Association between Subclinical Thyroid Dysfunction and Change in Bone Mineral Density in Prospective Cohorts

Running title: Thyroid Disease and Bone Loss

Authors:

Daniel Segna, MD [1], Douglas C Bauer, MD [2], Martin Feller, MD, MSc [1], Claudio Schneider, MD [1], Howard A. Fink, MD, MPH [3,4], Carole E Aubert, MD [1], Tinh-Hai Collet, MD [5], Bruno R da Costa, PhD [6], Karina Fischer, PhD [7,8], Robin P. Peeters, MD, PhD [9], Anne R. Cappola, MD, ScM [10], Manuel R Blum, MD [1], Hendrika A. van Dorland, PhD [1], John Robbins, MD, MHS [11], Kim Naylor, PhD [12], Richard Eastell, MD [12], André G. Uitterlinden, PhD [9], Fernando Rivadeneira Ramirez, MD, PhD [9], Apostolos Gogakos, MD, PhD [13], Jacobijn Gussekloo, MD, PhD [14], Graham R. Williams, MBBS, PhD [13], Ann Schwartz, PhD, MPH [2], Jane A. Cauley, DrPH [15], Drahomir A. Aujesky, MD, MSc [1], Heike A. Bischoff-Ferrari, MD, DrPH [7,8,], Nicolas Rodondi, MD, MAS [1,6,] for the Thyroid Studies Collaboration.

Affiliations:

[1] Department of General Internal Medicine, Inselspital, Bern University Hospital, University of Bern, Switzerland; [2] Departments of Medicine and Epidemiology & Biostatistics, University of California, San Francisco, United States; [3] Geriatric Research Education and Clinical Center, Minneapolis VA Health Care System, Minneapolis, MN, United States; [4] Department of Medicine, University of Minnesota, Minneapolis, United States, [5] Service of Endocrinology, Diabetes and Metabolism, University Hospital of Lausanne, Lausanne, Switzerland; [6] Institute of Primary Health Care (BIHAM), University of Bern, Bern, Switzerland; [7] Centre on Aging and Mobility, University of Zurich, Zurich, Switzerland; [8] Department of Geriatrics, University Hospital Zurich, Zurich, Switzerland; [9] Department of Internal Medicine & Department of Epidemiology, Erasmus Medical Center Rotterdam, The Netherlands; [10] University of Pennsylvania School of Medicine, Philadelphia, PA, United States; [11] Department of Medicine, University of California Davis, Sacramento, United States; [12] Department of Oncology and Metabolism, University of Sheffield, Sheffield, United Kingdom; [13] Department of Medicine, Imperial College London, London, United Kingdom; [14] Department of Public Health and Primary Care & Gerontology and Geriatrics, Leiden University Medical Center, Leiden, the Netherlands; [15] Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, United States

Key terms: Thyroid Disease, Hypothyroidism, Hyperthyroidism, Bone Density, Bone Loss, Prospective Studies

Word count: 2,318

Abstract count (without subheadings): 239

Tables / Figures: 2/1

Online-only Tables/Figures: 4/2

Corresponding Author: Nicolas Rodondi, MD, MAS, Department of General Internal Medicine, Inselspital, Bern University Hospital, University of Bern, 3010 Bern, Switzerland; Email: Nicolas.Rodondi@insel.ch,

Abstract

Background: Subclinical hyperthyroidism (SHyper) has been associated with increased risk for hip and other fractures, but the linking mechanisms remain unclear.

Objective: To investigate the association between subclinical thyroid dysfunction and bone loss.

Methods: Individual participant data analysis after a systematic literature search in MEDLINE/EMBASE (1946-2016). Two reviewers independently screened and selected prospective cohorts providing baseline thyroid status and serial bone mineral density (BMD) measurements. We classified thyroid status as euthyroidism (thyroid-stimulating hormone [TSH] 0·45-4·49mIU/l), SHyper (TSH<0.45mIU/l) and subclinical hypothyroidism (SHypo, TSH≥4·50-19·99mIU/l) both with normal free thyroxine levels. Our primary outcome was annualized percentage BMD change (%ΔBMD) from serial dual x-ray absorptiometry scans of the femoral neck, total hip and lumbar spine, obtained from multivariable regression in a random-effects two-step approach.

Results: Among 5,458 individuals (median age 72 years, 49·1% women) from 6 prospective cohorts, 451 (8·3%) had SHypo and 284 (5·2%) had SHyper. During 36,569 person-years of follow-up, those with SHyper had a greater annual bone loss at the femoral neck vs. euthyroidism: %ΔBMD=-0·18 (95%CI:-0·34,-0·02; I^2=0%), with a non-statistically significant pattern at the total hip: %ΔBMD=-0·14 (95%CI:-0·38,0·10; I^2=53%), but not at the lumbar spine: %ΔBMD=0·03 (95%CI:-0·30,0·36; I^2=25%). Especially participants with TSH<0·10mIU/l showed an increased bone loss in the femoral neck (%ΔBMD=-0·59;[95%CI:-0·99,-0·19]) and total hip region (%ΔBMD=-0·46 [95%CI:-1·05,-0·13]). In contrast, SHypo was not associated with bone loss at any site.

Conclusion: Among adults, SHyper was associated with increased femoral neck bone loss, potentially contributing to the increased fracture risk.

Introduction

Overt hyperthyroidism is a known risk factor for decreased bone mineral density (BMD) and fractures (1-3) whereas overt hypothyroidism is not, except during thyroxine overreplacement.(4) Compared to overt thyroid disease, subclinical thyroid dysfunction (SCTD) is a more common phenomenon, with a prevalence reaching 10% for subclinical hypothyroidism (SHypo) in the elderly (5) and 3·2% for subclinical hyperthyroidism (SHyper).(6)

Among 70,298 individual participant data (IPD) from prospective cohort studies, we found that SHyper (but not SHypo) was associated with an increased risk up to 36% of fractures compared to euthyroidism.(7)

Yet the underlying pathophysiologic mechanism remains unclear. Increased bone loss may mediate this association, and is best assessed with serial bone mineral density measurements to assess bone health and evaluate the future risk for osteoporotic fractures.(8, 9) However, data on the association between SCTD and bone loss are limited to one prospective cohort study conducted only in men.(10) To investigate the influence of SCTD on bone loss, a potential mediator in its association with fracture risk, we conducted a pooled IPD analysis from all population-based prospective cohort studies with baseline thyroid status and serial BMD assessments.

Methods

Search Strategy and Selection Criteria

We report this IPD analysis according to the PRISMA-IPD statement (11) and published the study protocol online in the International prospective register of meta-analyses (PROSPERO CRD42015019814).(12). We conducted a systematic literature search in EMBASE and

Medline from inception until September, 5th, 2016 without language restrictions, and searched bibliographies of key articles in the field. We included IPD from prospective cohorts with available baseline thyroid status and serial BMD measurements. We excluded studies assessing individuals with overt thyroid dysfunction only, or limited to participants pre-treated for either thyroid or bone diseases. Two physicians (DS, CEA) independently assessed each study's eligibility (title and abstract screen: Cohen's kappa coefficient [κ]=0·80; full-text search: κ=1·00), potential risks of bias, and study quality using the Newcastle-Ottawa Quality Assessment Scale.(13) Remaining uncertainties were solved with a third author (NR). Furthermore, we included unpublished IPD from the Thyroid Studies Collaboration,(7) an international network of high-quality prospective cohort studies. In case of unclear data issues (e.g. unreasonable outliers), we contacted the designated cohort contact persons.

Thyroid Status

All cohorts measured TSH using third-generation assays, whereas fT4 assay kits varied across studies. Similar to previous IPD analyses,(7, 14, 15) we used uniform TSH cutoff levels based on an expert consensus meeting of the Thyroid Studies Collaboration, expert reviews,(16, 17) and cohort-specific cut-offs for fT4 reference ranges (Appendix Table 1) for a better comparability. We defined euthyroidism as TSH 0.45-4.49mIU/l, SHypo as TSH between 4.50-19.99mIU/l with fT4 within reference range, SHyper as TSH <0.45mIU/l with fT4 within reference range. We excluded individuals with overt hypothyroidism (n=124) and hyperthyroidism (n=90), as well as other discordant thyroid function tests due to unclear cause/mechanisms (n=27).

Assessment of Bone- and Thyroid-Altering Medication

We collected data on anti-osteoporotic medication(18) and glucocorticoids(19) in all cohorts at baseline and during follow-up. Bone-altering medication comprised: bisphosphonates, calcitonin, teriparatide, proton pump inhibitors, selective estrogen receptor modulators, oral corticosteroids, thiazides, postmenopausal hormone therapy, contraceptives, androgens, anti-androgens, and fluorides. Similarly, we collected all available data on thyroid-altering medication: thyroxine, anti-thyroid drugs, lithium, and amiodarone.

Annualized Percentage Change in Bone Mineral Density (%ΔBMD)

Our primary outcome was the annualized percentage change between baseline and the last available follow-up measurements (%ΔBMD) at the femoral neck, total hip, and lumbar spine, in order to standardize BMD measurements across different cohorts, devices and follow-up durations, as in former study-level meta-analyses.(20, 21)

All BMD measurements were obtained from gold-standard dual x-ray absorptiometry (DXA, Appendix Table 1). The rationale for total hip, femoral neck, and lumbar spine as reference body sites was their high relevance to the risk assessment of major osteoporotic fractures.(22) To increase the accuracy and reproducibility for each body site, all cohorts implemented a strict quality control with cross-calibration using standardized phantoms to avoid inter-device variability and longitudinal shifts and drifts (Appendix Table 2).

In a previous publication, we observed an increased risk of hip fractures in participants with SHyper.(7) In the current work, we also examined whether this could be explained by the mediating effect of increased bone loss in this region. For this secondary analysis, every cohort provided us with both data on incident fractures and %ΔBMD. The definitions of fracture categories are detailed elsewhere.(7)

Data Analysis

Following recommendations for IPD analyses (23, 24) and previous studies,(7, 14) we used a random-effects two-step approach, first analyzing associations between thyroid status and %ΔBMD for each cohort using linear multivariable regression models controlling for age, sex, body mass index (BMI),(25) diabetes mellitus,(25) smoking,(26) and menopausal status.(27) Data were complete for age and sex, with rare missing data for BMI (0·2%), smoking (0·3%), menopausal status (0·3%), and diabetes mellitus (<0·01%). This approach yielded adjusted differences in %ΔBMD between euthyroid individuals and those with SHyper or SHypo, and respective standard errors. In a second step, we calculated pooled estimates with 95%CI using inverse-variance random-effects models,(28) and assessed the heterogeneity across cohorts by means of I^2 statistic.(29). Additional information is detailed in the Appendix.

Results

Out of 1,558 articles identified in our literature search and through contact with experts, six cohort studies met all inclusion criteria (Appendix Figure 1).(10) Two other cohorts were potentially eligible, but not included because of different BMD measurement techniques and devices.(30, 31) The final sample for our primary outcome comprised 5,458 individuals (median age 72 years, 49·1% female participants) with a median follow-up of 6·7 years and total observation of 36,569 patient-years (Table 1). 4,723 (86·5%) participants were euthyroid, 451 (8·3%) had SHypo, and 284 (5·2%) had SHyper, including 230 (4·2%) with low but not suppressed TSH (0·10-0·44 mIU/l) and 54 (1·0%) with suppressed TSH (<0·10mIU/l). According to the modified Newcastle-Ottawa Quality Assessment Scale,(13) study quality was good to excellent with three studies achieving the full score of seven,(32-34) and three studies with six points (Appendix Table 2).(10, 35, 36)

In euthyroid individuals, femoral neck BMD decreased 0·59% per year (95%CI:0·54, 0·63), total hip BMD decreased 0·55% per year (95%CI:0·49, 0·61), while spine BMD increased

0·32% per year (95%CI:-0·21, 0·84) in unadjusted models. In multivariable regression models, SHyper was associated with an increased bone loss at the femoral neck compared to euthyroidism: %ΔBMD=-0·18 (95%CI:-0·34, -0·02; I^2=0·0%, Appendix Figure 2), with a non-statistically significant pattern for total hip: %ΔBMD=-0·14 (95%CI:-0·38, 0·10, I^2=52·7%), but not for lumbar spine: %ΔBMD=0·03 (95%CI:-0·30, 0·36; I^2=24·8%) (Table 2). Among participants with SHyper and TSH <0·10 mUI/l, bone loss notably increased at the femoral neck [%ΔBMD=-0·59 (95%CI:-0·99,-0·19, I^2=0·0%)], with a similar pattern at the total hip [%ΔBMD=-0·46 (95%CI:-1·05,0·13, I^2=59·5%)] compared to euthyroidism. In contrast, SHypo was not associated with increased bone loss at any body site (Appendix Table 3). An analysis stratifying for cohort-specific fT4 quartiles resulted in a significantly increased hip bone loss in the highest vs. lowest fT4 quartile for both femoral neck %ΔBMD=-0·18 (95%CI:-0·29, -0·06, p<0·01) and total hip %ΔBMD=-0·20 (95%CI:-0·27, -0·12, p=0·02, Figure 1). In SHyper, bone loss was significantly increased for both men and women at the femoral neck (%ΔBMD=-0·33 [95%CI:-0·66, -0·01] vs. %ΔBMD=-0·14 [95%CI:-0·24, -0·05]) compared to euthyroidism, however without effect modification by gender (p for interaction 0·58), but not total hip (%ΔBMD=-0·38 [95%CI:-0·80, 0·03] vs. %ΔBMD=-0·05 [95%CI:-0·25, 0·14], p for interaction=0·43). There was a pattern for a larger bone decrease at the femoral neck among participants with SHyper ≥75 vs. < 75 years (%ΔBMD=-0·34 [95%CI:-0·52, -0·16] vs. %ΔBMD=-0·13 [95%CI:-0·22, -0·04], p for interaction=0·09), but not at the total hip (%ΔBMD=-0·28 [95%CI:-0·69, 0·12] vs. %ΔBMD=-0·15 [95%CI:-0·33, 0·04], p for interaction=0·77).

Most sensitivity analyses yielded similar results (Table 2), whereas exclusion of both thyroid- and bone-altering drug users at any time showed a greater bone loss in SHyper at the femoral neck and a comparable pattern for total hip, without significant changes for lumbar spine. When

excluding studies with >20% missing follow-up BMD, bone loss was significantly increased in SHyper at both hip measurement sites.

The adjusted HR for fractures associated with SHyper was 1·47 (95%CI:0·74, 2·91; p=0·27) for hip, 1·19 (95%CI:0·69, 2·03; p=0·53) for any, and 0·95 (95%CI:0·58, 1·57; p=0·85) for non-spine fractures. Compared to our previous publication,(7) confidence intervals were larger due to the smaller number of individuals with both fracture assessment and serial BMD scans (N=5,458 vs. N=70,298). Additional adjustment for baseline BMD and %ΔBMD in the total hip region yielded lower risk estimates, particularly for hip fractures (HR=1·28; 95%CI:0·64, 2·54; p=0·49). Additionally, there was no significant effect modification by thyroid status (SHyper vs. euthyroidism) in the association between %ΔBMD in the hip region and the risk for hip, non-spine and any fractures (Appendix Table 4).

Discussion

In our IPD analysis of 5,458 individuals from six population-based prospective cohorts, SHyper was associated with a moderately increased annualized bone loss at the femoral neck with a similar, non-significant trend for total hip, but not for lumbar spine, which may be influenced by the development of degenerative arthritis and vascular calcification. Bone loss at the femoral neck and total hip was largest among individuals with TSH levels <0·10 mUI/l showing approximately a double to 3-fold annualized rate of hip bone loss. Moreover, participants in the highest fT4 quartile had a more pronounced hip bone loss than participants in the lowest fT4 quartile. Conversely, SHypo was not associated with increased bone loss compared to euthyroid controls.

Bone loss at the femoral neck and, to a lesser extent, at total hip, was even greater after excluding individuals on bone metabolism and/or thyroid function-altering medication at any time. These results suggest increased hip bone loss especially in endogenous forms of SHyper, and are compatible with a recent study-level meta-analysis with 78% higher fracture risk in

endogenous and 25% higher in exogenous forms of SHyper vs euthyroidism. (37) A cross-sectional study among 88 postmenopausal women reported significantly lower hip and lumbar spine BMD levels in endogenous, but not exogenous SHyper.(38) Longer exposure to decreased TSH levels in endogenous SHyper could be an explanation,(37, 39) as exogenous SHyper is usually quickly corrected with regular TSH monitoring.

Although there was no evidence of interaction by age or sex on the association between SHyper and hip bone loss, point estimates for femoral neck/total hip %ΔBMD in SHyper were lower in men than in women. These results are compatible with our previous publication showing a higher HR for hip fractures in men than in women with SHyper compared to euthyroid controls ([HR=1·92, 95%CI:1·26, 2·94] vs. [HR=1·29, 95%CI:1·08, 1·55], p for interaction 0·09).(7)

Our study found a potential mediating effect of hip bone loss in the association between SHyper and increased risk for hip fractures, as shown by the decreased HRs after additional adjustment for %ΔBMD and baseline BMD at the total hip. However, confidence intervals were large and the association was not statistically significant , as power was limited by the relatively low number of hip fractures (265 in the present analysis compared to 2,975 in our previous article).(7) Additionally, we found no clear interaction of thyroid status (SHyper vs euthyroidism) in the association between %ΔBMD in the hip region and fracture risk. Therefore, there may be additional mediators such as bone turnover and neuromuscular function in the association between SHyper and fracture risk. SHyper has been associated with reduced muscle strength,(40) increased frailty,(41) and an increased cardiovascular morbidity(15) in previous prospective cohorts, which all may result in an increased risk for falls and subsequent low-traumatic fractures.

Our study has the following strengths. It is the first analysis on the association between SCTD and bone loss including a large proportion of IPD from six prospective population-based cohort studies from five different countries with a balanced gender distribution. Compared to study-

level meta-analyses, an IPD analysis increases the power and accuracy of aggregated evidence by providing highly standardized and confounder-adjusted results from different cohort studies and reliable data on subgroups without ecological fallacy.(23) Although causality and the role of a drug intervention cannot be established in a cohort study, these data represent the best available evidence, as there is no published or ongoing randomized controlled trial on this topic to our knowledge. We could exclude individuals on thyroid- and bone-altering medication at any time point in our main and sensitivity analyses reducing the possibility of treatment bias.

However, our study has some limitations. First, we could not assess the association between persistent SCTD and bone loss, as serial thyroid hormone measurements were obtained only in one cohort. SHyper has an annual spontaneous progression rate of only 1-2%,(16) and SHypo of 3-4%(6) to overt thyroid disease. In a sensitivity analysis, we accounted for this issue excluding both bone- and thyroid-altering drug users at any time, which found an even faster bone loss at the femoral neck in SHyper. Second, the etiology of SHyper was not systematically assessed which precluded further subgroup analyses. Third, available information on drug treatment varied somewhat in detail and time span. However, missingness for thyroid- or bone-altering drugs at baseline was negligible (thyroid replacement therapy [0·75%], anti-osteoporotic agents [1·06%], oral corticosteroids [0·76%]). Fourth, our study population was older than the general population, which may reduce the generalizability of our results to younger individuals with SHyper. Fifth, only the OPUS(36) offered information on triiodothyronine (T3) levels, which made a uniform exclusion of participants with abnormal T3 values impossible. Thus, some individuals suffering from T3-toxicosis or non-thyroidal illness may have been included in the subgroup of SHyper. Finally, although we observed a potential mediating effect of total hip %ΔBMD in the association between SHyper and hip fractures, this secondary analysis was subject to limited power shown by large confidence intervals.

Conclusion

Hip bone loss was increased in individuals with SHyper, especially in those with TSH <0·10mIU/l, high-normal fT4 levels, and SHyper of potentially endogenous etiology, compared to euthyroidism. These results suggest that individuals with SHyper may be exposed to a greater osteoporosis risk due to accelerated hip bone loss. Although bone loss may not solely be responsible for the increased fracture risk, SHyper would represent a treatable risk factor.

Acknowledgements

Financial Support

This work was supported by grants from the Swiss National Science Foundation (SNSF 320030-150025 and 320030-172676 to Prof. Rodondi). Dr. Collet's research is supported by grants from the Swiss National Science Foundation (P3SMP3-155318, PZ00P3-167826). **Cardiovascular Health Study (CHS):** This research was supported by contracts HHSN268201200036C, HHSN268200800007C, N01HC55222, N01HC85079, N01HC85080, N01HC85081, N01HC85082, N01HC85083, N01HC85086, and grant U01HL080295 from the National Heart, Lung, and Blood Institute (NHLBI), with additional contribution from the National Institute of Neurological Disorders and Stroke (NINDS). Additional support was provided by R01AG023629 from the National Institute on Aging (NIA). A full list of principal CHS investigators and institutions can be found at CHS-NHLBI.org. **Health, Aging and Body Composition (Health ABC) study:** The Health, Aging, and Body Composition study is supported by contracts N01-AG-6-2101, N01-AG-6-2103, and N01-AG-6-2106 from the National Institute on Aging; grant R01-AG028050 from the National Institute on Aging; grant R01-NR012459 from the National Institute of Nursing Research; and by the Intramural Research Program of the National Institutes of Health, National Institute on Aging. **Osteoporotic Fractures in Men (MrOS) Study:** The Osteoporotic Fractures in Men (MrOS) Study is supported by National Institutes of Health funding. The following institutes provide support: the National Institute on Aging (NIA), the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS), the National Center for Advancing

Translational Sciences (NCATS), and NIH Roadmap for Medical Research under the following grant numbers: U01 AG027810, U01 AG042124, U01 AG042139, U01 AG042140, U01 AG042143, U01 AG042145, U01 AG042168, U01 AR066160, and UL1 TR000128. **Osteoporosis and Ultrasound Study (OPUS):** supported by Sanofi-Aventis, Eli Lilly, Novartis, Pfizer, Proctor and Gamble Pharmaceuticals and Roche. **Rotterdam Study:** funded by Erasmus MC and Erasmus University, Rotterdam, the Netherlands; the Netherlands Organisation for Scientific Research (NWO); the Netherlands Organisation for the Health Research and Development (ZonMw); the Research Institute for Diseases in the Elderly (RIDE); the Ministry of Education, Culture and Science; the Dutch Ministry for Health, Welfare and Sports; the European Commission (DG XII); and the Municipality of Rotterdam. **Sheffield Study:** funded by Arthritis Research United Kingdom

Contribution

Study design: Segna, Bauer, Rodondi; statistical analyses: Segna, Bauer, Rodondi, Collet, da Costa, Feller, Bischoff-Ferrari, Fischer; literature search: Segna, Aubert; manuscript writing: Segna, Rodondi, Bauer, Feller; data collection and preparation: Segna, Rodondi, Bauer, Eastell, Williams, Peeters, Uitterlinden, Rivadeneira Ramírez, Gogakos, Naylor, Cauley; critical review of the manuscript: Schneider, Fink, Aubert, Collet, da Costa, Fischer, Peeters, Cappola, Blum, van Dorland, Robbins, Naylor, Eastell, Uitterlinden, Rivadeneira Ramírez, Gogakos, Gussekloo, Williams, Schwartz, Cauley, Aujesky, Bischoff-Ferrari.

Conflict of interest

Dr. Rodondi and Dr. Gussekloo report funding for a randomized controlled trial on subclinical hypothyroidism (TRUST trial) from the European Commission FP7-HEALTH-2011, Specific Programme "Cooperation" – Theme "Health" Investigator-driven clinical trials for therapeutic interventions in elderly populations (Proposal No: 278148-2). Dr. Peeters reports lecture and/or advisory board fees from Genzyme B.V., EISAI, IPSEN, and Goodlife Fertility; and grant support from Veracyte, all outside of the submitted work. Dr. Eastell reports grants and personal fees from Amgen, grants from Department of Health, grants from AstraZeneca, grants, personal fees and non-financial support from

Immunodiagnostic Systems, grants from ARUK/MRC Centre for Excellence in Musculoskeletal Ageing Research, grants from National Institute for Health Research, grants from MRC/AZ Mechanisms of Diseases Call, grants from MRC , grants and personal fees from Alexion, grants and other from National Osteoporosis Society, grants, personal fees and other from Roche, personal fees and other from Eli Lilly, other from European Calcified Tissue Society, other from IOF CSA, other from IBMS, other from ASBMR, personal fees from D-STAR, personal fees from GSK Nutrition, outside the submitted work; Dr Robbins reports funding from NHLBI during the conduct of the study. Dr Collet reports grants from Swiss National Science foundation during the conduct of the study.

References

1. Cummings SR, Nevitt MC, Browner WS, Stone K, Fox KM, Ensrud KE, et al. Risk factors for hip fracture in white women. Study of Osteoporotic Fractures Research Group. N Engl J Med. 1995;332(12):767-73. Epub 1995/03/23. doi: 10.1056/nejm199503233321202. PubMed PMID: 7862179.
2. El Hadidy el HM, Ghonaim M, El Gawad S, El Atta MA. Impact of severity, duration, and etiology of hyperthyroidism on bone turnover markers and bone mineral density in men. BMC Endocr Disord. 2011;11:15. Epub 2011/08/09. doi: 10.1186/1472-6823-11-15. PubMed PMID: 21819612; PubMed Central PMCID: PMCPMC3176471.
3. Vestergaard P, Mosekilde L. Hyperthyroidism, bone mineral, and fracture risk--a meta-analysis. Thyroid. 2003;13(6):585-93. Epub 2003/08/22. doi: 10.1089/105072503322238854. PubMed PMID: 12930603.
4. Abrahamsen B, Jorgensen HL, Laulund AS, Nybo M, Bauer DC, Brix TH, et al. The excess risk of major osteoporotic fractures in hypothyroidism is driven by cumulative hyperthyroid as opposed to hypothyroid time: an observational register-based time-resolved cohort analysis. J Bone Miner Res. 2015;30(5):898-905. Epub 2014/11/29. doi: 10.1002/jbmr.2416. PubMed PMID: 25431028.
5. Hollowell JG, Staehling NW, Flanders WD, Hannon WH, Gunter EW, Spencer CA, et al. Serum TSH, T(4), and thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III). J Clin Endocrinol Metab. 2002;87(2):489-99. Epub 2002/02/12. doi: 10.1210/jcem.87.2.8182. PubMed PMID: 11836274.
6. Biondi B, Cooper DS. The clinical significance of subclinical thyroid dysfunction. Endocr Rev. 2008;29(1):76-131. Epub 2007/11/10. doi: 10.1210/er.2006-0043. PubMed PMID: 17991805.
7. Blum MR, Bauer DC, Collet TH, Fink HA, Cappola AR, da Costa BR, et al. Subclinical thyroid dysfunction and fracture risk: a meta-analysis. JAMA. 2015;313(20):2055-65. Epub 2015/05/27. doi: 10.1001/jama.2015.5161. PubMed PMID: 26010634; PubMed Central PMCID: PMCPMC4729304.
8. Johnell O, Kanis JA, Oden A, Johansson H, De Laet C, Delmas P, et al. Predictive Value of BMD for Hip and Other Fractures. Journal of Bone and Mineral Research. 2005;20(7):1185-94. doi: 10.1359/jbmr.050304.

9. Cummings SR, Black DM, Nevitt MC, Browner W, Cauley J, Ensrud K, et al. Bone density at various sites for prediction of hip fractures. The Study of Osteoporotic Fractures Research Group. Lancet. 1993;341(8837):72-5. Epub 1993/01/09. PubMed PMID: 8093403.
10. Waring AC, Harrison S, Fink HA, Samuels MH, Cawthon PM, Zmuda JM, et al. A prospective study of thyroid function, bone loss, and fractures in older men: The MrOS study. J Bone Miner Res. 2013;28(3):472-9.
11. Stewart LA, Clarke M, Rovers M, Riley RD, Simmonds M, Stewart G, et al. Preferred Reporting Items for Systematic Review and Meta-Analyses of individual participant data: the PRISMA-IPD Statement. Jama. 2015;313(16):1657-65. Epub 2015/04/29. doi: 10.1001/jama.2015.3656. PubMed PMID: 25919529.
12. Segna D. The relationship between subclinical thyroid dysfunction and bone mineral density: an individual participant data analysis. http://wwwcrdyorkacuk/PROSPERO/display_recordasp?ID=CRD42015019814. 2016.
13. Wells GA SB, O'Connell D,Peterson J, Welch V , Losos M, Tugwell P. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. http://wwwohrica/programs/clinical_epidemiology/oxfordasp.
14. Rodondi N, den Elzen WP, Bauer DC, Cappola AR, Razvi S, Walsh JP, et al. Subclinical hypothyroidism and the risk of coronary heart disease and mortality. Jama. 2010;304(12):1365-74.
15. Collet TH, Gussekloo J, Bauer DC, den Elzen WP, Cappola AR, Balmer P, et al. Subclinical hyperthyroidism and the risk of coronary heart disease and mortality. Arch Intern Med. 2012;172(10):799-809. Epub 2012/04/25. doi: 10.1001/archinternmed.2012.402. PubMed PMID: 22529182; PubMed Central PMCID: PMCPmc3872478.
16. Surks MI, Ortiz E, Daniels GH, Sawin CT, Col NF, Cobin RH, et al. Subclinical thyroid disease: scientific review and guidelines for diagnosis and management. Jama. 2004;291(2):228-38.
17. Helfand M. Screening for Subclinical Thyroid Dysfunction in Nonpregnant Adults: A Summary of the Evidence for the U.S. Preventive Services Task Force. Annals of Internal Medicine. 2004;140(2):128-41. doi: 10.7326/0003-4819-140-2-200401200-00015.
18. Reid IR. Short-term and long-term effects of osteoporosis therapies. Nature reviews Endocrinology. 2015;11(7):418-28. Epub 2015/05/13. doi: 10.1038/nrendo.2015.71. PubMed PMID: 25963272.
19. Seibel MJ, Cooper MS, Zhou H. Glucocorticoid-induced osteoporosis: mechanisms, management, and future perspectives. The lancet Diabetes & endocrinology. 2013;1(1):59-70. Epub 2014/03/14. doi: 10.1016/s2213-8587(13)70045-7. PubMed PMID: 24622268.
20. Li D, Hitchcock CL, Barr SI, Yu T, Prior JC. Negative spinal bone mineral density changes and subclinical ovulatory disturbances--prospective data in healthy premenopausal women with regular menstrual cycles. Epidemiol Rev. 2014;36:137-47. Epub 2013/11/28. doi: 10.1093/epirev/mxt012. PubMed PMID: 24275546.
21. Chang KV, Hung CY, Chen WS, Lai MS, Chien KL, Han DS. Effectiveness of bisphosphonate analogues and functional electrical stimulation on attenuating post-injury osteoporosis in spinal cord injury patients- a systematic review and meta-analysis. PLoS One. 2013;8(11):e81124. Epub 2013/11/28. doi: 10.1371/journal.pone.0081124. PubMed PMID: 24278386; PubMed Central PMCID: PMCPMC3838359.
22. Leslie WD, Brennan-Olsen SL, Morin SN, Lix LM. Fracture prediction from repeat BMD measurements in clinical practice. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA. 2015. Epub 2015/08/06. doi: 10.1007/s00198-015-3259-y. PubMed PMID: 26243362.
23. Riley RD, Lambert PC, Abo-Zaid G. Meta-analysis of individual participant data: rationale, conduct, and reporting. Bmj. 2010;340:c221. Epub 2010/02/09. doi: 10.1136/bmj.c221. PubMed PMID: 20139215.
24. Simmonds MC, Higgins JP, Stewart LA, Tierney JF, Clarke MJ, Thompson SG. Meta-analysis of individual patient data from randomized trials: a review of methods used in practice. Clinical trials (London, England). 2005;2(3):209-17. Epub 2005/11/11. PubMed PMID: 16279144.

25. Drake MT, Murad MH, Mauck KF, Lane MA, Undavalli C, Elraiyah T, et al. Clinical review. Risk factors for low bone mass-related fractures in men: a systematic review and meta-analysis. J Clin Endocrinol Metab. 2012;97(6):1861-70. Epub 2012/04/03. doi: 10.1210/jc.2011-3058. PubMed PMID: 22466344.
26. Ward KD, Klesges RC. A meta-analysis of the effects of cigarette smoking on bone mineral density. Calcified tissue international. 2001;68(5):259-70. Epub 2001/10/31. PubMed PMID: 11683532.
27. Cauley JA. Bone health after menopause. Current opinion in endocrinology, diabetes, and obesity. 2015;22(6):490-4. Epub 2015/10/30. doi: 10.1097/med.0000000000000204. PubMed PMID: 26512774.
28. DerSimonian R, Laird N. Meta-analysis in clinical trials. Controlled clinical trials. 1986;7(3):177-88. Epub 1986/09/01. PubMed PMID: 3802833.
29. da Costa BR, Juni P. Systematic reviews and meta-analyses of randomized trials: principles and pitfalls. European heart journal. 2014;35(47):3336-45. Epub 2014/11/25. doi: 10.1093/eurheartj/ehu424. PubMed PMID: 25416325.
30. Svare A, Nilsen TI, Asvold BO, Forsmo S, Schei B, Bjoro T, et al. Does thyroid function influence fracture risk? Prospective data from the HUNT2 study, Norway. Eur J Endocrinol. 2013;169(6):845-52. Epub 2013/09/14. doi: 10.1530/eje-13-0546. PubMed PMID: 24031093.
31. Ceresini G, Ceda GP, Lauretani F, Maggio M, Usberti E, Marina M, et al. Thyroid status and 6-year mortality in elderly people living in a mildly iodine-deficient area: the aging in the Chianti Area Study. J Am Geriatr Soc. 2013;61(6):868-74. Epub 2013/05/08. doi: 10.1111/jgs.12267. PubMed PMID: 23647402; PubMed Central PMCID: PMCPmc3686888.
32. Barbour KE, Zmuda JM, Boudreau R, Strotmeyer ES, Horwitz MJ, Evans RW, et al. The effects of adiponectin and leptin on changes in bone mineral density. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA. 2012;23(6):1699-710. Epub 2011/08/31. doi: 10.1007/s00198-011-1768-x. PubMed PMID: 21877199; PubMed Central PMCID: PMCPMC3536828.
33. Finigan J, Greenfield DM, Blumsohn A, Hannon RA, Peel NF, Jiang G, et al. Risk factors for vertebral and nonvertebral fracture over 10 years: a population-based study in women. J Bone Miner Res. 2008;23(1):75-85. Epub 2007/09/06. doi: 10.1359/jbmr.070814. PubMed PMID: 17784843.
34. van der Deure WM, Uitterlinden AG, Hofman A, Rivadeneira F, Pols HA, Peeters RP, et al. Effects of serum TSH and FT4 levels and the TSHR-Asp727Glu polymorphism on bone: the Rotterdam Study. Clin Endocrinol. 2008;68(2):175-81.
35. Garin MC, Arnold AM, Lee JS, Robbins J, Cappola AR. Subclinical thyroid dysfunction and hip fracture and bone mineral density in older adults: the cardiovascular health study. J Clin Endocrinol Metab. 2014;99(8):2657-64.
36. Murphy E, Gluer CC, Reid DM, Felsenberg D, Roux C, Eastell R, et al. 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. J Clin Endocrinol Metab. 2010;95(7):3173-81. Epub 2010/04/23. doi: 10.1210/jc.2009-2630. PubMed PMID: 20410228.
37. Yan Z, Huang H, Li J, Wang J. Relationship between subclinical thyroid dysfunction and the risk of fracture: a meta-analysis of prospective cohort studies. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA. 2016;27(1):115-25. Epub 2015/08/01. doi: 10.1007/s00198-015-3221-z. PubMed PMID: 26223189.
38. Belaya ZE, Melnichenko GA, Rozhinskaya LY, Fadeev VV, Alekseeva TM, Dorofeeva OK, et al. Subclinical hyperthyroidism of variable etiology and its influence on bone in postmenopausal women. Hormones (Athens, Greece). 2007;6(1):62-70. Epub 2007/02/28. PubMed PMID: 17324919.

39. Biondi B. Natural history, diagnosis and management of subclinical thyroid dysfunction. Best practice & research Clinical endocrinology & metabolism. 2012;26(4):431-46. Epub 2012/08/07. doi: 10.1016/j.beem.2011.12.004. PubMed PMID: 22863386.
40. Brennan MD, Powell C, Kaufman KR, Sun PC, Bahn RS, Nair KS. The impact of overt and subclinical hyperthyroidism on skeletal muscle. Thyroid. 2006;16(4):375-80. Epub 2006/05/02. doi: 10.1089/thy.2006.16.375. PubMed PMID: 16646684.
41. Virgini VS, Rodondi N, Cawthon PM, Harrison SL, Hoffman AR, Orwoll ES, et al. Subclinical Thyroid Dysfunction and Frailty Among Older Men. J Clin Endocrinol Metab. 2015;100(12):4524-32. Epub 2015/10/27. doi: 10.1210/jc.2015-3191. PubMed PMID: 26495751; PubMed Central PMCID: PMCPmc4667157.
42. Orwoll E, Blank JB, Barrett-Connor E, Cauley J, Cummings S, Ensrud K, et al. Design and baseline characteristics of the osteoporotic fractures in men (MrOS) study--a large observational study of the determinants of fracture in older men. Contemp Clin Trials. 2005;26(5):569-85. Epub 2005/08/09. doi: 10.1016/j.cct.2005.05.006. PubMed PMID: 16084776.

Correspondence to:

Prof. Nicolas Rodondi, MD, MAS, Head of Ambulatory Care, Department of General Internal Medicine, University of Bern, Bern University Hospital, Freiburgstrasse 4, 3010 Bern, Switzerland. Email: nicolas.rodondi@insel.ch. Tel: + 41 (31) 632 41 63. Fax: +41 (31) 632 88 85

Backup contact:

Daniel Segna, MD, Department of General Internal Medicine, Bern University Hospital, Bern, Switzerland. Email: daniel.segna@insel.ch

Table 1: Baseline Characteristics of Included Cohort Studies

General					Follow-up for Bone Mineral Density			Thyroid Status		Thyroid Drugs *		Anti-osteoporotic Drugs †
Cohort	**Sample characteristics**	**N**	**Age** (median,IQR) years	**Female** (%)	**Baseline,** years	**Median** (IQR), years	**Person-years**	**SHyper (%)**	**SHypo (%)**	**Baseline**	**Follow-up ‡**	**Follow-up ‡**
Cardiovascular Health Study(35)	CDA aged ≥65y with Medicare eligibility in 2 US communities §	425	75·0 (73·0-78·0)	229 (53·9%)	1994-1995	4·0 (4·0-4·0)	1,700	17 (4·0%)	42 (9·9%)	50 (11·8%)	63 (14·8%)	29 (6·8%)
Health ABC Study(32) ‖	CDA aged 70-79y with Medicare eligibility in 2 US communities	1,772	74·0 (72·0-77·0)	709 (40·0%)	1997-1998	8·8 (4·9-9·0)	15,594	49 (2·8%)	228 (12·9%)	142 (8·0%)	227 (12·8%)	153 (8·6%)
Osteoporotic Fractures in Men (MrOS) Study(10)	CDMs aged ≥ 65y in 6 US clinical centers	910	72·0 (68·0-76·0)	0 (0%)	2000-2002	6·7 (6·5-6·9)	6,097	11 (1·2%)	77 (8·5%)	51 (5·6%)	97 (10·7%)	64 (7·0%)
Osteoporosis and Ultrasound Study (OPUS)(36)	Women aged 20-80y, Germany, France, UK	665	63·6 (39·5-70·4)	665 (100%)	1999-2001	6·0 (5·8-6·2)	3,990	102 (15·3%)	4 (0·6%)	0 (0·0%)	31 (4·7%)	29 (6·8%)
Rotterdam Study(34)	Adults aged 55y+, Netherlands	1,531	68·1 (62·6-73·9)	924 (60·4%)	1990-1993	7·0 (2·9-11·1)	10,717	101 (6·6%)	84 (5·5%)	36 (2·4%)	36 (2·4%)	22 (1·4%)
Sheffield Study(33)	Women aged 50-85y, Sheffield, UK	155	63·5 (57·7-68·8)	155 (100%)	1990-1991	10·0 (5·1-10·0)	1,550	4 (2·6%)	16 (10·3%)	0 (0·0%)	9 (5·8%)	23 (14·8%)
Overall	6 cohorts	5,458	72 (67·0-76·0)	2,682 (49·1%)	1990-2001	6·7 (4·8-8·9)	36,569	284 (5·2%)	451 (8·3%)	279 (5·1%)	463 (8·5%)	328 (6·0%)

Footnote:

Values given in absolute numbers and percentages for participants with serial dual-x-ray-absorptiometry (DXA) scans. For medication, percentage is related either to total number at baseline or follow-up, as appropriate. Abbreviations: BMD: bone mineral density at any site of interest (femoral neck, total hip, lumbar spine). CA: California; CDA: community-dwelling adults; CDM: community-dwelling men; IQR: interquartile range; MD: Maryland; NC: North Carolina; PA: Pennsylvania; UK: United Kingdom; US: United States; y: years.

Baseline characteristics for main analysis after exclusion of participants with one, or a combination of, bone-altering medication at baseline: hormone replacement therapy (n=878), anti-osteoporotic treatment (n=226, including bisphosphonates, calcitonin, teriparatide, selective estrogen receptor modulators, fluoride), proton pump inhibitors (n=177), oral corticosteroids (n=78), contraceptives (n=28), androgens (n=8), anti-androgens (n=2),

* Thyroid-altering medication includes thyroid hormone replacement therapy and anti-thyroid drugs. OPUS and Sheffield Study did not record anti-thyroid drugs. Additional thyroid-altering drugs vary across studies and are considered in our main analysis

† Anti-osteoporotic medication includes: bisphosphonates, calcitonin parathyroid hormone, selective estrogen receptor modulators, fluoride substitution. Additional bone-altering agents vary across studies and are considered in our main analysis.

‡ Different follow-up durations for BMD across studies and participants, therefore individual information provided for each patient.

§ Baseline and follow-up DXA scans from the study site in Pittsburgh, PA

‖ Thyroid status measured one year after 1st BMD measurement

Table 2: Sensitivity Analyses for the Multivariable-Adjusted* Association between Subclinical Hyperthyroidism and Annualized Change in Bone Mineral Density

	N SHyper/Euthyroidism	%ΔBMD	95%CI	I^2	*p*
Femoral Neck					
Main analysis: Exclusion of bone drug users at baseline	**283/4700**	-0·18	-0·34; -0·02	0·0%	0·44
and no history of osteoporosis, and/or previous, and/or incident fractures	222/3517	-0·23	-0·45; <-0·01	23·2%	0·26
Exclusion of bone drug users† at any time	234/3559	-0·18	-0·36; <-0·01	0·0%	0·48
Exclusion of both thyroid‡- and bone-influencing drug users at any time	184/3348	-0·36	-0·71; <-0·01	45·9%	0·10
Exclusion of cohorts with >20% missing follow-up BMD §	154/2968	-0·36	-0·63; -0·09	0·0%	0·56
Inclusion of participants with TSH<0.10mIU/l only	54/4700	-0·59	-0·99; -0·19	0·0%	0·44
Total Hip					
Main analysis: Exclusion of bone drug users at baseline	**232/4122**	-0·14	-0·38; 0·10	52·7%	0·06
and no history of osteoporosis, and/or previous, and/or incident fractures	181/3013	-0·17	-0·53; 0·19	74·5%	<0·01
Exclusion of bone drug users† at any time	184/3037	-0·16	-0·47; 0·15	60·8%	0·03
Exclusion of both thyroid‡- and bone-influencing drug users at any time	141/2844	-0·40	-0·96; 0·16	81·9%	<0·01
Exclusion of cohorts with >20% missing follow-up BMD §	103/2389	-0·38	-0·65; -0·10	15·8%	0·31
Inclusion of participants with TSH<0.10mIU/l only	42/4122	-0·46	-1·05; 0·13	59·5%	0·04
Lumbar Spine					
Main analysis: Exclusion of bone drug users at baseline	**163/2974**	0·03	-0·30; 0·36	24.8%	0·26
and no history of osteoporosis, and/or previous, and/or incident fractures	121/1985	-0·06	-0·42; 0·29	19·5%	0·29
Exclusion of bone drug users† at any time	128/2069	0·33	-0·35; 1·00	64·6%	0·04
Exclusion of both thyroid‡- and bone-influencing drug users at any time	101/1930	0·39	-0·47; 1·25	64·7%	0·04
Exclusion of cohorts with >20% missing follow-up BMD §	53/1619	0·36	-0·55; 1·28	62·5%	0·10
Inclusion of participants with TSH<0.10mIU/l only	23/2974	0·44	-1·12; 0·24	0·0%	0·52

Footnote: Abbreviations: %ΔBMD: annualized percentage change in bone mineral density compared to euthyroid individuals, I^2: I^2 statistics, 95%CI: 95% confidence intervals; N: number of participants; p = p for heterogeneity, SHyper: subclinical hyperthyroidism.

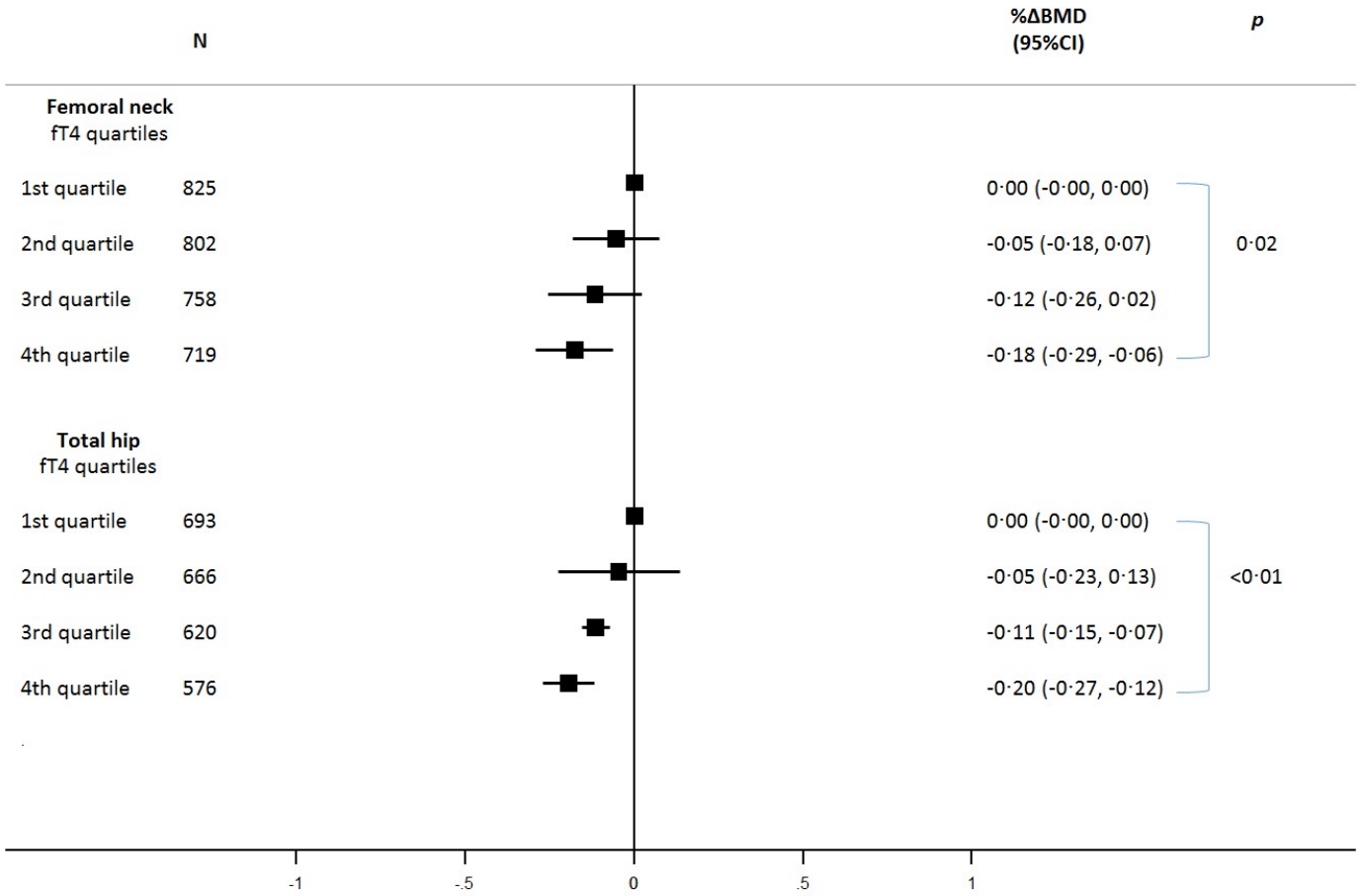
* Multivariable adjustment for age, sex, bone mass index, smoking and menopausal status, history of diabetes. Values presented as mean difference in annualized percentage change in BMD, as compared to euthyroid controls.

† Bone-altering drug users with intake of either bisphosphonates, calcitonin, teriparatide, selective estrogen receptor modulators, oral corticosteroids, thiazide diuretics, androgens, anti-androgens, hormone replacement therapy or proton pump inhibitors.

‡ Thyroid-altering drug users with intake of either thyroxine, antithyroid drugs, amiodarone, or lithium.

§ Exclusion of the Cardiovascular Health Study (35), Osteoporotic Fractures in Men (MrOS) Study(42) and Osteoporosis and Ultrasound Study (OPUS)(36) for the sensitivity analysis of %ΔBMD at the femoral neck and total hip. Additionally, no data available for %ΔBMD at the lumbar spine in Rotterdam Study.(34)

Figure 1: Annualized Percentage Change in Hip Bone Mineral Density Stratified by Cohort-Specific fT4 Quartiles



Legend: Multivariable adjustment for age, sex, bone mass index, smoking and menopausal status, history of diabetes. Values presented as mean difference in annualized percentage change in BMD (%ΔBMD). 95%CI: 95% confidence intervals; fT4: free thyroxine; quartiles obtained from each cohort, p for difference in %ΔBMD between the highest and lowest fT4 quartile.

Appendix Table 1: Definition of Thyroid Status* and Measurement Techniques/Devices for Bone Mineral Density

Study	Subclinical hypothyroidism	Subclinical hyperthyroidism	Measurement technique	Devices	Body sites
Cardiovascular Health Study (35)	TSH ≥4·5mIU/l & TSH <20mIU/l, normal fT4 0·7-1·7ng/dl (9-22pmol/l) or missing fT4 (0/42, 0·0%)	TSH <0·45mIU/l & normal fT4 0·7-1·7ng/dl (9-22pmol/l) or missing fT4 (0/17, 0·0%)	DXA	Hologic QDR 2000 (Hologic, Bedford, MA, USA)	Total hip, femoral neck
Health ABC Study (32) †	TSH ≥4·5mIU/l & TSH <20mIU/l, normal fT4 0·8-1·8ng/dl (10-23pmol/l) or missing fT4 (0/228, 0·0%)	TSH <0.45mIU/l & normal fT4 0.8-1.8ng/dl (10-23pmol/l) or missing fT4 (0/49, 0·0%)	DXA	Hologic QDR 4500, (Hologic, Bedford, MA, USA)	Total hip, femoral neck, lumbar spine (lumbar spine sub-region)
Osteoporotic Fractures in Men (MrOS) Study (10)	TSH ≥4·5mIU/l & TSH <20mIU/l, normal fT4 0·7-1·85ng/dl (9-24pmol/l) or missing fT4 (0/77, 0·0%)	TSH <0·45mIU/l & normal fT4 0·7-1·85ng/dl (9-24pmol/l) or missing fT4 (0/11, 0·0%)	DXA	Hologic QDR 4500, (Hologic, Bedford, MA, USA)	Total hip, femoral neck, lumbar spine
Osteoporosis and Ultrasound Study (OPUS) (36)	TSH ≥4·5mIU/l & TSH <20mIU/l, normal fT4 0·7-1·8ng/dl (9-23pmol/l) or missing fT4 (0/4, 0·0%)	TSH <0·45mIU/l & normal fT4 0·7-1·8ng/dl (9-23pmol/l) or missing fT4 (0/102, 0·0%)	DXA	Hologic QDR 4500, (Hologic, Bedford, MA, USA/ Lunar Expert XL (GE Lunar Corp., Madison, WI)	Total hip, femoral neck, lumbar spine
Rotterdam Study (34)	TSH ≥4·5mIU/l & TSH <20mIU/l, normal fT4 0·9-1·9ng/dl (11-25pmol/l) or missing fT4 (29/84, 34·5%)	TSH <0·45mIU/l & normal fT4 0·9-1·9ng/dl (11-25pmol/l) or missing fT4 (22/101, 21·8%)	DXA	Lunar DPX, (Madison, WI, USA)	Total hip, femoral neck
Sheffield Study (33)	TSH ≥4·5mIU/l & TSH <20mIU/l, normal fT4 0·9-1·7ng/dl (12-22pmol/l) or missing fT4 (0/16, 0·0%)	TSH < 0·45mIU/l & normal fT4 0·9-1·7ng/dl (12-22pmol/l) or missing fT4 (0/4, 0·0%)	DXA	Lunar DPX, (Madison, WI, USA)	Total hip, femoral neck, lumbar spine

Legend: * For a better comparability, we used a homogenous definition of TSH ranges based on an expert consensus meeting of the Thyroid Studies Collaboration (International Thyroid Conference, Paris, 2010), Individual free thyroxine (fT4) cut-off values for each cohort. based on an expert consensus meeting of the Thyroid Studies Collaboration (International Thyroid Conference, Paris, 2010), All BMD values were analyzed in g/cm^2. Abbreviations: BMD: bone mineral density; DXA: dual x-ray absorptiometry; fT4: free thyroxine; L: lumbar vertebra; TSH: thyroid-stimulating hormone. † fT4 was measured only in participants with TSH ≤ 0.10mIU/l or TSH ≥ 7.00mIU/l

Appendix Table 2: Quality Assessment for Thyroid Hormone and Bone Mineral Density Measurements

Study	Blinding to thyroid status*	Ascertainment of exposure	Assessment of most important covariates	Assessment of relevant co-medication at baseline	Standardization techniques for BMD measurements	Completeness of follow-up †	Newcastle Ottawa Quality Assessment Scale ‡	Duration of follow-up, median (IQR) in main analysis
Cardiovascular Health Study (CHS) (35)	Yes	Third generation TSH assay	Age, sex, BMI, diabetes mellitus, smoking and menopausal status	Anti-osteoporotic medication, oral corticosteroids, hormone replacement therapy, proton pump inhibitors, thyroxine, antithyroid medication, lithium, amiodarone	Regular cross-calibration of devices/sites with anthropometric phantoms. Regular longitudinal change calibration with phantom	59·2% §	6	4·0 (4·0-4·0)
Health, Aging and Body Composition (Health ABC) study (32)	Yes	Third generation TSH assay	Age, sex, BMI, diabetes mellitus, smoking and menopausal status	Anti-osteoporotic medication, oral corticosteroids, hormone replacement therapy, thyroxine, antithyroid medication, lithium, amiodarone	Regular cross-calibration of devices/sites with anthropometric spine phantoms. Longitudinal change calibration weekly with hip phantom	92·9%	7	8·8 (4·9-9·0)
Osteoporotic Fractures in Men (MrOS) Study (10)	Yes	Third generation TSH assay	Age, sex, BMI, diabetes mellitus, smoking status,	Anti-osteoporotic medication, oral corticosteroids, proton pump inhibitors, androgens, thyroxine, antithyroid medication, lithium, amiodarone	Regular cross-calibration of devices/sites, and calibration for longitudinal changes using identical standardized phantoms	75·1%	6	6·7 (6·5-6·9)
Osteoporosis and Ultrasound Study (OPUS) (36)	Yes	Third generation TSH assay	Age, sex, BMI, diabetes mellitus, smoking and menopausal status	Anti-osteoporotic medication, oral corticosteroids, hormone replacement therapy, proton pump inhibitors, thyroxine	Regular cross-calibration of devices/sites, and calibration for longitudinal changes using European spine phantoms	62·2%	6	6·0 (5·8-6·2)
Rotterdam Study (34)	Yes	Third generation TSH assay	Age, sex, BMI, diabetes mellitus, smoking and menopausal status	Anti-osteoporotic medication, oral corticosteroids, hormone replacement therapy, proton pump inhibitors, thyroxine, antithyroid medication, lithium, amiodarone	Cross calibration of devices by performing repeated measurements on 100 individuals.	96·1%	7	7·0 (2·9-11·1)
Sheffield Study (33)	Yes	Third generation TSH assay	Age, sex, BMI, diabetes mellitus, smoking and menopausal status	Anti-osteoporotic medication, oral corticosteroids, hormone replacement therapy, thyroxine	Cross-calibration of devices, and calibration for longitudinal changes using an aluminum spine phantom.	83·2%	7	10·0 (5·1-10·0)

Legend:

Abbreviations: BMD: bone mineral density at any site of interest (femoral neck, total hip, lumbar spine); BMI: body mass index; DXA: dual x-ray absorptiometry; IQR: interquartile range; TSH: thyroid-stimulating hormone;

[*] Blinding of participants, treating physicians, study nurses and investigators involved in BMD measurements and fracture adjudication.

[†] no serial measurements of bone mineral density at the femoral neck, total hip and lumbar spine region at any point during follow-up.

[‡] Quality assessment using a slightly modified Newcastle-Ottawa Quality Assessment Scale –Cohort Studies (13) including following criteria (1 point each): "Representativeness of the exposed cohort", "Selection of the non-exposed cohort", "Ascertainment of exposure", "Comparability of cohorts on the basis of the design or analysis", "Assessment of outcome", "Was follow-up long enough for outcomes to occur", "Adequacy of follow-up of cohorts". The criterion "Demonstration that outcome of interest was not present at start of study" could not be considered as bone loss is a continuous outcome. Therefore, a maximum score of 7 points can be achieved.

[§] Baseline and follow-up DXA scans from the study site in Pittsburgh, PA.

Appendix Table 3: Sensitivity Analyses for the Multivariable-Adjusted* Association between Subclinical Hypothyroidism and Annualized Change in Bone Mineral Density

	N SHypo/Euthyroidism	%ΔBMD	95% CI	I^2	*p*
Femoral Neck					
Main analysis: Exclusion of bone drug users† at baseline	**448/4700**	0·00	-0·12; 0·13	0·0%	0·52
and no history of osteoporosis, and/or previous, and/or incident fractures	327/3517	-0·03	-0·17; 0·12	0·0%	0·47
Exclusion of bone drug users at any time	330/3559	0·08	-0·06; 0·23	0·0%	0·50
Exclusion of both thyroid‡- and bone-influencing drug users at any time	222/3348	0·08	-0·10; 0·27	1·9%	0·40
Exclusion of cohorts with >20% missing follow-up BMD §	326/2968	0·01	-0·15; 0·17	0·0%	0·59
Total Hip					
Main analysis: Exclusion of bone drug users† at baseline	**411/4122**	0·02	-0·08; 0·12	0·0%	0·48
and no history of osteoporosis, and/or previous, and/or incident fractures	295/3013	-0·01	-0·12; 0·11	0·0%	0·52
Exclusion of bone drug users at any time	295/3037	0·10	-0·02; 0·22	0·0%	0·78
Exclusion of both thyroid‡- and bone-influencing drug users at any time	192/2844	0·14	-0·01; 0·28	0·0%	0·76
Exclusion of cohorts with >20% missing follow-up BMD §	288/2389	0·05	-0·09; 0·19	0·0%	0·98
Lumbar Spine					
Main analysis: Exclusion of bone drug users† at baseline	**323/2974**	-0·01	-0·34; 0·32	37·7%	0·19
and no history of osteoporosis, and/or previous, and/or incident fractures	216/1985	-0·10	-0·34; 0·14	0·0%	0·70
Exclusion of bone drug users at any time	220/2069	-0·08	-0·34; 0·18	0·0%	0·82
Exclusion of both thyroid‡- and bone-influencing drug users at any time	141/1930	-0·11	-0·43; 0·21	0·0%	0·80
Exclusion of cohorts with >20% missing follow-up BMD §	243/1619	-0·08	-0·31; 0·15	0·0%	0·54

Footnote: Abbreviations: %ΔBMD: annualized percentage change in bone mineral density compared to euthyroid individuals, I2: I2 statistics, 95%CI: 95% confidence intervals; N: number of participants; p = p for heterogeneity; SHypo: subclinical hypothyroidism.

* Multivariable adjustment for age, gender, body mass index, smoking and menopausal status, history of diabetes. Values presented as mean difference in annualized percentage change in BMD, as compared to euthyroid controls.

† Bone drug users with intake of either bisphosphonates, calcitonin, teriparatide, selective estrogen receptor modulators, oral corticosteroids, thiazide diuretics, androgens, anti-androgens, hormone replacement therapy or proton pump inhibitors.

‡ Thyroid-altering drug users with intake of either thyroxine, antithyroid drugs, amiodarone, or lithium.

§ Exclusion of the Cardiovascular Health Study,(35) Osteoporotic Fractures in Men (MrOS) Study,(10) and Osteoporosis and Ultrasound Study (OPUS)(36) for the sensitivity analysis of %ΔBMD at the femoral neck and total hip. Additionally, no data available for %ΔBMD at the lumbar spine in Rotterdam Study.(34)

Appendix Table 4: Secondary Analyses on the Association between Annualized Percentage Change in Bone Mineral Density (BMD) as a Continuous Variable and Fracture Risk and Effect Modification by Thyroid Status (SHyper vs. Euthyroidism)

	%ΔBMD femoral neck			%ΔBMD total hip		
	HR	**95%CI**	***p* for interaction**	**HR**	**95%CI**	***p* for interaction**
Hip fractures *						
adjusting for age and gender						
SHyper	1·08	0·80-1·47	0·24	1·21	0·77-1·89	0·07
Euthyroidism	0·90	0·83-0·97		0·79	0·71-0·88	
multivariable adjustment §						
SHyper	1·08	0·80-1·46	0·27	1·12	0·72-1·74	0·13
Euthyroidism	0·91	0·84-0·98		0·79	0·71-0·89	
Any fractures †						
adjusting for age and gender						
SHyper	0·94	0·80-1·10	0·60	0·88	0·74-1·04	0·57
Euthyroidism	0·90	0·85-0·94		0·83	0·77-0·90	
multivariable adjustment §						
SHyper	0·94	0·81-1·10	0·56	0·88	0·75-1·04	0·58
Euthyroidism	0·90	0·86-0·94		0·84	0·77-0·90	
Non-spine fractures						
adjusting for age and gender						
SHyper	0·93	0·78-1·10	0·85	0·89	0·74-1·09	0·80
Euthyroidism	0·94	0·90-0·99		0·92	0·85-1·00	
multivariable adjustment §						
SHyper	0·94	0·79-1·11	0·87	0·90	0·75-1·09	0·86
Euthyroidism	0·95	0·90-1·00		0·92	0·85-1·00	

Footnote: Abbreviations: BMD: bone mineral density; %ΔBMD: annualized percentage change in bone mineral density compared to euthyroid individuals, HR: hazard ratio, SHyper: subclinical hyperthyroidism, 95%CI: 95% confidence interval.

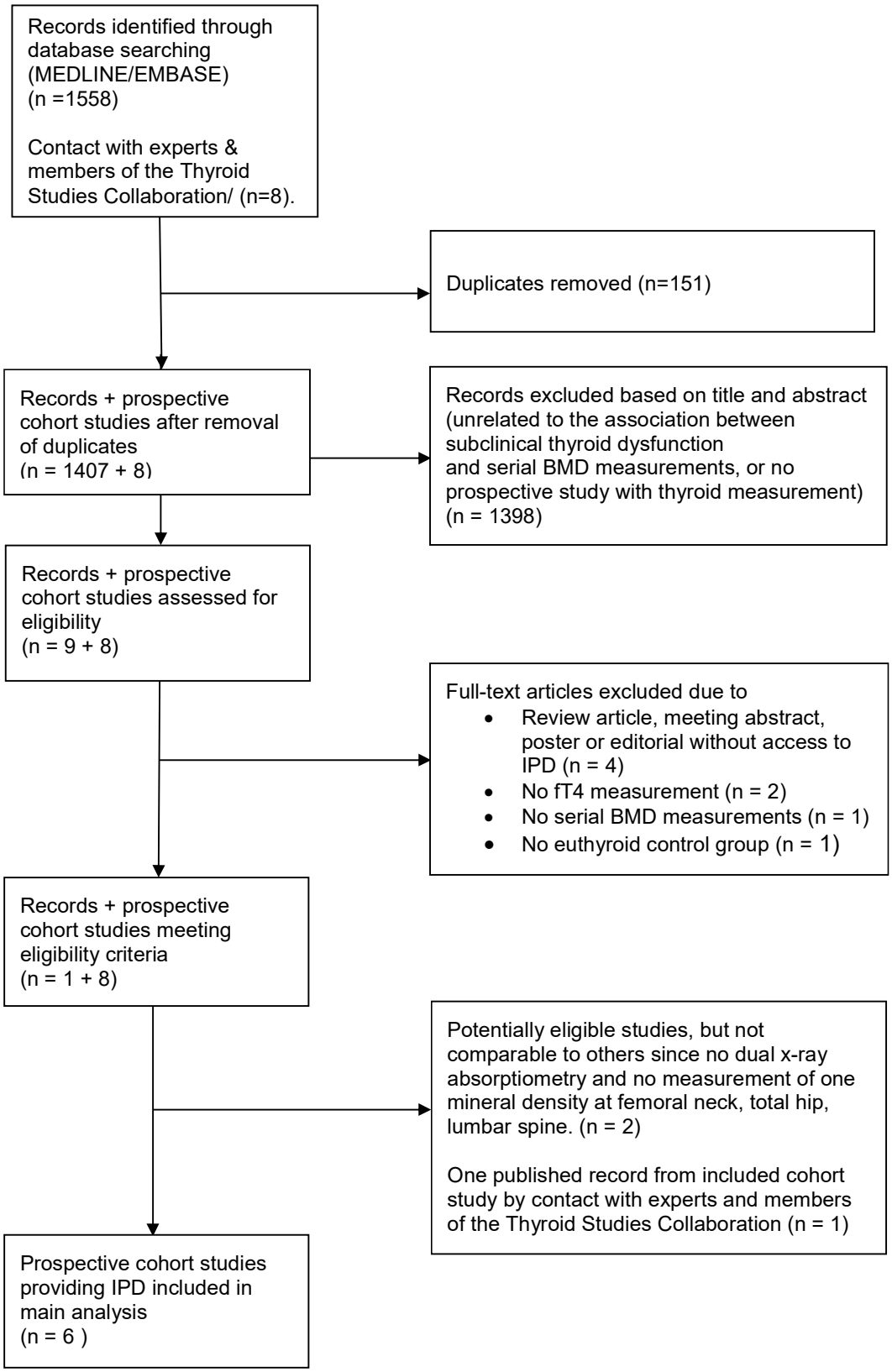
* Hip fractures comprise pertrochanteric, subtrochanteric and femoral neck fractures. Exclusion of periprosthetic and pathologic fractures in this region

† Any fractures comprise both non-spine and radiologically confirmed spine fractures. The Cardiovascular Health Study could not contribute due to missing information on spine and non-spine fractures other than hip fractures.

‡ Incident non-spine fractures defined as hip or any other non-pathologic fractures excluding the spinal, cranial/facial and acral fractures. The Cardiovascular Health Study, Sheffield and Osteoporosis and Ultrasound (OPUS) studies could not contribute due to missing assessment of any fractures.

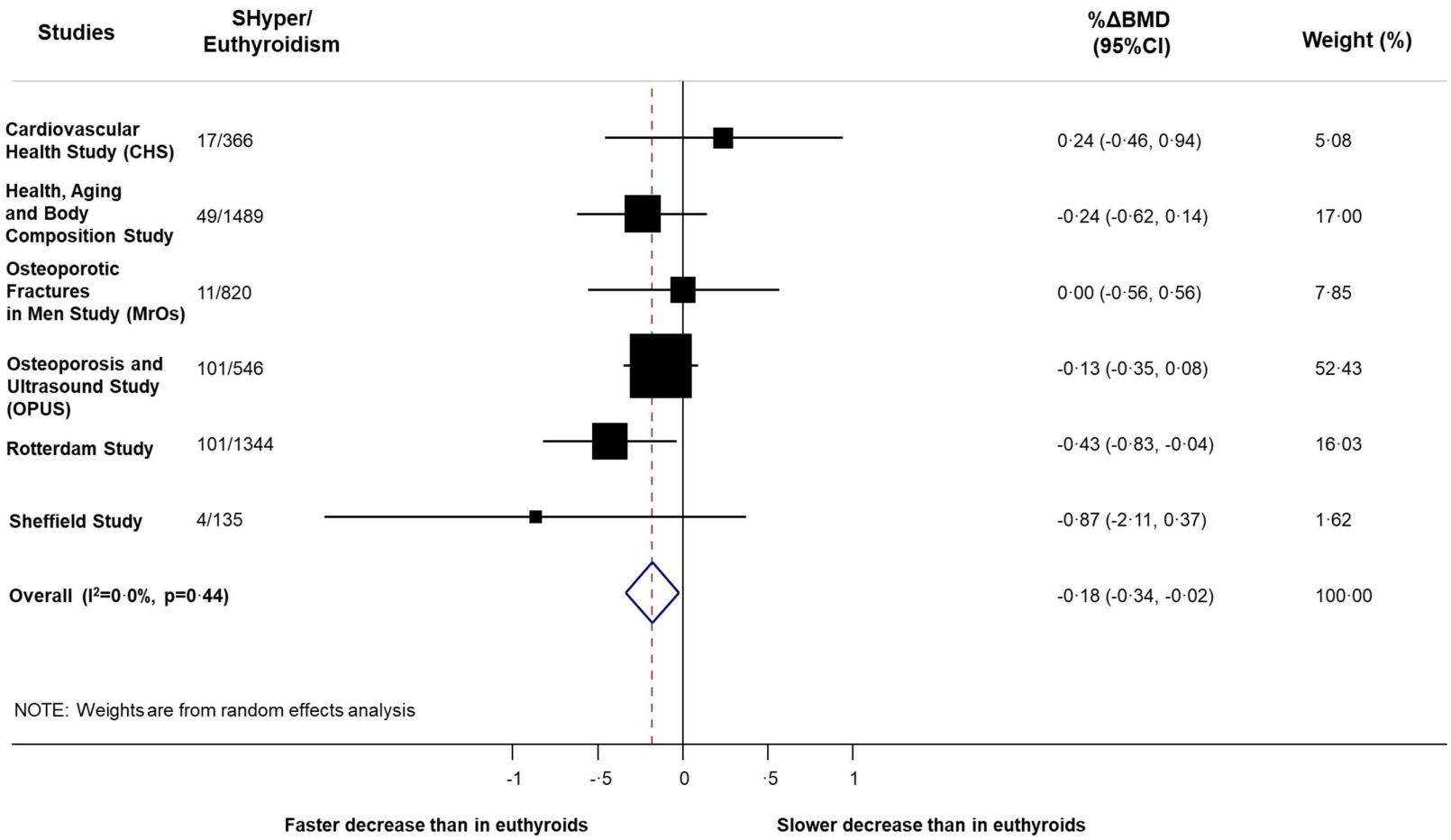
§ Multivariable adjustment for age, gender, body mass index, history of diabetes mellitus and smoking status

Appendix Figure 1: Flow chart of study selection



Legend: BMD: bone mineral density, fT4: free thyroxine, IPD: individual participant data, n= number of studies

Appendix Figure 2: Subclinical Hyperthyroidism and Annualized Percentage Change in Femoral Neck Bone Mineral Density Compared to Euthyroid Individuals



Legend: Multivariable adjustment for age, sex, bone mass index, smoking and menopausal status, history of diabetes. Values presented as mean difference in annualized percentage change in BMD (%ΔBMD), as compared to euthyroid controls. Abbreviations: I2: I2 statistics; p = p for heterogeneity; 95%CI: 95% confidence intervals

Appendix Methods:

Details of statistical approach

Considering the effects of bone- and thyroid-altering medication on BMD, we conducted the main analysis on individuals without bone-altering medication at baseline, and provided sensitivity analyses with 1) additional exclusion of participants with history of osteoporosis, and previous or incident hip, spine, or non-spine fractures during observation time, 2) bone-altering medication at any time (i.e. at baseline and/or follow-up visits), 3) exclusion of both thyroid- and bone-altering drug users at any time, 4) exclusion of cohorts with >20% missing follow-up BMD for %ΔBMD at any site (Appendix Table 2), and 5) selection of participants with SHyper and TSH<0·10 mUI/l in order to investigate those with suppressed TSH levels.

Similar to previous IPD analyses,(7, 14) we performed stratified analyses for sex, age, and cohort-specific fT4 quartiles using the same multivariate regression models as explained above.

When investigating %ΔBMD as a potential mediator in the association between SHyper and fractures, we used the same sample as for the main analysis, and conducted a one-step approach analysis, using a multivariable shared frailty Cox proportional hazards model controlling for the same covariates (age, sex, BMI, smoking status) as in Blum et al..(7) We added %ΔBMD and baseline BMD at the total hip as new covariates to the multivariable model in order to assess changes in risk estimates. Additionally, we assessed the association between %ΔBMD at the femoral neck/total hip (as a continuous variable), and fractures using a multivariable Cox regression model adjusting for 1) age and gender, 2) age, gender plus BMI, smoking status and diabetes mellitus). We then stratified the analysis according to thyroid status (SHyper vs. euthyroidism) in order to examine a potential effect modification by thyroid status.