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ORIGINAL RESEARCH

# High SHBG and Low Bioavailable Testosterone are Strongly Causally Associated with Increased Forearm Fracture Risk in Women: An MR Study Leveraging Novel Female-Specific Data

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## Abstract

The effects of androgens on women's bone health are not fully understood. Mendelian randomization (MR) studies using sex-combined data suggest that sex hormone-binding globulin (SHBG) and bioavailable testosterone (BioT) causally affect bone traits. Given significant sex differences in hormone regulation and effects, female-specific MR studies are necessary. In the current study, we explored the causal relationships of SHBG, BioT, and total testosterone (TT) with forearm fracture (FAFx) risk in women using two-sample MR analyses. We utilized a unique female-specific FAFx outcome dataset from three European biobanks (UFO, HUNT, Estonian Biobank) comprising 111,351 women and 8823 FAFx cases, along with female-specific genetic instruments of SHBG, BioT, and TT identified in the UK Biobank. We also assessed bone mineral density (BMD) at the forearm (FA), femoral neck (FN), and lumbar spine (LS) using female-specific GWAS data from the GEFOS consortium. High SHBG (odds ratio per standard deviation increase (OR/SD): 1.53, 95% confidence intervals (CIs): 1.34–1.75), low BioT (OR/SD: 0.77, 0.71–0.84) and low TT (OR/SD 0.90, 0.83–0.98) were causally associated with increased FAFx risk. BioT was positively, and SHBG inversely, causally associated with especially FA-BMD, but also LS-BMD and FN-BMD, while TT was only significantly positively associated with FA-BMD and LS-BMD. We propose that endogenous androgens and SHBG are important for women's bone health at distal trabecular-rich bone sites such as the distal forearm and may serve as predictors for FAFx risk.

**Keywords** Mendelian randomization analysis · Female · Fractures · Bone · Sex hormone-binding globulin · Testosterone · Bone density

## Introduction

It is estimated that one-third of postmenopausal women in Europe will sustain a fragility fracture during their remaining lifetime, a risk on par with that of cardiovascular disease [1]. The economic impact of osteoporotic fractures is substantial, with associated costs being up to six times higher than those for patients without fractures in the US population [2].

The beneficial effects of estrogens on bone disease in women are well established [3]. The effects of androgens, however, remain less well understood. Androgens can influence bone directly through their interaction with androgen receptors, or indirectly by binding to estrogen receptors α and β following aromatization in fat or other tissues [4]. Preclinical data from studies involving female mice devoid of androgen receptors and ovariectomized rats treated with dihydrotestosterone (DHT), a non-aromatizable androgen, suggest that androgen receptor signaling is of importance for maintaining the health of trabecular bone in females [5–7]. Interestingly, in women diagnosed with complete androgen insensitivity syndrome (CAIS)—a condition caused by a loss of function mutation in the gene encoding the androgen receptor—decreased bone mineral density (BMD) is more commonly observed at the trabecular-rich lumbar spine than the femoral neck, which mainly consists of cortical bone [8, 9].

Only a few percent of circulating testosterone (T) circulates freely, with the majority bound to and transported by sex hormone-binding globulin (SHBG) and albumin [10]. The free hormone hypothesis, though still somewhat

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controversial, states that it is mostly the free hormone concentration that accounts for the hormone's biological activity and thus best correlates with any biological effects. Although several observational studies have demonstrated that low levels of circulating SHBG [11–16] or high levels of circulating free T (or bioavailable T, BioT) [11, 13, 17–22] are associated with bone health parameters in women, results are far from consistent [12, 18, 23–27]. As for circulating total T (TT), the findings are even less compelling [13, 15, 17, 18, 23, 25, 28–31], with only a handful of observational studies indicating a relationship [11, 21, 32].

Thus, while preclinical findings suggest that androgens play a protective role in bone health, the effects of androgens on bone health in women still remain unclear as discrepancies of observational studies could be explained by biases, such as residual confounding (e.g., age, comorbidities and medication) and reverse causality. Mendelian randomization (MR) can overcome several shortcomings in observational studies by combining genetic information fixed at birth (eliminating reverse causation bias) and observational data to create possible unbiased causal associations between an exposure and outcome [33]. Because germline genetic variants are selected at random during meiosis, MR is less likely to be influenced by environmental and lifestyle factors.

Recently, a few sex-combined MR studies have suggested that SHBG or BioT may causally affect BMD [34–36], osteoporosis risk [37, 38], or fracture risk [39, 40]. However, as the regulation of circulating levels of these androgen-related hormones (exposures in MR) and their effects (outcomes in MR) differ substantially between men and women, sometimes showing opposing effects between sexes [41], it is essential to conduct female-specific MR analyses to understand the effects of androgens on bone traits in women. Such analyses require both female-specific genetic instruments and availability of female-specific genome-wide association study (GWAS) summary data for the outcomes being investigated in the MR.

In the present study, our aim was to use a two-sample MR framework to specifically explore the causal relationship between female androgen levels and bone health in women. As instruments we employed strong female-specific genetic signals for circulating SHBG, BioT, and Total Testosterone (TT) from the UK Biobank [41]. These signals were combined with a large and unique collection of female forearm fracture cases derived from three Nordic biobanks. Forearm fractures are common, occur relatively early in life, and show high heritability, providing increased statistical power and more precise estimates compared to other osteoporotic fractures [42]. Additionally, as a secondary aim, we assessed the causal association between SHBG, BioT, and TT and female-specific measures of femoral neck BMD, lumbar spine BMD, and forearm BMD. For sensitivity analyses, we further refined our MR analyses by focusing exclusively on cis single-nucleotide polymorphisms (SNPs) related to SHBG. Cis-SNPs, which are positioned close to or within the gene encoding the protein in question, are thought to offer a more accurate assessment of the causal role of a circulating protein in relation to a disease or trait [43]. Restricting an MR analysis to cis-SNPs thus reduces the risk of horizontal pleiotropy, where a genetic variant affects multiple unrelated traits through different biological pathways, compared to using trans-SNPs that are located farther from the gene and therefore are more likely to exert broader, unrelated effects.

## Methods

### Study Design

We performed a two-sample MR study using only female exposure and outcome data to assess the causal association of TT, SHBG, and BioT (exposures) with forearm fracture risk (primary outcome) and BMD (secondary outcome). As instrumental variables, we used female-specific SNPs from the largest available GWAS of circulating levels of TT, SHBG, and BioT in women [41]. As outcome measures, we used female-specific case–control data of forearm fractures collected from four large biobanks (*Estonian Biobank* from Estonia, *HUNT* from Norway, *UFO* from Sweden, and *UK Biobank* from the United Kingdom), and female-specific DXA-derived BMD GWAS summary statistics available from the GEFOS consortium (Table 1) [44, 45]. For a flowchart of the study methodology, see Supplementary Fig. 1. The current MR study relies on three core assumptions: The genetic instruments (1) are associated with the exposure of interest (relevance), (2) are not associated with confounders of the association between the exposure and the outcome (independence), and (3) only influence the outcome through the exposure and not directly or through alternative (horizontally pleiotropic) pathways (exclusion restriction). The paper is reported on the basis of recommendations by the Strengthening the Reporting of Observational Studies in Epidemiology Using Mendelian Randomization Guidelines (STROBE-MR) [46].

### Exposures

#### Data Source and Population

We obtained genetic predictors for the exposures from the largest available female-specific GWAS meta-analysis of TT, SHBG, and BioT [41]. This GWAS used data from the UK Biobank, a large, ongoing, prospective cohort study that comprises over 500000 participants, recruited from 2006 to 2010 across the United Kingdom at the age of 37

**Table 1** Description of female-specific data used for each phenotype

| Variable | Exposure/Outcome | Source | Ancestry | Sample size | Cases |
|---|---|---|---|---|---|
| **Sex hormones** | | | | | |
| SHBG | Exposure | UKBB, Ruth et al., PMID: 32,042,192 | EUR | 189,473 | - |
| Bioavailable testosterone | Exposure | UKBB, Ruth et al., PMID: 32,042,192 | EUR | 188,507 | - |
| Total testosterone | Exposure | UKBB, Ruth et al., PMID: 32,042,192 | EUR | 230,454 | - |
| **Fractures** | | | | | |
| Forearm fractures | Outcome, one-sample MR | UKBB | EUR | 237,572 | 11,564 |
| Forearm fractures | Outcome, two-sample MR | EstBB; HUNT; UFO | EUR | 111,351 | 8823 |
| Forearm fractures | Outcome, overlap-sample MR | UKBB; EstBB; HUNT; UFO | EUR | 348,923 | 20,387 |
| **BMD** | | | | | |
| Femoral neck BMD | Outcome, two-sample MR | GEFOS, Estrada et al., PMID: 22,504,420 | Mainly EUR | 22,900 | - |
| Lumbar spine BMD | Outcome, two-sample MR | GEFOS, Estrada et al., PMID: 22,504,420 | Mainly EUR | 22,177 | - |
| Forearm BMD | Outcome, two-sample MR | GEFOS, Zheng et al., PMID: 26,367,794 | EUR | 7848 | - |

*EstBB* Estonian Biobank, *HUNT* The Trøndelag Health Study, *UFO* Umeå Fracture and Osteoporosis Study, *UKBB* UK Biobank, *GEFOS* Genetic Factors for Osteoporosis consortium, *MR* Mendelian randomization, *BMD* Bone mineral density, *EUR* European ancestry

to 73 years [47]. Participants who self-reported by questionnaire as being of an ancestry other than white European were excluded in the GWAS as well as women who self-reported taking hormone-based medication at the time of the initial visit [41].

### Measurements of TT, SHBG and BioT

SHBG and TT were measured in nmol/L by chemiluminescent immunoassay analyses on a Beckman Coulter DXI 800 [48]. BioT was calculated from TT, SHBG, and albumin (measured in g/L by a colorimetric analysis on a Beckman Coulter AU5800) using the Vermeulen equation [41]. The regression model for the effect sizes of the genetic variants on the exposures included several covariates: All models for TT, SHBG, and BioT included genotyping chip, age at baseline, and ten genetically derived principal components. TT also included fasting time and assessment center as covariates. SHBG and BioT also included batch, dilution, menopause status, operation status, minutes since blood draw, and time of blood draw. Additionally, the main SHBG model included body mass index (BMI) to increase statistical power by reducing trait variance [41]. Similarly, as suggested by Ruth et al., we used genetic variants from the BMI-adjusted analyses but applied effect estimates from BMI-unadjusted analyses [41]. TT and SHBG were transformed using rank-based inverse normal transformation, whereas BioT was transformed using the natural log. To ease interpretation, we estimated standardized effect estimates for natural log BioT using available natural log effect estimates and standard errors, allele frequencies, and number of women as described by Rietveld et al. [49].

### Genetic Predictors of TT, SHBG, and BioT

The female-specific GWAS revealed 254 independent ($r^2 < 0.05$) genome-wide significant ($P$-value $< 5 \times 10^{-8}$) genetic instruments for TT ($N = 230{,}454$), 359 independent instruments for SHBG ($N = 189{,}473$), and 180 independent instruments for BioT ($N = 188{,}507$) [41]. All genetic variants had an imputation quality score > 0.5 and minor allele frequency > 0.1%. The genetic correlation between SHBG and TT was weak ($r_g = -0.06$), but there was a strong negative correlation between BioT and SHBG ($r_g = -0.74$) and a similar positive correlation between TT and BioT ($r_g = 0.65$). Quantifying instrument strength using the t-statistic approximation of the F-statistic, each genetic instrument showed an F-statistic greater than 10, indicating strong genetic instruments. Harmonization with the outcome data was performed using the TwoSampleMR package, with attempt to infer positive strand alleles using allele frequencies for palindromes. In case genetic instruments were missing in the outcome data, suggested proxies in the supplement of Ruth et al. were used [41]. As cis-SNPs for SHBG, we selected three commonly occurring variants utilized in previous studies: rs6761, rs1799941, and rs858519 [41, 50, 51]. For outcomes restricted to HapMap variants, we used rs12150660 as a proxy for rs1799941 and rs727428 as a proxy for rs858519.

## Outcomes

### Forearm Fractures

For the two-sample MR analyses of forearm fractures, we utilized summary statistics derived from individual-level outcome data sourced from three large Northern European

biobanks (Estonian Biobank from Estonia, HUNT from Norway, and UFO from Sweden). We also performed one-sample MR analyses using summary statistics calculated from individual-level data from the UK Biobank. In addition, we performed combined one-and two-sample MR using data from all four cohorts (overlap-sample MR). All four contributing biobanks obtained written informed consent from all participants and received approval from their respective research ethics committees. The UK Biobank has ethical approval from the Northwest Multicentre Research Ethics Committee (11/NW/0382). The UFO study was approved by the local research ethics committee at Umeå University (Umu dnr 03-426; EPN 2012-254-32 M; 2011/32-32 M; 2011-251-32 M). The HUNT study has been approved by the Regional Ethics Committee for Medical Research in Norway (REK 2015/615; REK 2014/144). Estonian Biobank has ethical approval from the Estonian Committee on Bioethics and Human Research at the Ministry of Social Affairs (No 1.1-12/624). For detailed information on the four contributing biobanks, please see Supplementary Table S1. Forearm fracture cases were identified by International Classification of Diseases (ICD) codes (ICD10, S52; ICD9, 813), for UK Biobank in combination with self-reported data. No self-reported forearm fracture data were used in Estonian Biobank, HUNT, or UFO (Table 2). Genotyping was performed in each cohort using Illumina genome-wide genotyping chips or in the case of UK Biobank, the UK Biobank Axiom Array, or the UK BiLEVE Array. Imputation was performed to ensure accurate ascertainment of common genetic variations above a minor allele frequency threshold of 1%. Logistic models, using the SAIGE (UK Biobank, Estonian Biobank, HUNT) or PLINK (UFO) software, were used to estimate the SNP association with fracture in each cohort, adjusting for age (simple and quadratic terms), height, weight, principal components, study site (when necessary), and family structure (if feasible). Menopausal status at the time of forearm fracture was not available for participants in some of the included cohorts, preventing us from adjusting or stratifying the analyses by menopausal status. However, the median age at fracture varied between 58 and 64 years in the included cohorts (Table 2), suggesting that most of the forearm fractures occurred after menopause. The effect estimates from the individual cohorts were then combined using fixed-effect inverse variance weighted meta-analysis. In the analyses, we only included female subjects of European descent with available data on genotypes and relevant covariates. For the cohorts from Estonian Biobank, HUNT, and UFO, individual participant-level data were available for in total 111 351 women, including 8 823 forearm fracture cases. For UK Biobank, data were available for 237 572 women, including 11 564 forearm fractures.

### Lumbar Spine BMD and Femoral Neck BMD

Effect estimates for the associations between the genetic instruments and lumbar spine BMD (LS-BMD; $n = 22\,177$) and femoral neck BMD (FN-BMD; $n = 22\,900$) were derived from female-specific GWAS summary statistics available from the GEnetic Factors for OSteoporosis (GEFOS) consortium (www.gefos.org) [44]. These data originate from a meta-analysis of 17 populations across North America, Europe, East Asia, and Australia with a variety of epidemiological designs and subject characteristics. The mean age of women across the cohorts varied between 33 and 76 years with an overall mean age of 59 years. Genotyping was performed by each individual study following standard protocols, and imputation was then carried out on ~ 2.5 million single-nucleotide polymorphisms (SNPs) from HapMap Phase 2 release 22. BMD of lumbar spine (L1-4) (LS) and femoral neck (FN) was measured by Dual-energy X-ray absorptiometry (DXA) [44]. The effect of each gene variant on standardized BMD was adjusted for age, weight, and four principal components. As female-specific beta values and standard errors are not given for all SNPs in this publicly available dataset, we estimated these parameters from *P*-values, allele frequencies, and number of women as described by Rietveld et al. [49].

**Table 2** Fracture details for individual forearm fracture studies

| Study | Fracture assessment method | Fracture cases (*n*) | Controls (*n*) | All (*n*) | Median age at fracture (yrs) |
|---|---|---|---|---|---|
| UKBB | Medical records* and self-reported | 11,564 | 226,008 | 237,572 | 60.5 |
| UFO | Medical records* and radiographic verification | 850 | 856 | 1706 | 61.4 |
| HUNT | Medical records* | 4447 | 31,581 | 36,028 | 64 |
| EstBB | Medical records* | 3526 | 70,091 | 73,617 | 58 |
| Total | | 20,387 | 328,536 | 348,923 | |

*EstBB* Estonian Biobank, *HUNT* The Trøndelag Health Study, *UFO* Umeå Fracture and Osteoporosis Study, *UKBB* UK Biobank

*ICD codes: ICD10, S52; ICD9, 813

### Forearm BMD

For effect estimates of associations between genetic instruments and forearm BMD (FA-BMD; n = 7 848), we used non-sex-specific GWAS summary statistics available from the GEFOS consortium (www.gefos.org) [45]. Although the dataset included a low number of male subjects, the female contribution was as high as 96%, and we thus consider these summary statistics as female-specific in our analyses. The FA-BMD data originate from a meta-analysis of five populations across North America, Europe, and Australia. The mean age of women across the cohorts varied between 48 and 71 years with an overall mean age of 61 years. Genotyping was performed by each individual cohort following standard protocols and the data were meta-analyzed using the software GWAMA [45]. BMD was measured using DXA. The effect of each gene variant was adjusted for sex, age, age$^2$, weight, and standardized to have a mean of zero and a standard deviation of one.

## Statistical Analysis

### Causal Effects

The primary MR analyses were performed using a two-sample summary statistics MR approach, where we obtained SNP-specific Wald estimates and then meta-analyzed them using inverse variance weighting (IVW) with random effects (RE), thereby estimating the total effect of the exposures (BioT, SHBG, and TT) on forearm fracture risk (using data from the Estonian Biobank, HUNT, and UFO), LS-BMD, FN-BMD, and FA-BMD. IVW-RE lessens the reliance of core MR assumption number three, that genetic variants should not act through additional parallel biological pathways (horizontal pleiotropy) and relies on the assumptions that pleiotropic effects among SNPs are random (balanced pleiotropy) and that their magnitude is independent of the magnitude of the corresponding genetic-exposure (G-X) effects, known as the InSIDE assumption [52]. In addition to the two-sample MR framework in the analyses of forearm fractures, we also included one-sample MR analyses using data from only the UK biobank, as well as an overlap-sample MR using outcome data from all four cohorts, i.e., Estonian Biobank, HUNT, UFO, and UK Biobank.

We also performed several sensitivity analyses using MR methods even less sensitive to horizontal pleiotropy. In these sensitivity analyses, we used (1) the weighted median method, which provides a causal estimate if at least 50% of the weight in the analysis is derived from valid instrumental variables [53]; (2) a mode-based estimation method, which assumes that the largest weighted contribution of "similar" (i.e., identical in infinite samples) SNP-specific MR estimates comes from valid instruments, thereby providing natural robustness against outlier variants (and hence likely are pleiotropic) [54]; (3) MR Egger method, which can detect and adjust for a non-zero average pleiotropic effect under the assumption that the instrument strength is independent of direct (pleiotropic) effects (InSIDE assumption) [55]; (4) MR-pleiotropy residual sum and outlier (MR-PRESSO) method, which uses a leave-one-out approach to detect pleiotropy and estimates causality after removing invalid IVs [56]; (5) MR-Lasso method, which models pleiotropic effects and then removes variants with non-zero pleiotropic effects [57]; and (6) MR-Robust Adjusted Profile Score (RAPS) that penalizes genetic variants that are pleiotropic and is robust to systematic and idiosyncratic pleiotropy. We also performed analyses after Steiger filtering, removing genetic variants that demonstrate higher correlation with the outcome than the exposure, thus filtering instruments that are likely to be arising due to reverse cause or potentially through horizontal pleiotropy [58, 59] (Supplementary Tables S3 and S4).

To ensure the robustness of our estimated associations, we performed additional sensitivity analyses by excluding SNPs associated with potential confounders. Confounders were identified based on variables from the FRAX® fracture risk assessment tool, including smoking, alcohol consumption, glucocorticoid use, rheumatoid arthritis, and body mass index (BMI) [60]. We also considered estradiol levels, physical activity, grip strength, educational attainment, and household income as confounders, acknowledging that some might also act as mediators in the exposure-outcome relationship. Any SNPs significantly associated with these confounders were excluded if they reached the Bonferroni-corrected P-value threshold of $7 \times 10^{-5}$ (0.05/732, adjusted for the 732 unique exposure SNPs). We assessed the associations of the exposure SNPs with potential confounders using GWAS sourced from The MRC IEU OpenGWAS data infrastructure [61]. For a detailed list of the specific GWAS referenced, see Supplemental Table S6.

To assess heterogeneity between the instrumental variable estimates of different SNPs, we visualized the data with funnel and scatter plots [62]. Funnel plots (individual instrumental variable estimates plotted against the standard errors of the instrumental variable estimate) allow for assessment of unbalanced directional pleiotropy, any asymmetry in the funnel plot is a sign of directional pleiotropy and could lead to bias in the results. Scatter plots (genetic associations with the outcome plotted against the genetic associations with the risk factor) highlight the heterogeneity of the Wald ratios. If all variants are valid IVs, a dose–response relationship should be observed in the scatterplot, and if a majority of variants are valid IVs, pleiotropic variants may be detected. In addition to visual assessments, we analyzed the heterogeneity in the MR estimates across the genetic variants using the I2 index and the Q heterogeneity test [52].

For SHBG, we investigated the associations of cis-SNPs with all examined outcomes. Utilizing a cis-SNP design is thought to reduce the risk of confounding effects caused by pleiotropy [43]. We employed Wald ratios to test individual associations of each cis-SNP with forearm fracture and BMD. Due to the inclusion of only HapMap variants in the LS-BMD and FN-BMD data, rs6761 was the only available cis-SNP, thus requiring us to use proxy SNPs for rs1799941 and rs858519. We also performed combined analyses of all three SHBG cis-SNPs using IVW. Given a moderate correlation among the cis-SNPs (linkage disequilibrium $R^2$ between 0.35 and 0.53), we applied a fixed-effect IVW method that accounts for this correlation [63].

All statistical analyses were performed using the software R (www.R-project.org) version 4.2.2 using the TwoSampleMR [64] and the MendelianRandomization [65] packages.

### Power Calculations

We performed power calculations for the two-sample MR analyses of forearm fractures based on R code from an online power calculation tool (https://github.com/kn3in/mRnd/) [66]. The main determinants of power estimation in MR analysis are the sample size, the genetic variance explained by the instruments, and the size of the causal effect [66]. As the GWAS of TT, SHBG, and BioT did not provide information on the genetic variance explained by each SNP on TT, SHBG, or BioT, we estimated this measure using formula derived by Shim et al. [67]. With this approximation, the 180 SNPs associated with BioT explain 7.0% of the variance of BioT, the 359 SNPs associated with SHBG explain 17,6% of the variance, and the 254 SNPs associated with TT explain 9.3% of the variance. For forearm fractures, using an available sample size of 111 351 and 8 823 cases, we had 80% power to detect a causal association of odds ratio (OR) 0.89 per standard deviation (SD) increase in BioT, OR 1.07 per SD increase in SHBG, and OR 0.91 per SD increase in TT (Supplementary Fig. S2).

## Results

The median age at the time of fracture was consistent across all four female forearm fracture cohorts, ranging between 58 and 64 years (Table 2). Baseline characteristics, such as weight and height, were also comparable among the fracture cohorts (Supplementary Table S2). Both exposure and outcome data included only women, were predominantly derived from population-based studies of European descent, and involved participants within a similar age bracket. In our primary analyses, there was no overlap between the participants in the exposure and outcome datasets. These characteristics collectively reduce the likelihood of sample differences in pleiotropic pathways or linkage disequilibrium patterns and reinforce the validity of our causal inferences.

### Effect of TT, bioT, and SHBG on Forearm Fractures

In the primary two-sample MR analyses, using an IVW method, we observed that high SHBG (OR per SD increase: 1.61, 95% confidence intervals (CIs) 1.39–1.87, $P = 2.6 \times 10^{-10}$) and low BioT (OR per SD increase: 0.76, 95% CI 0.68–0.84, $P = 4.3 \times 10^{-07}$) were causally associated with an increased risk of forearm fracture, while the inverse causal association of TT with fracture risk was comparatively weaker (OR per SD increase 0.90, 95% CI 0.82–0.99, 0.039) (Fig. 1, Supplementary Table S3). Analyses using UK Biobank in a one-sample MR setting and combined analysis of all four cohorts (overlap-sample MR) revealed similar effect estimates (Fig. 1, Supplementary Table S3).

When applying several alternative MR methodologies (Weighted median MR, Weighted mode MR, MR Egger, MR-PRESSO, MR LASSO, MR RAPS, and Steiger filtered analyses) in sensitivity analyses, the effect estimates for the causal associations with forearm fractures remained consistent (Supplementary Table S3). After excluding SNPs associated with potential confounders, the causal associations with forearm fractures also remained consistent (Supplementary Table S5). Scatter and funnel plots displayed moderate heterogeneity (Supplementary Fig. S3-S4), which also overall was confirmed by I2 index and the Q heterogeneity tests (Supplementary Table S3); however, the balanced distribution of individual estimates around the overall estimates suggests balanced pleiotropy, a finding supported by MR Egger analyses.

Analyses of three SHBG cis-SNPs yielded comparable and statistically significant Wald ratios in the two-sample and mixed-sample settings with slightly lower estimates in the one-sample setting. Combined analysis of all three cis-SNPs produced an SHBG IVW OR of 1.59 (95% CI 1.16–2.16) in the two-sample setting, closely matching estimates from analyses using all SHBG signals.

### Effect of TT, bioT, and SHBG on BMD

Analyses of BMD were in agreement with the effects seen on forearm fractures. In two-sample MR IVW analyses, BioT demonstrated a positive causal association with FN-BMD, LS-BMD, and FA-BMD, while SHBG exhibited an inverse causal relationship with all BMD measurements (Fig. 2, Supplementary Table S4). TT had somewhat smaller effect estimates and showed a significant positive causal association with FA-BMD and LS-BMD, but not with FN-BMD (Fig. 2, Supplementary Table S4). For all exposures, the effect estimates for FA-BMD were consistently larger than

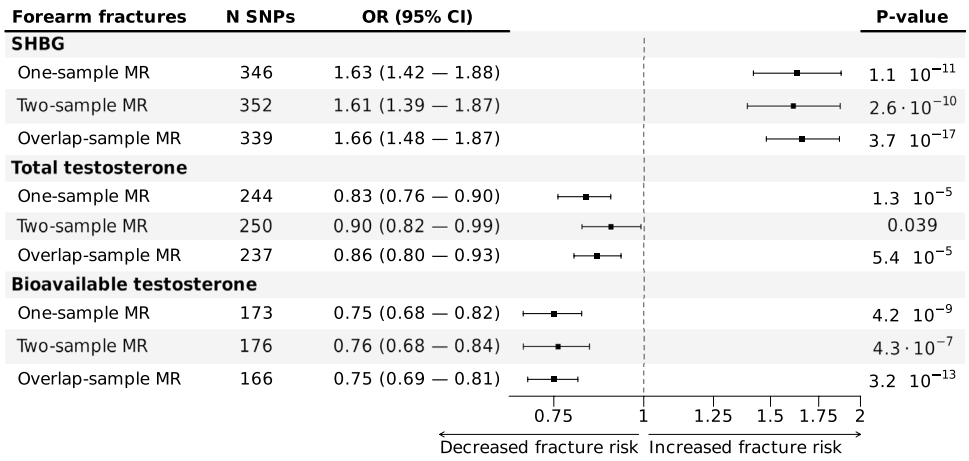


**Fig. 1** Forest plot of IVW MR associations of SHBG, TT, and BioT with forearm fractures. Inverse-variance weighted (IVW) causal effects of sex hormone-binding globulin (SHBG), total testosterone (TT), and bioavailable testosterone (BioT) on forearm fractures in women using three different outcome sets. One-sample MR consisting of data from the UK Biobank, two-sample MR consisting of data from Estonian Biobank (EstBB), The Trøndelag Health Study (HUNT), Umeå Fracture and Osteoporosis Study (UFO), and overlap-sample MR consisting of data from all four cohorts (UK Biobank, EstBB, HUNT, UFO). OR estimates and 95% CIs are given in OR change per 1 SD increase in SHBG, BioT, or TT. Abbreviations: IVW, inverse variance weighting; MR, Mendelian randomization; CI, confidence interval; SNPs, single-nucleotide polymorphisms

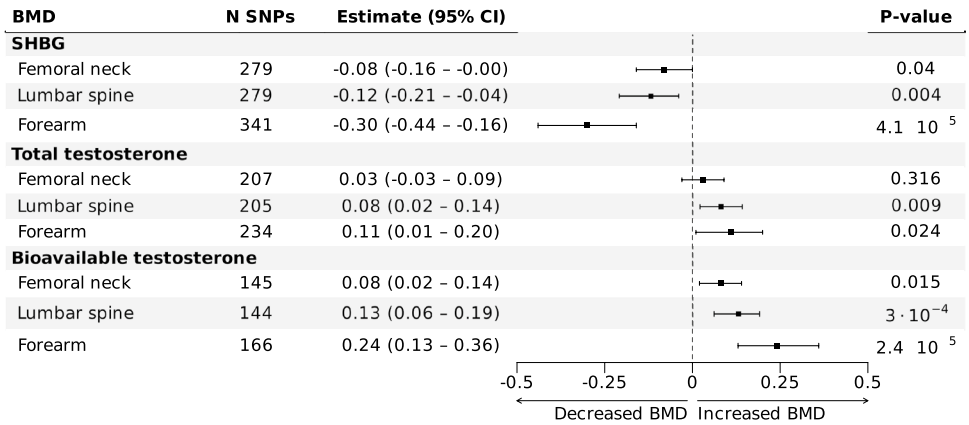


**Fig. 2** Forest plot of IVW MR associations of SHBG, TT, and BioT with bone mineral density. Inverse-variance weighted (IVW) causal effects of sex hormone-binding globulin (SHBG), total testosterone (TT), and bioavailable testosterone (BioT) on three different bone mineral density (BMD) measurements in women. Estimates and 95% CIs are given in SD change of BMD per 1 SD increase in SHBG, BioT, or TT. Abbreviations: SD, standard deviation; MR, Mendelian randomization; CI, confidence interval; SNPs, single-nucleotide polymorphisms

for LS-BMD and the effect estimates for LS-BMD were larger than for FN-BMD.

Sensitivity analyses employing multiple MR methodologies revealed a similar trend, albeit with some variations in estimated effect sizes (Supplementary Table S4). Upon excluding SNPs associated with potential confounders, the significant causal estimates observed in the primary analyses remained essentially stable (Supplementary Table S5). Visual examination of scatter and funnel plots for BMD outcomes revealed greater heterogeneity compared to forearm fracture analyses. Nevertheless, the overall balanced distribution of estimates implies balanced pleiotropy, a conclusion supported by MR Egger analyses (Supplementary Fig. S3-S4).

Analysis using the three SHBG cis-SNPs did not reveal statistically significant results for FN-BMD or LS-BMD in either individual or combined analyses. For FA-BMD analyses, one of the three SNPs (rs1799941) showed a statistically significant association (*P*-value: 0.014). Combined analyses of all three SNPs suggested a trend towards statistical significance, with a causal effect estimate of − 0.25 SD BMD change per 1 SD increase in SHBG (95% CI − 0.53 to 0.03,

*P*-value: 0.074), which is comparable to estimates observed with trans-SNPs.

## Discussion

The role of androgens for bone health in females is unclear. We, herein, used female-specific genetic instruments to determine the effect of androgens and SHBG for forearm fracture risk specifically in females. We demonstrate that increased SHBG and decreased BioT and TT levels are causally linked with increased forearm fracture risk and lower BMD in women.

In the present study, we opted to examine forearm fractures as our fracture outcome based on a several considerations. First, among osteoporotic fractures, forearm fractures are notably more frequent and emerge earlier in women [68], making them the most suitable for achieving robust analytical power in our European cohorts, where participants' average baseline age varied between 44 and 68 years. Secondly, both preclinical [5–7] and clinical data [18, 69] suggest that testosterone may preferentially affect trabecular bone. A majority of forearm fractures occurs in the distal forearm, which is a site of predominantly trabecular bone [70, 71], thus forearm fracture could likely be more susceptible to differences in androgen levels. Third, in a prior MR study, we established that decreased concentration of dehydroepiandrosterone sulfate (DHEAS), an adrenal sex steroid precursor, causes an increased risk of forearm fractures in females [72]. As there is an association of genetically predicted TT and BioT with genetically predicted DHEAS levels in women [41], we hypothesized that there is a likely upstream effect of DHEAS on TT, potentially influencing the risk of forearm fracture.

Though our study uniquely leverages both female-specific testosterone-related exposures and female-specific forearm fracture outcomes in a two-sample MR setting, our estimates of SHBG and BioT agree with earlier sex-combined MR studies [39, 40]. Sun et al. used sex-combined exposures for SHBG, derived from the UK Biobank, in two-sample MR analyses evaluating different sex-combined fracture outcomes using data from the FinnGen consortium [40]. In analogy with our results, high SHBG was associated with increased forearm fracture risk in that sex-combined study (OR = 1.23 per SD increase) but the effect estimate was more moderate compared with the effect estimate from the present female-specific MR analyses (OR = 1.61 per SD increase). Based on the combined findings from these two studies, we propose that the causal effect of high SHBG on forearm fracture risk may be stronger in women compared with men.

Our MR analyses reveal that BioT, TT, and SHBG causally affect BMD in females, exerting the greatest influence on FA-BMD and the smallest or no effect on FN-BMD, with LS-BMD estimates somewhere in between. These results indicate that at least part of the effect of BioT, TT, and SHBG on forearm fracture risk could be mediated by BMD. An earlier sex-combined two-sample MR study of SHBG, including only 11 genetic markers, observed a marked effect on forearm BMD, similar in size to our own finding for SHBG, however, this previous study found no significant effect on femoral neck or lumbar spine BMD, possibly related to using fewer genetic instruments and non-sex-specific exposure data [35]. Likewise, a later MR study noted significant effects of SHBG on female heel estimated BMD, that, similarly to the forearm, is a distal trabecular-rich bone site [36].

The present study has several strengths. We used exclusively female-specific datasets for both the selection of genetic instruments and for the exposures and outcomes in a solid two-sample MR framework. We obtained genetic instruments from the largest available female-specific GWAS of testosterone and SHBG to date, ensuring solid genetic instruments. Our MR analyses used a unique female-specific outcome dataset with well-defined forearm fracture cases that was well powered to detect also moderate effect sizes. We explored the MR assumptions by using multiple sensitivity analyses more robust to pleiotropy, used Steiger filtering to avoid problems of reverse causality, and excluded SNPs associated with potential confounders to ascertain that our effect estimates were consistent and reliable. By leveraging the protein nature of SHBG, unlike the sex steroid testosterone, we were also able to implement MR analyses using only cis-SNPs. This approach, which minimizes the risk of pleiotropic confounding [73], yielded consistent effect size estimates, further validating our results.

There are also some limitations of our study. First, our study focused solely on individuals of white ancestry, limiting the generalizability of our results to populations of other ancestries. Second, the large number of SNPs led to considerable heterogeneity in all our analyses. To ensure robust estimates, we employed a random-effects model as our primary method and supplemented it with several secondary methods based on different assumptions. All methods consistently indicated a strong association between SHBG, BioT, and forearm fracture risk, even when excluding SNPs associated with several potential confounders and when analyzing only SHBG cis-SNPs. Still, residual confounding or pleiotropy cannot be completely ruled out. Third, menopausal status of participants at the time of forearm fracture was not known for some of the included cohorts, preventing adjustment or stratification by menopausal status in the MR analyses. However, with the median age at forearm fracture ranging from 58 to 64 years, most fractures likely occurred after menopause. Moreover, because genetic variants used in Mendelian randomization remain consistent throughout life, the

associated hormonal effects may have influenced skeletal features and forearm fracture susceptibility throughout life, including crucial periods such as puberty, the menopausal transition period, and after menopause. Thus, although forearm fractures mainly occur after menopause, it is challenging using standard MR methodology to pinpoint at which life stage testosterone and SHBG levels exert the most significant impact on forearm fracture risk. Fourth, a few of our outcome GWAS datasets lacked some exposure SNPs, a problem that persisted even after proxy SNPs was included. Consequently, some of these SNPs had to be left out of our MR analyses, especially in analyses of the LS-BMD and FN-BMD data which are limited to HapMap variants. Though the absence of some exposure SNPs in the outcome GWAS dataset may impair statistical power and potentially increase variability in sensitivity analyses, we are confident that we have included a reasonable number of SNPs to ensure the validity of our MR analyses.

In conclusion, our results establish a causal relationship of genetically predicted increases in SHBG and decreases in BioT with significantly increased forearm fracture risk in women. These effects of SHBG and BioT on forearm fracture risk were also associated with marked effects on forearm BMD, as well as more moderate effects on lumbar spine and femoral neck BMD in women. The impact of TT on these bone health outcomes appears to be less pronounced. Based on these results and previous studies, we propose that androgens and SHBG levels are important for women's bone health of the distal forearm and that high SHBG and low BioT may serve as predictors for forearm fracture risk.

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**Author Contributions** The study was designed by collaboration between J.Q., M.N, and C.O. M.N., E.C., E.R., R.M., U.P-K, K.H., were either responsible for the different included cohorts or performed cohort-specific forearm fracture GWAS. J.Q. performed MR analyses and prepared all figures. J.Q. and C.O. wrote the first draft of the manuscript. All authors contributed to subsequent drafts of the manuscript and made the decision to submit the manuscript for publication.

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**Data Availability** Access to UK biobank data can be obtained by application to UK biobank (https://www.ukbiobank.ac.uk/). Access to individual-level data from HUNT requires collaboration with a Norwegian principal investigator. Researchers wishing to access HUNT data must apply through the HUNT Research Centre (https://www.ntnu.edu/hunt) and secure project approval from the Regional Committee for Medical and Health Research Ethics. Information on the application and conditions for data access in HUNT is available at https://www.ntnu.edu/hunt/data. Individual level Estonian Biobank data are available under restricted access administered by the Estonian Genome Centre of the University of Tartu (EGCUT) in accordance with the regulations of the Estonian Human Genes Research Act. Access can be obtained by application at https://genomics.ut.ee/en. For information on accessing data from the UFO, contact Ulrika Pettersson-Kymmer (ulrika.pettersson@umu.se). Summary statistics for bone mineral density measurements used in this article is publicly available via GEFOS: http://www.gefos.org.

## Declarations

**Conflict of interest** C.O. has filed patent applications in the field of effects of probiotics on osteoporosis. The remaining authors declare no competing interests.

**Human and Animal Rights and Informed Consent** All individual-level data used in this study have been obtained from four Nordic biobanks: The UK Biobank, The UFO Study, The HUNT study, and the Estonian Biobank. These biobanks obtained written informed consent from all participants and received approval from their respective research ethics committees. The UK Biobank has ethical approval from the Northwest Multicentre Research Ethics Committee (11/NW/0382). The UFO study was approved by the local research ethics committee at Umeå University (Umu dnr 03-426; EPN 2012-254-32 M; 2011/32-32 M; 2011-251-32 M). The HUNT study has been approved by the Regional Ethics Committee for Medical Research in Norway (REK 2015/615; REK 2014/144). Estonian Biobank has ethical approval from the Estonian Committee on Bioethics and Human Research at the Ministry of Social Affairs (No 1.1-12/624). The current study was carried out in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. The study did not involve experimental animals.

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