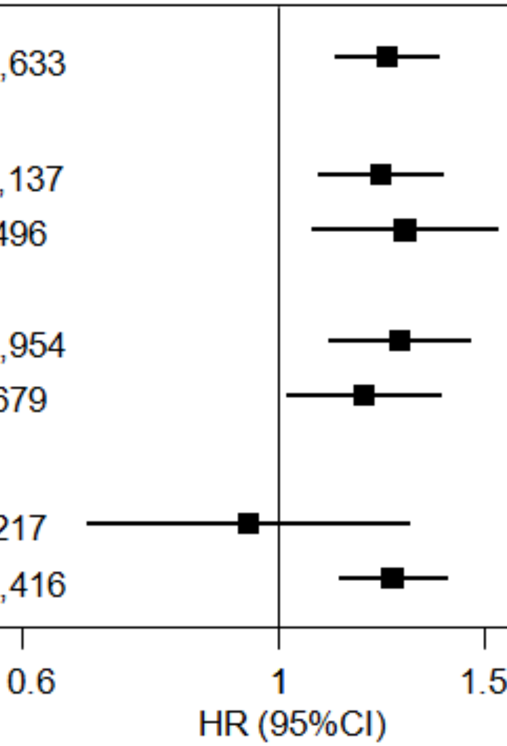
Figure 1. Risk of hip fracture according to thyroid-stimulating hormone categories



Abbreviations: CI, confidence interval; HR; hazard ratio; No., number; TSH, thyroid-stimulating hormone.
Data on hip fractures were available for 12 studies (all except PROSPER).

Figure 2. Risk of hip fracture per one standard deviation increase in free thyroxine, overall and stratified by sex, age, and duration of follow-up

	No. with fracture	No. of participants	Hazard ratio (95% CI)	*P*-value for interaction
Overall	542	20,633	1.24 (1.12-1.37)	-
Sex				
Women	372	11,137	1.23 (1.08-1.39)	0.67
Men	170	9,496	1.29 (1.07-1.55)	
Age				
<75 years	329	17,954	1.27 (1.11-1.46)	0.50
≥75 years	213	2,679	1.18 (1.02-1.38)	
Duration of follow-up				
<5 years	47	1,217	0.94 (0.68-1.30)	0.10
≥5 years	495	19,416	1.25 (1.12-1.40)	



Abbreviations: CI, confidence interval; FT4, free thyroxine; Health ABC, Health, Aging and Body Composition; HR; hazard ratio; No., Number; SOF, Study of Osteoporotic fractures, TSH, thyroid-stimulating hormone.
The analysis stratified for sex was adjusted for age. All other analyses were adjusted for age (as a continuous variable) and sex.
FT4 was measured in all studies but SOF and Health ABC Study (FT4 not measured in participants with TSH within the reference range).
Data on hip fractures were available for 10 studies with measured FT4 (all except PROSPER).