

Evidence of a Causal Effect of Estradiol on Fracture Risk in Men

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Context: Observational studies indicate that serum estradiol (E2) is more strongly associated with bone mineral density (BMD) than serum testosterone (T) is, whereas both E2 and T associate with fracture risk in men.

Objective: To evaluate the possible causal effect of serum E2 and T on fracture risk in men.

Design, Setting, and Participants: A Mendelian randomization (MR) approach was undertaken using individual-level data on genotypes, BMD as estimated by quantitative ultrasound of the heel (eBMD), fractures (n = 17,650), and relevant covariates of 175,583 unrelated men of European origin from the UK Biobank. The genetic instruments for serum E2 and T were taken from the most recent large-scale genome-wide association study meta-analyses on these hormones in men.

Results: MR analyses demonstrated a causal effect of serum E2 on eBMD and fracture risk. A 1 SD (or 9.6 pg/mL) genetically instrumented decrease in serum E2 levels was associated with a 0.38 SD decrease in eBMD (P value: 9.7×10^{-74}) and an increased risk of any fracture (OR: 1.35; 95% CI: 1.18, 1.55), nonvertebral major osteoporotic fractures (OR: 1.75; 95% CI: 1.35, 2.27), and wrist fractures (OR: 2.27; 95% CI: 1.62, 3.16). These causal effects of serum E2 levels on fracture risk were robust in sensitivity analyses and remained unchanged in stratified analyses for age, body mass index, eBMD, smoking status, and physical activity. MR analyses revealed no evidence of a causal effect of T levels on fracture risk.

Conclusion: Our findings provide evidence of a robust causal effect of serum E2, but not T, on fracture risk in men. (*J Clin Endocrinol Metab* 104: 433–442, 2019)

It is well recognized that sex steroids are crucial for male bone health (1). Many experimental animal studies as well as both human observational association studies and intervention studies provide evidence of a major role of estrogens over androgens on the maintenance of bone mass and strength in males (2–4). More specifically, serum estradiol (E2) levels correlate better than serum testosterone (T) levels with bone mineral density (BMD) at various sites, whereas both low E2 and low T levels are associated with increased fracture risk in men (1, 3,

5–10). However, the possible causal effect of E2 and T levels on fracture risk in men is unknown.

The principles of Mendelian randomization (MR) can be applied to test the role of biomarkers in disease etiology (11). MR uses genetic data to ascertain whether a given biomarker, such as E2 or T, is implicated in disease etiology, relying on a simple tenet: If a biomarker is etiologically involved in a disease process, then the genetic factors that influence the biomarker will influence disease risk (12). This established technique greatly limits

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Abbreviations: BMD, bone mineral density; BMI, body mass index; E2, estradiol; E2-GRS, genetic risk score for estradiol; eBMD, estimated bone mineral density; ER, estrogen receptor; GWAS, genome-wide association study; IVW, inverse variance-weighted; MR, Mendelian randomization; RCT, randomized controlled trial; SNP, single-nucleotide polymorphism; T, testosterone.

confounding because genotypes are expected to be randomly assorted at conception; furthermore, it is free of reverse causation because genotypes are always assigned before the onset of disease. Thus, MR studies overcome some of the limitations of observational studies and are conceptually similar to randomized controlled trials (RCTs) but provide a lifelong assessment of exposure to a biomarker, such as low serum E2 or T levels. Furthermore, recent advances in genotyping enable the application of the MR methodology in sample sizes that are not realistic for RCTs of T or E2 therapy in men. A recent medium-sized RCT (n = 211) demonstrated that 1-year treatment with T, augmenting both serum T and E2 levels, increased volumetric bone density and estimated bone strength in older men with low serum T levels (13). However, that study was underpowered for fracture analyses and could not separate causal effects of serum E2 and T on bone parameters.

We recently performed a large-scale genome-wide association study (GWAS) meta-analysis on serum E2 and identified significant genome-wide single-nucleotide polymorphisms (SNPs) associated with E2 levels in men (14). Subsequently, we used these SNPs in a two-sample MR analysis using summary statistics from large-scale GWAS meta-analyses on lumbar spine BMD and femoral neck BMD (15) and demonstrated a causal effect of serum E2 on BMD in men (14). However, individual-level data were not available in that study, precluding stratified analyses and evaluations of possible interactions with relevant covariates. In addition, we could not determine the causal effect of E2 on fracture risk. No previous MR analysis of the possible causal effect of serum T on BMD or fracture risk has been performed.

In the current study, we adopted an MR design to estimate the effect of genetically lowered E2 or T levels on estimated BMD (eBMD) and fracture risk in a two-sample MR analysis using individual-level data from unrelated men of European origin in the UK Biobank (n = 175,583, including 17,650 fractures), the largest male cohort worldwide. The MR instruments for serum E2 and T were the lead SNPs from the most recent and largest GWAS meta-analyses on these hormones in men of European descent (14, 16, 17).

Methods

Study population

UK Biobank

From 2006 to 2010, the UK Biobank recruited 502,647 individuals aged 37 to 76 years from across the United Kingdom (18). All participants provided information regarding their health and lifestyle via touch screen questionnaires, consented to physical measurements, and agreed to have their health

monitored. They also provided blood for future analysis. The UK Biobank has ethical approval from the Northwest Multi-centre Research Ethics Committee, and informed consent was obtained from all participants. For the current study, individual participant-level data of E2-associated and T-associated SNPs and evaluated phenotypes (eBMD using ultrasound of the heel and self-reported fractures) and relevant covariates [age, body mass index (BMI), smoking status, and physical activity] were available for 175,583 unrelated men of European origin (Table 1). For a physical activity parameter, we used vigorous physical activity (yes/no) because it is the dichotomous physical activity parameter that was most robustly associated with eBMD (14, 16, 17).

eBMD using ultrasound

Quantitative ultrasound of the heel was used to obtain a noninvasive eBMD that predicts fracture (19, 20). A Sahara Clinical Bone Sonometer (Hologic Corporation, Bedford, MA) was used for quantitative assessment of calcanei in UK Biobank participants. Details of the complete protocol are publicly available on the UK Biobank website (www.ukbiobank.ac.uk/). eBMD (g/cm^2) was derived as a linear combination of speed of sound and bone ultrasound attenuation ($\text{eBMD} = 0.0025926 \times (\text{bone ultrasound attenuation} + \text{speed of sound}) - 3.687$) (20).

Fractures

For achievement of maximal power in the fracture analyses, the primary analysis was the causal effect of E2 and T on all self-reported fractures (n = 17,650). Subanalyses according to fracture type were also performed for major nonvertebral osteoporotic fractures (n = 4379; wrist, arm, and hip) and wrist fractures (n = 2637), whereas the low number of hip (n = 341) and spine (n = 418) fractures precluded separate hip and spinal fracture analyses (Table 1).

Population stratification

To reduce the risk of influence from population stratification, we used only a subset of unrelated men of European origin in the UK Biobank. Pairs of individuals up to third-degree relatives were identified using the estimated kinship coefficients from KING's robust estimator (21).

Table 1. Baseline Characteristics of the Study Participants

	n = 175,583
Age, y	56.9 (8.1)
Weight, kg	86.2 (14.3)
Height, cm	175.9 (6.8)
BMI, kg/m^2	27.8 (4.2)
Current smoking, n (%)	21,597 (12.2)
Vigorous physical activity, n (%)	98,967 (56.0)
eBMD, g/cm^2	0.56 (0.12)
All fractures, n (%)	17,650 (10.0)
Major nonvertebral osteoporotic fractures, n (%)	4379 (2.5)
Wrist fractures, n (%)	2637 (1.5)

Values are given as mean (SD) or n (%). Major nonvertebral osteoporotic fractures are defined as wrist, arm, or hip fractures. eBMD was measured in the heel using ultrasound.

SNP selection and data sources

We selected the lead SNPs from identified loci associated with E2 (two independent SNPs from the base model) (14) or T (three independent SNPs) (16, 17) and the estimates of their effects from the most recent and largest GWAS meta-analyses on these hormones in men.

SNP validation

To validate the selected E2 and T SNPs as instruments for our MR analysis, we evaluated them for the three MR assumptions: strong association with exposure (E2 or T); absence of association with known confounders of the exposure-outcome association; and absence of pleiotropy, in which the genetic variant influences eBMD or fracture risk through mechanisms that are independent of E2 or T. Because of randomization of alleles at conception, confounding is greatly minimized in MR studies; however, we examined whether E2 or T SNPs influence important known confounders (age, physical activity, smoking status, or BMI; Supplemental Table 1) that may link E2 or T to BMD and/or fracture risk (4, 22–28). Pleiotropy may bias results if the chosen SNPs exert effects on eBMD or fracture risk independently of E2 or T. In this study, pleiotropy is less likely because all E2-associated and T-associated SNPs map to genes implicated in sex steroid physiology.

MR estimates

We assessed the effects of the SNPs on eBMD and fractures, weighting the effect of each SNP by the magnitude of its effect on E2 or T levels. In the absence of available data on E2 or T levels in the UK Biobank, we used effect estimates from the most recent GWASs as instrumental variable estimates of genetically determined E2 and T in a two-sample MR approach (29). To provide a summary measure for the effect, including all genome-wide significant SNPs for E2 or T, we combined weighted estimates using fixed-effects models in inverse variance-weighted (IVW) MR analyses. The effect size for the meta-analysis is reported as the effect of an SD change in E2 (1 SD = 9.6 pg/mL from the Framingham Heart Study) (14) or T (1 SD = 176 ng/dL from MrOS Sweden) (17), as this metric is more interpretable than an arbitrary difference (12). We also undertook power calculations to test whether our study was adequately powered to detect a clinically relevant change in fracture outcomes (30).

Power calculations

Our E2 MR analyses had a power of 100%, 72%, and 25% to detect an OR of 1.4, 1.2, and 1.1, respectively, for a 1 SD change in E2 on any fracture ($n = 175,583$; 17,650 fractures; setting α to 0.05). Our E2 MR analyses, excluding rs5934505 with an effect on both serum E2 and T, had a power of 100%, 66%, and 22% to detect an OR of 1.4, 1.2, and 1.1, respectively, for a 1 SD change in E2 on any fracture. Our T MR analyses had a power of 100%, 100%, and 70% to detect an OR of 1.4, 1.2, and 1.1, respectively, for a 1 SD change in T on any fracture. Our T MR analyses, excluding rs5934505, had a power of 100%, 99%, and 63% to detect an OR of 1.4, 1.2, and 1.1, respectively, for a 1 SD change in T on any fracture.

Data availability

The genetic and phenotypic UK Biobank data are available upon application to the UK Biobank to all *bona fide* researchers.

Results

SNP selection

E2 SNPs

The lead SNPs from genome-wide significant independent loci from the most recent and largest GWAS meta-analysis on E2 were selected (14). These included two E2-associated SNPs: rs727479 in *CYP19A1* (aromatase, the enzyme converting T to E2) and rs5934505 in *FAM9B* (a protein expressed exclusively in the testis) (Supplemental Table 2). Importantly, rs727479 in *CYP19A1* influences serum E2 but not serum T, whereas rs5934505 in *FAM9B* influences both serum E2 and T in the same direction. By comparing the effects of these two SNPs, it is possible to separate the causal effects of serum E2 from those of T (17).

T SNPs

The lead SNPs from genome-wide significant independent loci from the most recent and largest GWAS meta-analyses on T were selected (16, 17). These included three T-associated SNPs: rs5934505 in *FAM9B*, rs10822186 in *JMJD1C* (a testicular protein with transcriptional regulatory functions in the development of the testis and proposed to regulate T synthesis) (31), and rs12150660 in *SHBG* (this SNP is highly correlated with a penta-nucleotide repeat, which affects SHBG expression *in vitro*) (17, 32) (Supplemental Table 3).

LD, confounding, and pleiotropy assessment

We found no evidence of linkage disequilibrium between any of the E2 or T SNPs (pairwise $r^2 \leq 0.01$ for all).

In our literature search for potential confounders, BMI, physical activity, age, and smoking were identified as risk factors for low eBMD and/or fractures that have also been associated with E2 and/or T levels (4, 22–28). We found no significant association between the E2 or T SNPs and BMI, smoking status, age, or physical activity in the UK Biobank (Supplemental Table 1).

The E2-associated SNPs may also influence the risk of fractures independently of E2 through pleiotropy. Therefore, we performed sensitivity analyses excluding either the rs727479 in *CYP19A1* or the rs5934505 in *FAM9B*, yielding results very similar to those obtained when both SNPs were evaluated together. Because of the limited number of SNPs used as genetic instruments, Egger regression was not applicable.

Association of E2 and T SNPs with E2 and T serum levels

The associations of the two genome-wide significant E2 SNPs with E2 levels and the three T SNPs with T levels

are described in Supplemental Tables 2 and 3, respectively (14, 16, 17). Importantly, each extra C allele of rs727479 in *CYP19A1* (coding for aromatase, the enzyme converting T into E2) was associated with a 0.145 SD reduction in serum E2 levels (β : -0.145 SD E2/extra C allele; $P = 8.2 \times 10^{-30}$) (Supplemental Table 2), whereas it was not associated with serum T (β : 0.022 SD T/extra C allele; $P = 0.14$). Thus, this SNP is useful as an instrument to separate the causal effects of serum E2 from those of serum T. The proportion of the population-level variance in E2 levels explained by the two E2 SNPs and the proportion of variance explained by the three T SNPs are reflected by the *F*-statistics (*F*-statistics: E2 SNPs, 12.9 to 102.6; T SNPs, 53.6 to 338.2) (Supplemental Tables 2 and 3).

Association of E2 SNPs with eBMD and fracture susceptibility

The effect estimates of the associations of the two E2-associated SNPs with eBMD and fracture susceptibility were derived using data from the UK Biobank in models including age, height, and weight as continuous

parameters. The E2-decreasing alleles of both SNPs were significantly associated with reduced eBMD and increased risk of any fracture, nonvertebral major osteoporotic fractures, and wrist fractures (Supplemental Table 2).

MR analysis for the association of E2 and eBMD

We next performed IVW MR analyses of the association between E2 and eBMD. To estimate the association of genetically lowered E2 with eBMD, we used a fixed-effects model including the two independent E2-decreasing alleles. A decrease in E2 levels by 1 SD (or 9.6 pg/mL) was associated with a 0.38 SD decrease in eBMD (P value: 9.7×10^{-74}) (Fig. 1; Table 2). Age, BMI, smoking, and physical activity are associated with eBMD in men from the UK Biobank. The well-powered UK Biobank with individual-level data available for relevant covariates made it possible to evaluate the causal effect of serum E2 on eBMD stratified by age (divided by median age), BMI (overweight yes/no), smoking status (current smoking yes/no), and physical activity (vigorous physical activity yes/no) (Fig. 1). The causal effects of E2 on eBMD

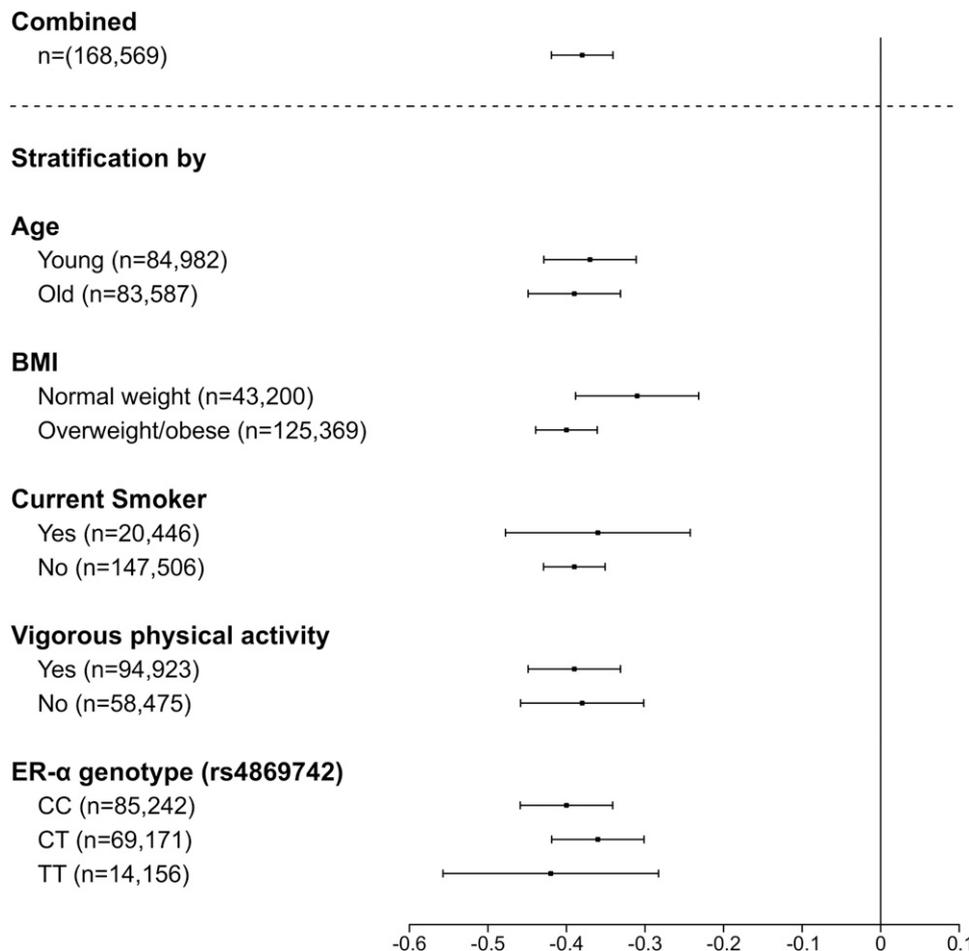


Figure 1. Stratified analyses of the estimated causal effect of E2 on eBMD. β for eBMD is given as SD eBMD per SD decrease in E2 (95% CIs). ER, estrogen receptor.

Table 2. Estimated Causal Effect of E2 on eBMD and Fractures

Genetic Instrument	Chr	Position	EA	OA	Freq EA	Gene	eBMD			All Fractures			Major Nonvertebral Osteoporotic Fractures			Wrist Fractures		
							β	SE	P	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P
rs5934505	23	8913826	T	C	0.73	FAM9B	-0.51	0.04	1.9E-38	1.34	(1.04, 1.73)	2.4E-02	2.36	(1.43, 3.90)	7.6E-04	2.85	(1.49, 5.44)	1.5E-03
rs727479	15	51534547	C	A	0.35	CYP19A1	-0.33	0.02	2.4E-40	1.36	(1.16, 1.59)	1.6E-04	1.56	(1.15, 2.12)	4.0E-03	2.09	(1.42, 3.08)	2.1E-04
IVW MR effect							-0.38	0.02	9.7E-74	1.35	(1.18, 1.55)	1.1E-05	1.75	(1.35, 2.27)	2.6E-05	2.27	(1.62, 3.16)	1.5E-06

β for eBMD is given as SD eBMD per SD decrease in E2. ORs for fractures are given per SD decrease in E2. Major nonvertebral osteoporotic fractures are defined as wrist, arm, or hip fractures. eBMD was measured in the heel using ultrasonography.

Abbreviations: Chr, chromosome; EA, effect allele; Freq EA, frequency of effect allele; OA, other allele.

were robust and remained unchanged in stratified analyses by age, BMI, smoking status, and physical activity. A weighted genetic risk score for E2 (E2-GRS) was significantly associated with eBMD ($P = 7.1 \times 10^{-63}$) (Supplemental Table 4). No significant interaction effect on eBMD was observed between this E2-GRS and age, BMI, smoking status, or physical activity (Supplemental Table 4). We observed similar causal effects of E2 on eBMD in the different genotype groups when stratified according to the previously reported BMD-associated estrogen receptor (ER)- α SNP rs4869742 (Fig. 1) (15). An additive but not synergistic effect (no significant interaction) of the ER α SNP rs4869742 (15) and the E2-GRS on eBMD was observed (Fig. 1; Supplemental Table 4).

MR analysis for the association of E2 and fracture susceptibility

IVW MR analyses revealed that a decrease in E2 levels by 1 SD was associated with an increased risk of any fracture (OR: 1.35; 95% CI: 1.18, 1.55), nonvertebral major osteoporotic fractures (wrist, arm and hip; OR: 1.75; 95% CI: 1.35, 2.27), and wrist fractures (OR: 2.27; 95% CI: 1.62, 3.16) (Fig. 2; Table 2). Sensitivity analyses excluding either rs727479 in *CYP19A1* (with an effect on serum E2 but not T) or rs5934505 in *FAM9B* (with an effect on both E2 and T in the same direction) yielded results

very similar to those obtained when both SNPs were evaluated together (Table 2). This finding indicates that E2 exerts a causal effect on fracture risk independently of serum T.

MR analyses stratified by age (divided by median age), BMI (overweight yes/no), eBMD (divided by the median), smoking status (current smoking yes/no), and physical activity (vigorous activity yes/no) revealed that the causal effect of E2 on fracture risk was robust and remained significant and of similar magnitude in stratified analyses (Fig. 3). The E2-GRS was significantly associated with fracture risk, and no significant interaction effect on fracture risk was observed for this E2-GRS and age, BMI, smoking status, or physical activity (Supplemental Table 4). The causal effect of E2 on fracture risk was also similar in the different genotype groups when stratified according to ER α SNP rs4869742 (Fig. 3). An additive effect, but not a synergistic effect (no significant interaction), of the ER α SNP rs4869742 (15) and the E2-GRS on fracture risk was observed (Fig. 3; Supplemental Table 4).

MR analysis for the association of T and fracture susceptibility

MR analyses including all three T SNPs (also rs5934505 in *FAM9B* with an effect on both E2 and T) indicated a modest causal effect of T on eBMD (a 0.14 SD decrease per SD decrease in T; Supplemental Table 5).

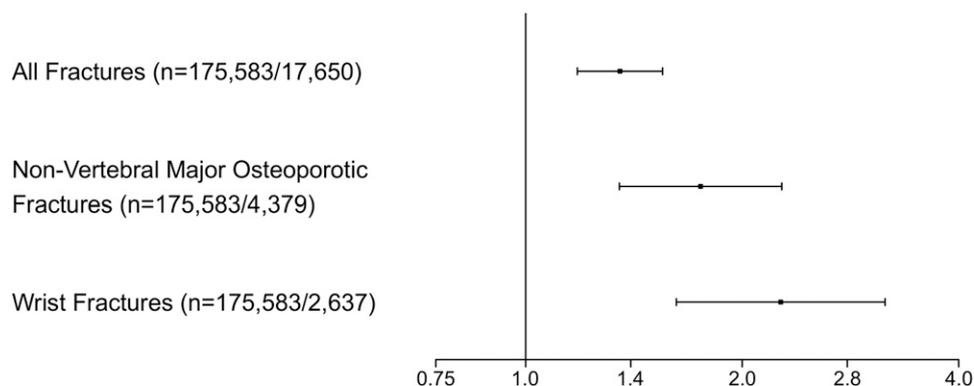


Figure 2. Estimated causal effect of E2 on fracture risk. ORs for fracture risk are given per SD decrease in E2 (95% CIs). Total numbers of subjects/fracture cases are given within parentheses.

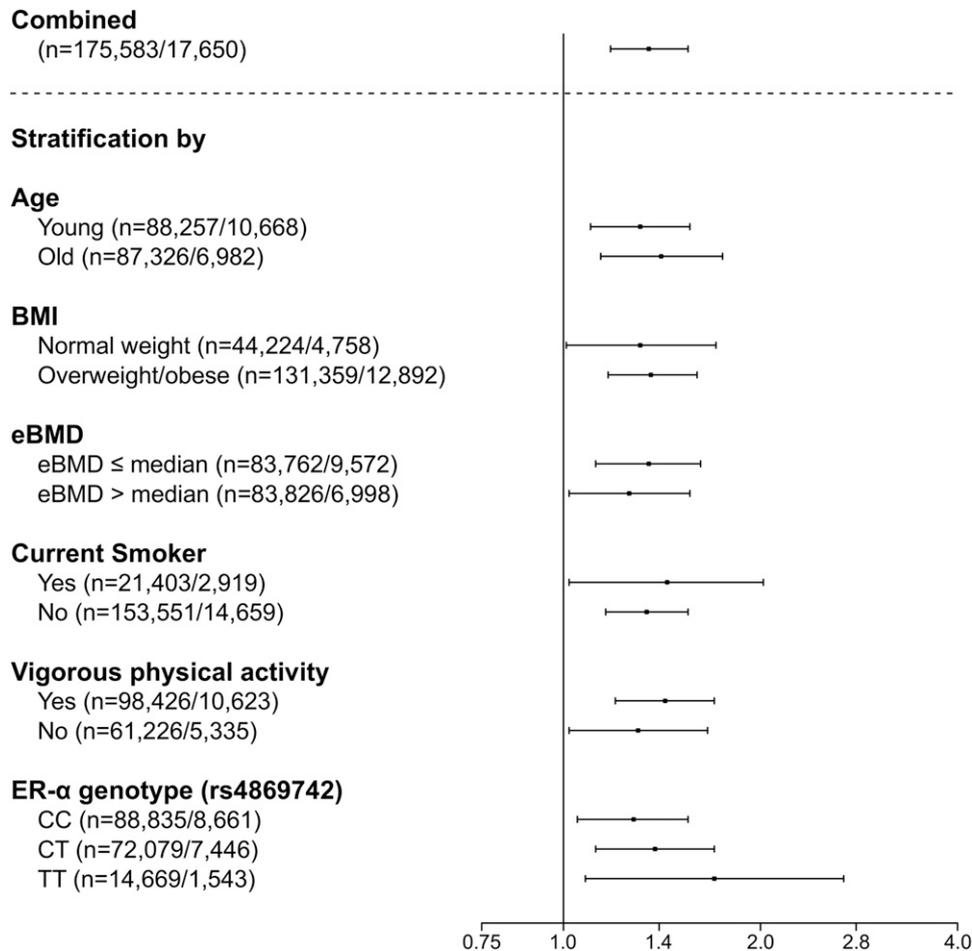


Figure 3. Stratified analyses of the estimated causal effect of E2 on any fracture. ORs for fracture risk are given per SD decrease in E2 (95% CIs). Total numbers of subjects/fracture cases are given within parentheses.

Sensitivity analyses excluding possible E2-mediated effects with removal of rs5934505 showed no evidence of a causal effect of T on eBMD (Supplemental Table 5). Neither MR analysis including all three T SNPs (OR: 1.03; 95% CI: 0.93, 1.15 per SD decrease in T for any fracture) nor MR analysis excluding rs5934505 revealed any significant causal effect of T on fracture risk (Supplemental Table 5).

Discussion

Both experimental animal studies and human interventional studies have revealed a major role of estrogens over androgens in the maintenance of bone mass in males (2–4). Observational studies have reported that both low serum T and low serum E2 levels are associated with increased fracture risk, but the possible causal effect of these hormones on fracture risk in men is unknown. Using MR analyses, we herein provide strong evidence of a causal effect of serum E2, but not T, on fracture risk in men of European descent.

Our finding of a causal effect of E2 on eBMD in the heel confirms a recent MR report demonstrating a causal

effect of serum E2 on both femoral neck and lumbar spine BMD (14). The magnitudes of the causal effects were similar: A 1 SD genetically instrumented decrease in serum E2 levels was associated with a 0.38 SD decrease in eBMD in the current study and 0.46 SD and 0.36 SD decreases in lumbar spine BMD and femoral neck BMD, respectively, in the previous MR report (14). A limitation with the previous MR report on the causal effect of E2 on dual-energy X-ray absorptiometry-derived BMD was the lack of individual-level data, precluding stratified analysis and evaluation of interactions (14). In the current study, the availability of the well-powered UK Biobank data set with individual-level data of relevant covariates enabled us to evaluate the causal effect of serum E2 on eBMD stratified by age, BMI, smoking status, and physical activity. The causal effects of E2 on eBMD were robust and remained unchanged in stratified analyses for parameters known to influence BMD. In addition, no significant interaction effect was observed for E2-GRS and age, BMI, smoking status, or physical activity on eBMD. Collectively, these findings demonstrate that serum E2 exerts a robust causal effect on eBMD independently of age, BMI, smoking status, and physical

activity. This strong evidence of a causal effect of E2 on eBMD is in accordance with the results from a recent medium-sized RCT ($n = 211$) demonstrating that 1-year treatment with T, augmenting both serum T and E2 levels, increased volumetric bone density and estimated bone strength in older men with low serum T levels (13).

Of note, in the current study we provide evidence of a robust causal effect of serum E2 on fracture risk. The magnitude of the causative effect of E2 on all fracture risks (OR: 1.35 per SD decrease in E2) in the current study is very similar to the observational association of E2 with any incident fracture (hazard ratio of 1.34 per SD decrease in E2) previously reported for elderly men in the MrOS Sweden cohort (9). Interestingly, the causal effects of E2 on nonvertebral major osteoporotic fracture risk (OR: 1.75 per SD decrease in E2) and wrist fracture risk (OR: 2.27 per SD decrease in E2) were substantial, suggesting that serum E2 has a major effect on clinically relevant osteoporotic fractures in men. A limitation of the current study is that the low number of hip and spine fractures precluded separate hip and spinal fracture analyses. The causal effects of E2 on fracture risk remained unchanged in stratified analyses for several parameters known to influence bone health, including age, BMI, eBMD, smoking status, and physical activity (4, 22–28). In addition, no significant interaction effect was observed for the E2-GRS and any of these parameters on fracture risk.

Sensitivity analyses including only rs727479, with an effect on serum E2 but not serum T, yielded results very similar to those obtained when both E2 SNPs were evaluated together. This finding indicates that E2 exerts a causal effect on fracture risk independently of serum T. Thus, serum E2 exerts a robust causal effect on eBMD and fracture risk that seems to be independent of many other known bone health-associated factors. On the basis of the findings in the current study, one may speculate that treatments augmenting serum E2, such as T supplementation, may reduce fracture risk in men. However, to avoid androgen receptor-related side effects, one might consider bone-specific selective ER modulators to reduce fracture risk in men. In addition, our findings indicate that nonaromatizable androgens, not augmenting E2, will be inefficient in reducing fracture risk in men. The finding of a causal effect of serum E2, but not T, on fracture risk might also be important for estimating possible skeletal side effects of endocrine therapies in men with prostate cancer. Our findings suggest that chemical castration with GnRH agonists as well as CYP17 inhibition using abiraterone will result in increased fracture risk in these patients, as both treatments will reduce serum T as well as serum E2 levels. This notion is supported by a substantially

increased fracture risk after chemical castration in patients who have prostate cancer (4, 33). In contrast, our data, showing no causal effect of T on fracture risk, indicate that endocrine prostate cancer treatment with the specific androgen receptor antagonist enzalutamide could avoid these skeletal side-effects. Nevertheless, it should be acknowledged that the present MR analyses are based on circulating levels of sex steroids. It is well known that sex steroids also are synthesized and metabolized locally in tissues (34). The resulting local tissue levels may be important for local sex steroid action, but their potential causal effects cannot be evaluated by the MR approach used in the current study.

Experimental animal studies as well as the case of a man with a mutation in $ER\alpha$ demonstrate that $ER\alpha$ is the major ER mediating the effect of E2 on the male skeleton (4, 35, 36). The $ER\alpha$ locus is one of very few loci that have been robustly associated with both BMD and fracture risk in GWAS meta-analyses (15). We hypothesized that genetic variants in $ER\alpha$ might interact with serum E2 (the ligand) for an effect on eBMD and fracture risk. However, the causal effect of serum E2 on eBMD and fracture risk was similar in the different genotypes of previously reported BMD-associated and fracture-associated $ER\alpha$ SNP rs4869742 (15). We observed an additive effect of rs4869742 and a serum E2 GRS on eBMD and fracture risk, but no significant interaction effect was observed. These findings indicate that genetic variants influencing the levels of the ligand (E2) and a genetic determinant in the $ER\alpha$ locus affecting ER responsiveness have an additive estrogenic activity in bone.

We observed no evidence of a causal effect of serum T on fracture risk in the present MR analyses. Although the present T MR analyses had 100% and 70% power to detect an OR of 1.2 and 1.1, respectively, per SD decrease in serum T for any fracture, we cannot exclude a minor causal effect of serum T on fracture risk. The lack of any clear causal effect of T on fracture risk in the present MR analyses contrasts with findings from a number of clinical and experimental animal studies (4, 8, 13, 37).

Strengths of this study include the large sample size with individual-level data available for SNPs used as genetic instruments, eBMD, fractures, and relevant covariates, making it possible to perform subgroup analyses. This study also has limitations. Although we controlled for pleiotropy in sensitivity analyses excluding SNPs, residual bias cannot be excluded because the exact function of the SNPs studied is unknown. However, all the SNPs lie in or near genes well validated for their role in sex steroid physiology. The null results for T could be explained by canalization, which is a phenomenon

resulting in compensatory feedback mechanisms (11). Another limitation with the current study is that serum sex steroid levels were not available in the UK Biobank. In addition, it should be noted that the GWAS, which identified the selected genetic instruments, used not only gold standard MS-based techniques (38) but also immunoassay-based techniques with a questionable specificity at the lower concentration range (39, 40) to assess sex steroid levels. Our MR analysis might also be limited in its ability to elucidate a possible causal role of biologically active T, as it is tightly bound to the high-affinity binding protein SHBG. Moreover, the effect of genetic variants in the androgen receptor and their possible interactions with serum T on fracture risk were not evaluated in the current study. A further limitation with the present MR approach is that the effects of endocrine disrupters, the variation in local aromatase activity in bone, and the interplay between free and bound fractions could not be evaluated. Our analyses were restricted to white populations of European ancestry, and further work will be required to investigate their relevance in populations of different ethnicity.

In conclusion, we provide MR evidence of a robust causal effect of serum E2, but not T, on fracture risk in men. Our findings may have direct clinical implications for patients who have prostate cancer when the possible skeletal side effects of endocrine treatments affecting both E2 and T levels are compared with those modulating only androgen receptor action.

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Disclosure Summary: The authors have nothing to disclose.

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