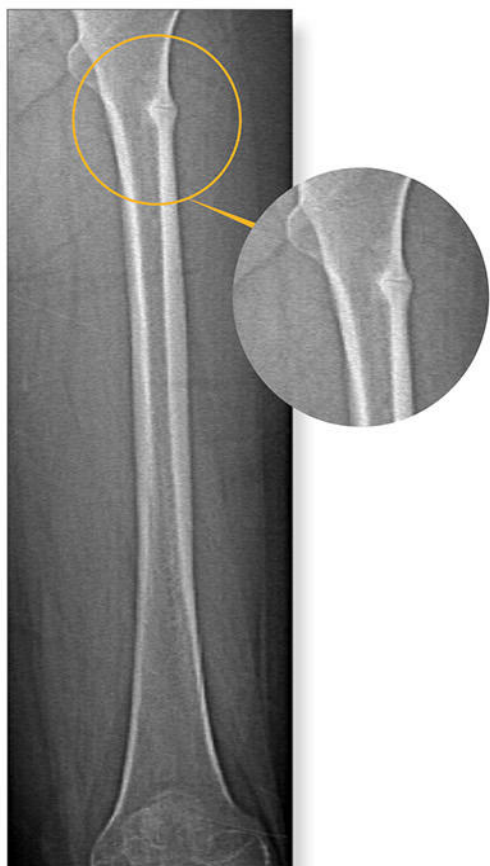
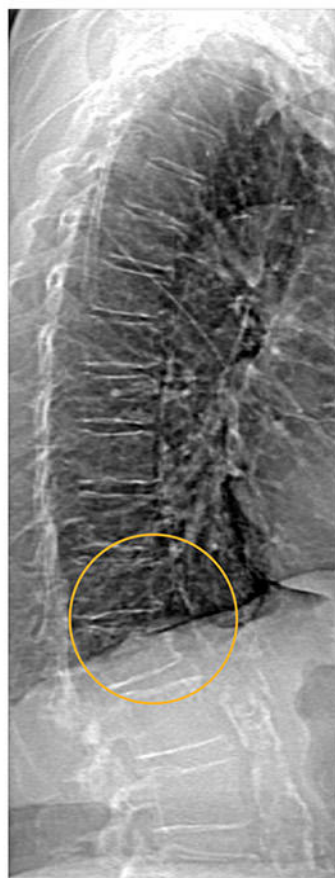




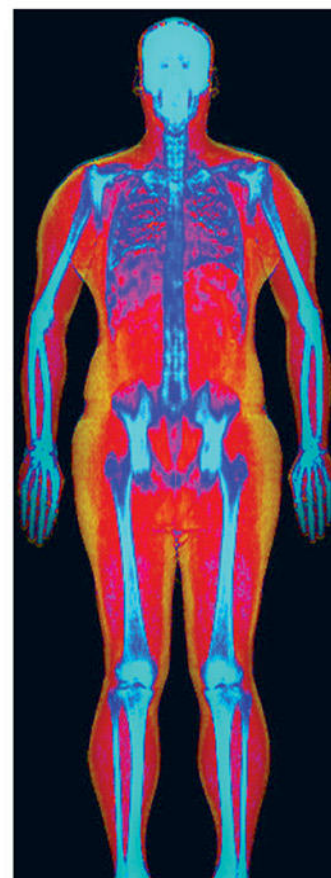
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# Thyroid Stimulating Hormone and Bone Mineral Density: Evidence From a Two-Sample Mendelian Randomization Study and a Candidate Gene Association Study

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## ABSTRACT

With population aging, prevalence of low bone mineral density (BMD) and associated fracture risk are increased. To determine whether low circulating thyroid stimulating hormone (TSH) levels within the normal range are causally related to BMD, we conducted a two-sample Mendelian randomization (MR) study. Furthermore, we tested whether common genetic variants in the TSH receptor (*TSHR*) gene and genetic variants influencing expression of *TSHR* (expression quantitative trait loci [eQTLs]) are associated with BMD. For both analyses, we used summary-level data of genomewide association studies (GWASs) investigating BMD of the femoral neck ( $n = 32,735$ ) and the lumbar spine ( $n = 28,498$ ) in cohorts of European ancestry from the Genetic Factors of Osteoporosis (GEFOS) Consortium. For the MR study, we selected 20 genetic variants that were previously identified for circulating TSH levels in a GWAS meta-analysis ( $n = 26,420$ ). All independent genetic instruments for TSH were combined in analyses for both femoral neck and lumbar spine BMD. In these studies, we found no evidence that a genetically determined 1-standard deviation (SD) decrease in circulating TSH concentration was associated with femoral neck BMD (0.003 SD decrease in BMD per SD decrease of TSH; 95% CI,  $-0.053$  to  $0.048$ ;  $p = 0.92$ ) or lumbar spine BMD (0.010 SD decrease in BMD per SD decrease of TSH; 95% CI,  $-0.069$  to  $0.049$ ;  $p = 0.73$ ). A total of 706 common genetic variants have been mapped to the *TSHR* locus and expression loci for *TSHR*. However, none of these genetic variants were associated with BMD at the femoral neck or lumbar spine. In conclusion, we found no evidence for a causal effect of circulating TSH on BMD, nor did we find any association between genetic variation at the *TSHR* locus or expression thereof and BMD. © 2018 The Authors. *Journal of Bone and Mineral Research* Published by Wiley Periodicals, Inc.

**KEY WORDS:** DXA; NEUROENDOCRINE; GENERAL POPULATION STUDIES; HUMAN ASSOCIATION STUDIES

## Introduction

Bone is a dynamic tissue that undergoes continuous remodeling to maintain its strength and integrity.<sup>(1)</sup> When bone remodeling is uncoupled and resorption exceeds formation, bone mineral density (BMD) progressively decreases and ultimately leads to osteoporosis.<sup>(2)</sup> To develop therapies that are more effective and accompanied by fewer side effects than current treatments, further research into the molecular mechanisms underlying the pathogenesis of osteoporosis is required.

One of these potential underlying mechanisms is thyroid status. Briefly, thyroid status is a composite measure of circulating thyroid stimulating hormone (thyrotropin, TSH)

and free thyroxine (fT4). In healthy individuals, circulating levels of TSH and fT4 are regulated by the hypothalamic-pituitary-thyroid axis (HPT axis) via feedforward and negative feedback mechanisms.<sup>(3)</sup> Therefore, circulating levels of TSH and fT4 are inversely related. However, the exact combination of circulating concentrations of TSH and fT4 is determined by the HPT axis set point, which is unique for each individual.<sup>(4)</sup> Thyroid hormones have a critical role in adult bone turnover and maintenance.<sup>(5)</sup> Hyperthyroidism (TSH concentration below the normal reference range and fT4 circulating level above the normal reference range) is associated with lower BMD and an increased risk of fracture, and is an established cause of secondary osteoporosis.<sup>(6)</sup> Furthermore, a similar relationship has been reported in

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individuals with subclinical hyperthyroidism (reduced circulating concentration of TSH but circulating fT4 within the normal reference range),<sup>(7,8)</sup> and in euthyroid individuals with a relatively low TSH and relatively high fT4 within the normal reference range.<sup>(9,10)</sup> Accordingly, individuals with subclinical hyperthyroidism had increased bone loss during prospective follow-up compared to euthyroid individuals.<sup>(11)</sup> In addition to effects of thyroid hormone on bone, some studies have suggested direct effects of TSH on bone.<sup>(12,13)</sup> However, others have not confirmed these findings.<sup>(14,15)</sup> Therefore, it remains unclear whether observed changes in bone mass and strength result from increased fT4 levels alone, or whether the associated decrease in TSH also contributes.

Mendelian randomization (MR) can be used to determine whether an association is causal, as it eliminates confounding and reverse causation. MR analysis uses genetic variants associated with an exposure as instrumental variables instead of direct measurements of the exposure.<sup>(16)</sup> Because genetic traits are inherited independently according to Mendel's second law, the determinant is randomly distributed in the population and independent of the outcome. In the present study, this assumes that genes associated with thyroid status and genes associated with BMD are inherited independently. Thus, analogous to randomized clinical trials, by using MR analysis the exposure can be assigned randomly and so associations between exposure (thyroid status) and outcome (BMD) can be investigated in the absence of confounding and reverse causation. MR studies can be performed on circulating TSH and fT4 levels within the normal reference range,<sup>(17)</sup> because these measures of thyroid status have been shown to be partly and independently genetically determined in large-scale genomewide association studies (GWASs).<sup>(18,19)</sup> Even though TSH and fT4 levels are highly correlated, different genetic variants were associated with circulating levels of TSH than with fT4.<sup>(18)</sup> The genetic independence of these traits highlights the individuality of the HPT-axis set point, and allows for separate analyses of TSH and fT4.

In the present study, we aimed to investigate whether thyroid status is causally associated with BMD through a two-sample Mendelian randomization study. However, due to the limited number of genetic instruments available for fT4 concentration in the largest meta-analysis to date on BMD (ie, lack of statistical power), only the relationship between TSH and BMD could be investigated rigorously. Additionally, to investigate the TSH receptor, which mediates TSH action in target cells, we explored the *TSHR* gene in a candidate gene study to determine whether genetic variation at this locus or expression thereof is associated with BMD.

## Materials and Methods

### Genetic variants for TSH

We selected single nucleotide polymorphisms (SNPs) for all genetic loci independently associated with circulating levels of TSH ( $p$  value  $< 5 \times 10^{-8}$ ) identified by the largest GWAS meta-analysis to date.<sup>(18)</sup> All participants included in the GWAS were of European ancestry, and individuals with known thyroid pathologies, taking thyroid medication, who underwent thyroid surgery and with circulating levels of TSH  $< 0.4$  mIU/L or  $> 4.0$  mIU/L were excluded from the analyses. For comparability of the different cohorts, the circulating levels of TSH were log-transformed, and standardized to Z-values. Due to these

transformations, the additive beta estimates of the SNPs can be interpreted as the per-allele standard deviation (SD) change in logTSH concentration. In total, 20 loci for TSH were identified in 26,420 participants. Overall, the mean age of participants ranged from 42.5 to 79.0 years, 44% of the participants were men. As an illustration, the descriptives of the two largest included cohorts (PROSPER and Sardinia) were as follows. In PROSPER, 49.1% were men and the mean age was 75.3 years with 3.4 years SD. In the Sardinia cohort, 46.9% were men and the mean age was 42.5 years with 17.7 years SD. The mean TSH concentration was 1.9 mIU/L and 1.7 mIU/L respectively (SD was 0.8 mIU/L for both cohorts).

### Data sources and outcome definition

We used publicly available data from the largest meta-analysis to date on BMD from the Genetic Factors of Osteoporosis (GEFOS) consortium,<sup>(20)</sup> which identified novel loci for BMD at the femoral neck, lumbar spine, and forearm, sites of the three most common osteoporotic fractures. Forearm BMD data were not used in the present study, because of the relatively low number of participants ( $n = 8143$ ). The meta-analysis on femoral neck BMD comprised 32,735 participants from nine cohorts of European ancestry and the meta-analysis on lumbar spine BMD comprised 28,498 participants from eight cohorts of European ancestry. The mean age in the participating cohorts ranged from 17.7 to 80.2 years, and 34% of the participants in the meta-analysis were men. From these data, we extracted the per-allele beta estimates of the SNPs previously identified in relation to circulating levels of TSH on femoral neck BMD and lumbar spine BMD, accompanied by the standard errors and the effect alleles.

### Power calculation

The statistical power for the MR analyses for TSH on BMD was calculated using a publicly available power calculator.<sup>(21)</sup> For the femoral neck BMD and the lumbar spine BMD there was sufficient power (femoral neck power = 0.85, and lumbar spine power = 0.80) to detect a causal association with a coefficient of 0.07 SD of BMD per decrease of 1 SD of TSH when using the data from GEFOS.<sup>(20)</sup>

### Statistical analyses

Methods for MR analysis of summary-level data have been described previously.<sup>(17,22,23)</sup> Briefly, associations between individual genetic instruments for circulating levels of TSH and BMD were estimated, after taking into account multiple testing via Bonferroni correction based on the number of genetic instruments tested. We combined effects of the individual genetic instruments using inverse-variance-weighted (IVW) analyses,<sup>(23)</sup> resulting in a weighted mean estimate of the effect of genetically determined 1-SD decrease in circulating level of TSH on BMD of the femoral neck and the lumbar spine in SD. However, this method could suffer from bias, because of potential pleiotropic effects of the genetic variants on other apparently unrelated phenotypes. If genetic variants have pleiotropic effects that influence outcome (eg, BMD) via alternative pathways, the observed associations can be biased. Therefore, MR-Egger regression<sup>(24)</sup> was conducted as sensitivity analysis to account for potential pleiotropy and to formally test the presence of directional pleiotropy. Additionally, we performed weighted median estimator (WME) analyses,<sup>(25)</sup> which estimate a weighted median effect of genetically determined 1-SD decrease in

circulating level of TSH on BMD. Similarity of the IVW and WME effect estimates indicates that the results are robust.<sup>(25)</sup> We also performed additional sensitivity analyses to account for possible regression dilution of analyses. In two-sample MR analyses, the reliability of the results depends on the precision of the previously measured association between the genetic variants and the exposure (ie, circulating TSH concentration). If the reported effect sizes for TSH do not reflect the true effect of the genetic variants, the association between the genetically determined levels of TSH and BMD will be erroneous. One of the available tests to assess the resulting imprecision of the MR is the  $I^2_{GX}$  statistic.<sup>(26)</sup> Preferably the  $I^2_{GX}$  statistic is close to 1, but an  $I^2_{GX}$  statistic  $\geq 0.9$  is still acceptable.<sup>(26)</sup> If the  $I^2_{GX}$  statistic is lower, the effect estimate is likely to be diluted, which means the observed association is an underestimation of the true effect. This type of bias can be corrected by simulation extrapolation (SIMEX).<sup>(27)</sup> This method simulates estimates of the investigated association with increasing imprecision to extrapolate a more precise estimate.

The combined effects of the genetic variants were calculated using the codes in R that were provided online by the authors.<sup>(24–26)</sup> Results are presented as the mean effect per 1-SD genetically determined decrease in circulating TSH level together with the 95% confidence interval (CI); a two-sided  $p$  value of less than 0.05 was considered statistically significant.

### Candidate gene association study on the TSH receptor

To investigate whether variation in the TSH receptor gene (*TSHR*) is associated with BMD, we conducted a candidate gene association study using the same publicly available summary level data of the GEFOS consortium.<sup>(20)</sup> SNPs were indexed if located in the *TSHR* gene or within 50,000 base pairs upstream or downstream. Additionally, previously reported SNPs influencing expression of *TSHR* (expression quantitative loci [eQTLs]) were included in the study. We excluded SNPs with a minor allele frequency (MAF) lower than 5% or if the SNP was absent from the GEFOS datasets. To determine an appropriate threshold for statistical significance, we based the correction factor on the number of independent genetic variants, meaning those not in linkage disequilibrium (LD). The number of independent genetic variants was calculated using the web-based tool LDlink (considering an  $R^2 < 0.4$ ).<sup>(28)</sup> A cutoff of  $R^2 < 0.4$  was chosen, to limit the number of independent variants to a minimum, resulting in a smaller chance of false negative results. A  $-\log(p$  value) plot was constructed using R package ggplot2<sup>(29)</sup> for both femoral neck and lumbar spine BMD, with a Bonferroni-corrected significance threshold ( $p = 0.05/\text{number of independent variants}$ ) and a nominal threshold ( $p = 0.05$ ).

## Results

### Effect of individual genetic instruments for TSH

The associations between individual genetic instruments for circulating concentration of TSH and BMD are summarized in Table 1. Of the 20 SNPs previously associated with circulating TSH level, 19 were available in the BMD datasets; for rs6885099 in *PDE8B* we used rs2046045 as a proxy SNP ( $R^2 = 1.00$ ,  $D' = 1.00$ ). None of the individual genetic instruments for circulating concentration of TSH were associated with femoral neck BMD ( $p$  values  $> 0.05$ ) (Fig. 1A) or lumbar spine BMD ( $p$  values  $> 0.05$ ) (Fig. 1B).

### Combined effect of the genetic instruments for TSH

Using the IVW analyses (Table 2), we found no evidence for an association between genetically determined lower circulating levels of TSH and femoral neck BMD (0.003 SD decrease in BMD per SD decrease in TSH; 95% CI,  $-0.053$  to  $0.048$ ;  $p = 0.92$ ) (Fig. 1C) or lumbar spine BMD (0.010 SD decrease in BMD per SD decrease in TSH; 95% CI,  $-0.069$  to  $0.049$ ;  $p = 0.73$ ) (Fig. 1D). The estimates from MR-Egger regression and WME analyses were consistent with these results. Because the  $I^2_{GX}$ -statistic of the combined genetic variants for TSH was 0.81, we performed additional SIMEX of the MR-Egger estimate, which did not materially change the observations. Moreover, MR-Egger did not indicate the presence of directional pleiotropy given the absence of evidence of deviation of the regression line from the intercept.

### Common genetic variants in the TSH receptor locus and expression loci

A total of 755 common SNPs were mapped either in the *TSHR* locus or in eQTLs. In the GEFOS dataset, 706 of the mapped SNPs were available, amounting to 44 independent loci (Supporting Table 1).  $-\log(p$  value) plots are shown for the *TSHR* SNPs and BMD of the femoral neck (Fig. 2A) and the lumbar spine (Fig. 2B). At the nominal significance of  $p < 0.05$ , five SNPs were associated with femoral neck BMD and three with lumbar spine BMD. However, none of these associations remained statistically significant following Bonferroni correction for multiple testing.

## Discussion

We used Mendelian randomization to determine whether lower circulating levels of TSH within the normal range are causally associated with reduced BMD. Despite interrogating the largest publicly available GWAS meta-analyses,<sup>(20)</sup> we were unable to demonstrate an association between genetic instruments for circulating levels of TSH and femoral neck or lumbar spine BMD. Furthermore, no significant association was found between common genetic variants within the *TSHR* gene or expression regulating regions thereof and BMD. Thus, we found no evidence for a causal relationship between lower circulating levels of TSH within the normal range and reduced BMD, or for any association between genetic variance in the *TSHR* gene or *TSHR* expression and BMD.

These findings add to previous research regarding the role of TSH in the skeleton, which has yielded contrasting results. In osteoblasts of rodent and of human origin TSH receptors were identified,<sup>(30)</sup> although no expression of the  $TSH\alpha$  or  $TSH\beta$  subunits was observed.<sup>(31)</sup> The reported effects of TSH on osteoblasts in vitro are contradictory because inhibition,<sup>(32)</sup> stimulation,<sup>(33–35)</sup> and no effect<sup>(31,36)</sup> of TSH on differentiation and function have all been observed. Furthermore, in human osteoblasts, TSH receptor expression and cAMP responses to TSH are low, making physiologically relevant actions of TSH unlikely.<sup>(37)</sup> In osteoclasts the findings have been more consistent, with the majority reporting TSH inhibiting osteoclast formation and function whereas others have shown no effect.<sup>(32,35,36)</sup>

In vivo, thyroid hormone treated *TSHR*-knockout mice displayed decreased BMD and bone strength,<sup>(32,38)</sup> but importantly, this phenotype also reflects the consequences of profound congenital hypothyroidism and delayed thyroid hormone replacement.<sup>(31)</sup> Consistent with this, adult rodents,

**Table 1.** Associations of Individual Genetic Instruments for TSH With BMD of the Femoral Neck and the Lumbar Spine

Gene	SNP	Chromosome	Position	Effect allele	EAF	Effect on TSH in SD	F-statistic	Femoral neck BMD in SD	Lumbar spine BMD in SD
<i>NR3C2</i>	rs10032216	4	149669506	T	0.781	0.087	63	0.0069	0.0052
<i>FGF7</i>	rs10519227	15	49746364	A	0.245	-0.072	43	0.0005	-0.0123
<i>CAPZB</i>	rs10799824	1	19841174	A	0.161	-0.113	89	-0.0056	-0.0244
<i>ITPK1</i>	rs11624776	14	93595591	A	0.660	-0.064	34	-0.0021	0.0026
<i>VEGFA</i>	rs11755845	6	43904780	T	0.266	-0.065	42	-0.0097	-0.0023
<i>IGFBP5</i>	rs13015993	2	217625523	A	0.736	0.078	61	0.0068	0.0014
<i>MBIP</i>	rs1537424	14	36574018	T	0.608	-0.052	33	0.0108	0.0069
<i>GLIS3</i>	rs1571583	9	4267209	A	0.249	0.057	32	-0.0023	0.0015
<i>PRDM11</i>	rs17723470	11	45227567	T	0.279	-0.065	42	0.0042	0.0036
<i>MIR1179</i>	rs17776563	15	89119104	A	0.322	-0.060	36	0.0039	0.0100
<i>NFIA</i>	rs334699	1	61620496	A	0.052	-0.141	45	0.0009	-0.0091
<i>MAF/LOC440389</i>	rs3813582	16	79749353	T	0.674	0.082	67	-0.0125	-0.0016
<i>INSR</i>	rs4804416	19	7223848	T	0.569	-0.057	40	0.0015	-0.0063
<i>ABO</i>	rs657152	9	136139265	A	0.343	0.058	42	-0.0026	-0.0018
<i>PDE8B</i>	rs6885099	5	76530349	A	0.594	-0.141	245	-0.0033	0.0125
<i>PDE10A</i>	rs753760	6	166046483	C	0.691	0.100	100	-0.0008	0.0099
<i>NRG1</i>	rs7825175	8	32416274	A	0.210	-0.066	36	-0.0035	0.0099
<i>VEGFA</i>	rs9472138	6	43811762	T	0.285	-0.079	62	-0.0115	-0.0028
<i>SASH1</i>	rs9497965	6	148521292	T	0.415	0.051	32	-0.0034	-0.0008
<i>SOX9</i>	rs9915657	17	70127536	T	0.541	-0.064	51	0.0063	-0.0040

Data presented as beta coefficients per effect allele.

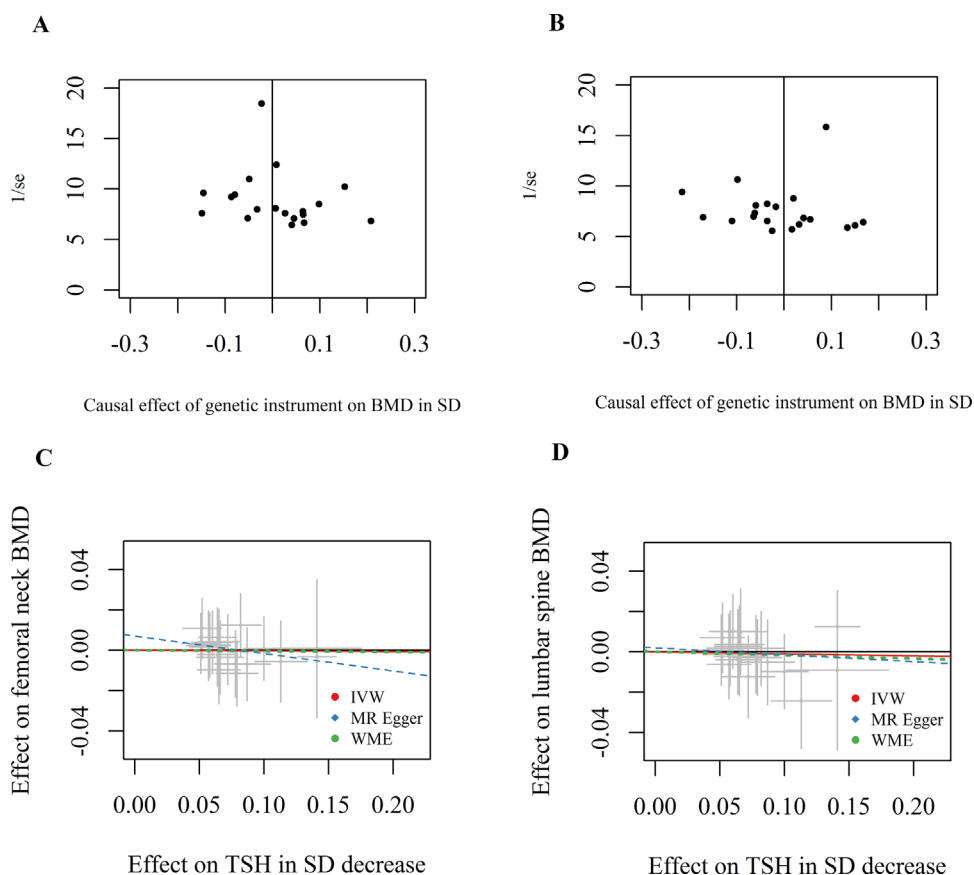
TSH = thyroid stimulating hormone; BMD = bone mineral density; SNP = single-nucleotide polymorphism; EAF = effect allele frequency; SD = standard deviation.

treated with TSH doses insufficient to alter systemic T3 or T4 level, showed suppressed bone resorption and increased formation.<sup>(35,39,40)</sup> By contrast, a similar skeletal phenotype of delayed bone development<sup>(31)</sup> was reported in two contrasting mouse models for congenital hypothyroidism (i) *Pax8*-knockout mice with no T4 or T3 but grossly elevated TSH in the presence of a fully functional TSH receptor and (ii) *TSHR*-knockout mice with no T4 or T3 but grossly elevated TSH in the absence of a functional TSH receptor. Although these results do not support a predominant role for the TSH receptor in bone, the effects of TSH could be masked by the severely reduced T4 and T3 levels.

Human observational studies have shown strong indications for an association between higher thyroid status within and outside the normal range and lower BMD.<sup>(6,8,9)</sup> Importantly, in observational studies in humans, no conclusions can be drawn on relative roles of TSH or thyroid hormones because they are maintained by the HPT axis in a physiological reciprocal relationship.<sup>(41)</sup> In two genetic studies investigating the relationship between TSH and BMD, the nonsynonymous Asp727Glu polymorphism in the human *TSHR* gene (rs1991517) has been associated with higher mean BMD.<sup>(42,43)</sup> However, this observation has not been replicated by other studies and no other common *TSHR* genetic variants have been associated with BMD.

In this study we investigated the effect of circulating TSH levels, within the normal range, on BMD in the absence of confounding, by using genetic variants associated with circulating TSH level as instrumental variables in a two-sample MR analysis using summary level data. This highly efficient method allows for large sample sizes to be used, but has the disadvantage that stratified analyses, for example by sex, age, or menopausal status, are not possible. Analyses in specific subgroups such as postmenopausal women would also have

been of interest, due to their increased risk of developing osteoporosis.<sup>(44)</sup> Furthermore, in previous observational studies stronger associations between thyroid status and BMD were observed in women compared to men.<sup>(8,45)</sup> Therefore, we cannot conclude that no association between TSH levels and BMD is present in more vulnerable subgroups. Nonetheless, in the general population as a whole, we found no causal association between TSH and BMD. Another potential limitation of our study is overlap between GWAS meta-analyses of thyroid function parameters and GWAS meta-analyses of BMD; three out of nine cohorts (Framingham Heart Study, TwinsUK study, and Rotterdam Study) were included in both studies. If weak instruments were used, this overlap in study populations could lead to bias.<sup>(46)</sup> Because all genetic instruments were selected from among the top hits of the largest published GWAS on thyroid function to date, the instrument strength was assumed to be sufficient based on previous studies.<sup>(47)</sup> Therefore, potential effects of weak instrument bias can be expected to be negligible.<sup>(46)</sup> Furthermore, the genetic instrument identification and the MR were performed in cohorts of European ancestry, which may limit generalizability to non-European populations. A potential limitation of our combined genetic variants for circulating TSH level could be that they also reflect the circulating levels of ft4, due to the reciprocal physiological relationship between TSH and ft4 in healthy individuals.<sup>(41)</sup> However, the GWAS that identified the variants for TSH had identified different genetic variants for ft4.<sup>(18)</sup> Reciprocal associations of TSH SNPs with ft4 were assessed in sensitivity analyses, yet, as stated by the authors, the study was underpowered to detect any statistically significant associations.<sup>(18)</sup> Even though no certain conclusions can be drawn, the results of the sensitivity analyses did not imply strong reciprocal associations with circulating ft4 levels for the SNPs associated



**Fig. 1.** The effect of genetic instruments for TSH levels on BMD. Results are presented as the beta coefficients. (A, B) The x-axis presents the per-allele effect on BMD for each individual genetic variant; the y-axis presents the 1/standard error (1/SE) for each effect estimate. The association between 20 individual genetic variants for TSH levels and (A) femoral neck BMD in standard deviations and (B) lumbar spine BMD in standard deviations. (C, D) The x-axis presents the decrease in TSH in standard deviations; the y-axis presents the effect on BMD. The modeled association between genetic instruments for TSH and BMD using IVW analysis, MR Egger, and WME are shown for (C) femoral neck BMD in standard deviations and (D) lumbar spine BMD in standard deviations. IVW = inverse-variance weighted; WME = weighted median estimator.

with circulating TSH. Therefore, the results for the MR study on lower circulating TSH might be influenced by slightly higher levels of fT4, yet these effects appear to be small. A final limitation of using genetic variants for TSH identified by this GWAS meta-analyses is the euthyroid state of the included participants. Because circulating TSH levels in clinical thyroid dysfunction are unlike the individual set point,<sup>(48)</sup> we cannot

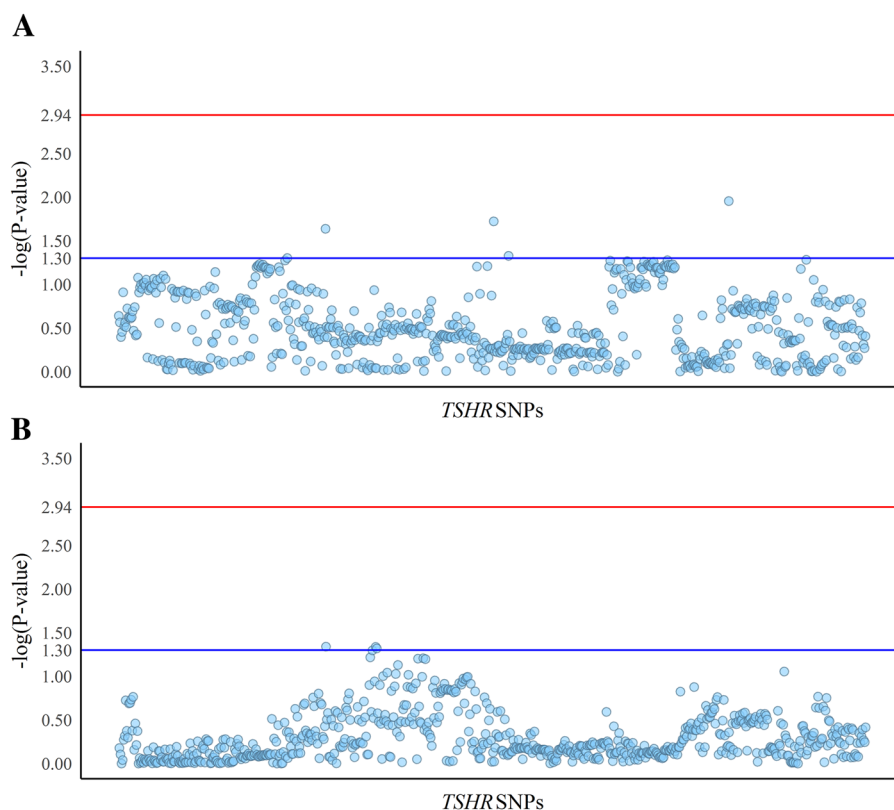
extrapolate our results to individuals with TSH outside the reference range. Thus, our results are only applicable to adults with circulating TSH levels within the reference range.

In addition, we investigated the association of common genetic variants in the *TSHR* gene locus and the expression loci with BMD in a candidate gene study. For this analysis we used 706 common genetic variants (44 independent loci), which

**Table 2.** Mendelian Randomization Estimates for TSH on BMD

	Femoral neck BMD in SD	<i>p</i>	Lumbar spine BMD in SD	<i>p</i>
Inverse-variance weighted	0.00 (−0.05; 0.05)	0.92	−0.01 (−0.07; 0.05)	0.73
MR-Egger				
Estimate	−0.09 (−0.23; 0.08)	0.28	−0.03 (−0.20; 0.15)	0.71
Intercept	0.01 (−0.01; 0.02)	0.15	0.00 (−0.01; 0.02)	0.75
MR-Egger+SIMEX				
Estimate	−0.10 (−0.13; 0.03)	0.16	−0.04 (−0.21; 0.14)	0.68
Intercept	0.01 (−0.00; 0.02)	0.15	0.00 (−0.01; 0.02)	0.75
Weighted median	0.00 (−0.08; 0.07)	0.90	−0.02 (−0.10; 0.07)	0.67

Data presented as beta coefficients with 95% confidence interval per standard deviation decrease of serum level thyrotropin (TSH). TSH = thyroid stimulating hormone; BMD = bone mineral density; SD = standard deviation; SIMEX = simulation extrapolation.



**Fig. 2.**  $-\log(p)$  plot of the candidate gene analysis. Results are presented as the  $-\log$  of the  $p$  value for each SNP organized by position number. The lower horizontal line at 1.30 corresponds to the  $-\log$  of  $p$  value = 0.05 indicating nominal significance, the upper horizontal line at 2.94 corresponds to the  $-\log$  of  $p$  value =  $1.14 \times 10^{-3}$  indicating the Bonferroni-corrected threshold of statistical significance. The association between *TSHR* SNPs and (A) femoral neck BMD and (B) lumbar spine BMD.

covered the majority of the common variation in *TSHR* and the eQTLs of this gene. A limitation of this method is the unknown effect of the tested genetic variants inside the *TSHR* gene on the TSH receptor and on thyroid status, yet we observed no indication for biologically relevant associations between this gene and BMD. Furthermore, an important limitation is the absence of 49 SNPs mapped to the *TSHR* gene in the summary-level data we used. Nevertheless, we found no association between common variation of the *TSHR* locus and BMD despite using the largest human dataset available for BMD of the femoral neck and lumbar spine.

## Conclusion

In summary, we found no evidence that circulating TSH levels in the normal range are causally associated with BMD nor did we find any association between common genetic variation in the *TSHR* gene or expression of *TSHR* and BMD. Therefore, the associations found in observational studies between low circulating TSH and lower BMD are possibly related to the reciprocal higher levels of ft4, due to residual confounding or reverse causality. In clinical treatment of thyroid disease, treatment is aimed at normalization of TSH levels into the normal range and alleviation of symptoms. Based on our current results, we found no indications for inappropriateness of current guidelines aimed at restoration of TSH within the normal reference range with regard to bone health. In future research,

better genetic tools for ft4 levels are required to further interpret the effects of thyroid status on BMD. Additionally, more clinical end points could be investigated resulting in greater clinical applicability.

## Disclosures

DvH reports grants from the European Commission, during the conduct of the study. All other authors have nothing to disclose.

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## References

1. Raggatt LJ, Partridge NC. Cellular and molecular mechanisms of bone remodeling. *J Biol Chem*. 2010;285(33):25103–8.
2. Dempster DW, Lindsay R. Pathogenesis of osteoporosis. *Lancet*. 1993;341(8848):797–801.
3. Bowers J, Terrien J, Clerget-Froidevaux MS, et al. Thyroid hormone signaling and homeostasis during aging. *Endocr Rev*. 2013;34(4):556–89.
4. Andersen S, Bruun NH, Pedersen KM, Laurberg P. Biologic variation is important for interpretation of thyroid function tests. *Thyroid*. 2003;13(11):1069–78.
5. Bassett JH, Williams GR. Role of thyroid hormones in skeletal development and bone maintenance. *Endocr Rev*. 2016;37(2):135–87.
6. Vestergaard P, Mosekilde L. Hyperthyroidism, bone mineral, and fracture risk—a meta-analysis. *Thyroid*. 2003;13(6):585–93.
7. Blum MR, Bauer DC, Collet TH, et al. Subclinical thyroid dysfunction and fracture risk: a meta-analysis. *JAMA*. 2015;313(20):2055–65.
8. Yang R, Yao L, Fang Y, et al. The relationship between subclinical thyroid dysfunction and the risk of fracture or low bone mineral density: a systematic review and meta-analysis of cohort studies. *J Bone Miner Metab*. 2018 Mar;36(2): 209–20.
9. Murphy E, Gluer CC, Reid DM, 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.
10. Aubert CE, Floriani C, Bauer DC, et al. Thyroid Studies Collaboration. Thyroid function tests in the reference range and fracture: individual participant analysis of prospective cohorts. *J Clin Endocrinol Metab*. 2017 Aug 1;102(8): 2719–28.
11. Segna D, Bauer DC, Feller M, et al. Thyroid Studies Collaboration. Association between subclinical thyroid dysfunction and change in bone mineral density in prospective cohorts. *J Intern Med*. 2018 Jan;283(1): 56–72.
12. Martini G, Gennari L, De Paola V, et al. The effects of recombinant TSH on bone turnover markers and serum osteoprotegerin and RANKL levels. *Thyroid*. 2008;18(4):455–60.
13. Mazziotti G, Sorvillo F, Piscopo M, et al. Recombinant human TSH modulates in vivo C-telopeptides of type-1 collagen and bone alkaline phosphatase, but not osteoprotegerin production in postmenopausal women monitored for differentiated thyroid carcinoma. *J Bone Miner Res*. 2005;20(3):480–6.
14. Giusti M, Cecoli F, Ghiara C, et al. Recombinant human thyroid stimulating hormone does not acutely change serum osteoprotegerin and soluble receptor activator of nuclear factor-kappaBeta ligand in patients under evaluation for differentiated thyroid carcinoma. *Hormones (Athens)*. 2007;6(4):304–13.
15. Karga H, Papaioannou G, Polymeris A, et al. The effects of recombinant human TSH on bone turnover in patients after thyroidectomy. *J Bone Miner Metab*. 2010;28(1):35–41.
16. Lawlor DA, Harbord RM, Sterne JA, Timpson N, Davey Smith G. Mendelian randomization: using genes as instruments for making causal inferences in epidemiology. *Stat Med*. 2008;27(8):1133–63.
17. Bos MM, Smit RA, Trompet S, van Heemst D, Noordam R. Thyroid signaling, insulin resistance and type 2 diabetes mellitus: a Mendelian randomization study. *J Clin Endocrinol Metab*. 2017 Jun 1;102(6): 1960–70.
18. Porcu E, Medici M, Pistis G, et al. A meta-analysis of thyroid-related traits reveals novel loci and gender-specific differences in the regulation of thyroid function. *PLoS Genet*. 2013;9(2):e1003266.
19. Medici M, Visser WE, Visser TJ, Peeters RP. Genetic determination of the hypothalamic-pituitary-thyroid axis: where do we stand? *Endocr Rev*. 2015;36(2):214–44.
20. Zheng HF, Forgetta V, Hsu YH, et al. Whole-genome sequencing identifies EN1 as a determinant of bone density and fracture. *Nature*. 2015;526(7571):112–7.
21. Brion MJ, Shakhbuzov K, Visscher PM. Calculating statistical power in Mendelian randomization studies. *Int J Epidemiol*. 2013;42(5):1497–501.
22. Noordam R, Smit RA, Postmus I, Trompet S, van Heemst D. Assessment of causality between serum gamma-glutamyltransferase and type 2 diabetes mellitus using publicly available data: a Mendelian randomization study. *International journal of epidemiology*. 2016 Dec 1;45(6):1953–60.
23. Burgess S, Scott RA, Timpson NJ, Davey Smith G, Thompson SG. Using published data in Mendelian randomization: a blueprint for efficient identification of causal risk factors. *Eur J Epidemiol*. 2015;30(7):543–52.
24. Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. *Int J Epidemiol*. 2015;44(2):512–25.
25. Bowden J, Davey Smith G, Haycock PC, Burgess S. Consistent estimation in Mendelian randomization with some invalid instruments using a weighted median estimator. *Genet Epidemiol*. 2016;40(4):304–14.
26. Bowden J, Del Greco MF, Minelli C, Davey Smith G, Sheehan NA, Thompson JR. Assessing the suitability of summary data for two-sample Mendelian randomization analyses using MR-Egger regression: the role of the I<sup>2</sup> statistic. *Int J Epidemiol*. 2016 Dec 1;45(6):1961–74.
27. Cook JR, Stefanski LA. Simulation-extrapolation estimation in parametric measurement error models. *J Am Stat Assoc*. 1994;89(428):1314–28.
28. Machiela MJ, Chanock SJ. LDlink: a web-based application for exploring population-specific haplotype structure and linking correlated alleles of possible functional variants. *Bioinformatics*. 2015;31(21):3555–7.
29. Wickham H. *ggplot2: elegant graphics for data analysis*: New York: Springer-Verlag 2009.
30. Inoue M, Tawata M, Yokomori N, Endo T, Onaya T. Expression of thyrotropin receptor on clonal osteoblast-like rat osteosarcoma cells. *Thyroid*. 1998;8(11):1059–64.
31. Bassett JH, Williams AJ, Murphy E, et al. A lack of thyroid hormones rather than excess thyrotropin causes abnormal skeletal development in hypothyroidism. *Mol Endocrinol*. 2008;22(2):501–12.
32. Abe E, Mariani RC, Yu W, et al. TSH is a negative regulator of skeletal remodeling. *Cell*. 2003;115(2):151–62.
33. Baliram R, Latif R, Berkowitz J, et al. Thyroid-stimulating hormone induces a Wnt-dependent, feed-forward loop for osteoblastogenesis in embryonic stem cell cultures. *Proc Natl Acad Sci U S A*. 2011;108(39):16277–82.
34. Ramajayam G, Vignesh RC, Karthikeyan S, et al. Regulation of insulin-like growth factors and their binding proteins by thyroid stimulating hormone in human osteoblast-like (SaOS2) cells. *Mol Cell Biochem*. 2012;368(1–2):77–88.
35. Sampath TK, Simic P, Sendak R, et al. Thyroid-stimulating hormone restores bone volume, microarchitecture, and strength in aged ovariectomized rats. *J Bone Miner Res*. 2007;22(6):849–59.
36. Hase H, Ando T, Eldeiry L, et al. TNFalpha mediates the skeletal effects of thyroid-stimulating hormone. *Proc Natl Acad Sci U S A*. 2006;103(34):12849–54.
37. Tsai JA, Janson A, Bucht E, et al. Weak evidence of thyrotropin receptors in primary cultures of human osteoblast-like cells. *Calcif Tissue Int*. 2004;74(5):486–91.
38. Zhang W, Zhang Y, Liu Y, et al. Thyroid-stimulating hormone maintains bone mass and strength by suppressing osteoclast differentiation. *J Biomech*. 2014;47(6):1307–14.
39. Sun L, Vukicevic S, Baliram R, et al. Intermittent recombinant TSH injections prevent ovariectomy-induced bone loss. *Proc Natl Acad Sci U S A*. 2008;105(11):4289–94.
40. Domic-Cule I, Draca N, Luetic AT, et al. TSH prevents bone resorption and with calcitriol synergistically stimulates bone formation in rats with low levels of calcitropic hormones. *Horm Metab Res*. 2014;46(5):305–12.
41. Bassett JH, Williams GR. Critical role of the hypothalamic-pituitary-thyroid axis in bone. *Bone*. 2008;43(3):418–26.
42. van der Deure WM, Uitterlinden AG, Hofman A, 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.



43. Heemstra KA, van der Deure WM, Peeters RP, et al. Thyroid hormone independent associations between serum TSH levels and indicators of bone turnover in cured patients with differentiated thyroid carcinoma. *Eur J Endocrinol.* 2008;159(1):69–76.
44. Curtis E, Litwic A, Cooper C, Dennison E. Determinants of muscle and bone aging. *J Cell Physiol.* 2015;230(11):2618–25.
45. Tsourdi E, Wallaschofski H, Rauner M, et al. Thyrotropin serum levels are differentially associated with biochemical markers of bone turnover and stiffness in women and men: results from the SHIP cohorts. *Osteoporos Int.* 2016;27(2):719–27.
46. Haycock PC, Burgess S, Wade KH, Bowden J, Relton C, Davey Smith G. Best (but oft-forgotten) practices: the design, analysis, and interpretation of Mendelian randomization studies. *Am J Clin Nutr.* 2016;103(4):965–78.
47. Pierce BL, Ahsan H, Vanderweele TJ. Power and instrument strength requirements for Mendelian randomization studies using multiple genetic variants. *Int J Epidemiol.* 2011;40(3):740–52.
48. Hoermann R, Midgley JEM, Larisch R, Dietrich JWC. Advances in applied homeostatic modelling of the relationship between thyrotropin and free thyroxine. *PLoS One.* 2017;12(11):e0187232.