Endogenous DHEAS is Causally Linked with Lumbar Spine Bone Mineral Density and Forearm Fractures in Women

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Abstract

**Context:** A recent pooled analysis of four clinical trials demonstrated that treatment with dehydroepiandrosterone (DHEA) increases lumbar spine BMD (LS-BMD) in women. The causal effect of endogenous adrenal-derived DHEA-sulphate (DHEAS) on LS-BMD and fracture risk in women is unknown.

**Objective:** To determine whether circulating DHEAS is causally associated with LS-BMD and fracture risk in women.

**Methods:** A two-sample mendelian randomization study using genetic predictors of serum DHEAS derived from the largest available female-specific genome wide association study (GWAS) meta-analysis (n=8 565). Genetic associations with DXA-derived BMD (n=22 900) were obtained from female specific GWAS summary statistics available from the GEFOS consortium while individual-level data of 238 565 women of white ancestry from the UK Biobank were used for associations with fractures (11 564 forearm fractures, 2 604 hip fractures) and estimated heel BMD by ultrasound (eBMD).

**Results:** A 1 standard deviation (SD) genetically instrumented increase in log serum DHEAS levels was associated with a 0.21 SD increase in LS-BMD (P-value: 0.01) and a 0.08 SD increase in eBMD (P-value: <0.001). Genetically predicted DHEAS decreased forearm fracture risk (odds ratio (OR): 0.70, 95% confidence interval (CI): 0.55-0.88 per SD increase in DHEAS) while no significant causal association with hip fractures was observed.

**Conclusions:** Genetically predicted serum DHEAS increases LS-BMD and decreases forearm fracture risk in women. Based on the results of the present study and previous RCTs of DHEA treatment, we propose that both endogenous adrenal-derived DHEA(S) and pharmacological DHEA treatment improve bone health in women.

**Key Words**
Mendelian randomization, dehydroepiandrosterone sulfate, bone mineral density, fracture, osteoporosis, women
Introduction

Osteoporosis is a common skeletal disease, leading to a reduction in bone density and quality, and increased fracture risk. One in two elderly women will at some point suffer from an osteoporotic fracture (1,2). The incidence of fractures increases exponentially with age (3) and it is predicted that the number of people aged 80 years and older will nearly triple globally by the year 2050 (4).

The adrenal-derived hormones dehydroepiandrosterone (DHEA) and its sulphate ester (DHEAS) are the most abundant circulating steroid hormones. Many of the actions of DHEA(S) are mediated by the downstream metabolism into biologically active androgens and estrogens in peripheral target tissues. Adrenal-derived DHEA(S) is the main source of androgens in both pre- and post-menopausal women and the main contributor of estrogens in postmenopausal women (5,6). Circulating DHEA(S) levels decline with age (7,8), but the mechanism behind this decline and its implications for general health remain unclear. The age-related decline in DHEA(S) has led to speculations that a relative DHEA(S) deficiency may contribute to the development of common age-related diseases, such as osteoporosis (9–11). This notion is supported by findings from the largest available genome wide association study (GWAS) meta-analysis on serum DHEAS, demonstrating that genetic determinants of serum DHEA(S) are linked with biological pathways of ageing (12).

The possible link between DHEA(S) and bone disease in women has been studied with varying results. A recent well-performed pooled analysis using individual-level data from four randomized clinical trials demonstrated that treatment with DHEA for 12 months increases lumbar spine BMD (LS-BMD) but not femoral-neck BMD (FN-BMD) in postmenopausal women (11). No well-powered study, however, has evaluated the effect of DHEA treatment on fracture risk in postmenopausal women. Some but not all observational association studies have demonstrated that circulating DHEA(S) is directly associated with BMD or indirectly associated with BMD loss in postmenopausal women (13–20). However, the causal associations of endogenous adrenal-derived circulating DHEA(S) with LS-BMD and fracture risk in women is unknown.

The principles of Mendelian randomization (MR) can be applied to test the role of a circulating biomarker such as DHEA(S) in disease etiology (21). MR uses genetic data to ascertain whether a given biomarker, such as DHEAS, is implicated in disease etiology. If a biomarker is etiologically involved in a disease process, then the genetic factors that influence the biomarker will influence disease risk (22). This established technique greatly limits 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). Furthermore, recent advances in genotyping enable the application of the MR methodology in sample sizes that are not realistic for well-powered RCTs evaluating the effect of DHEA treatment on fracture risk in women.

A recent MR study evaluating the association between serum DHEAS and estimated BMD in the heel (eBMD) of women did not observe any statistically significant causal effect of DHEAS on this bone parameter (23). The possible causal associations of endogenous circulating DHEAS on LS-BMD or
fracture risk in women were however not evaluated. Furthermore, this study on eBMD in women displays several weaknesses. First, the selected genetic instruments for circulating DHEAS levels in women, were partly invalid. As a source for the genetic instruments of serum DHEAS in women, the authors used a female-specific GWAS of DHEAS that included a substantially lower number of women (n = 3,358) (24) compared with the largest available female specific DHEAS GWAS (n = 8,565) (12). Importantly, two of their selected genetic instruments were located at loci that did not show any genetic association with circulating DHEAS in women when using the conventional genome-wide significant threshold (p < 5 × 10^{-7} was selected as threshold instead of p < 5 × 10^{-8}). Furthermore, a genetic marker located in the locus of sulfotransferase 2A1 (SULT2A1), an enzyme known to be involved in the conversion of DHEA to DHEAS (5), was included as a genetic instrument. A previous study has demonstrated that DHEAS increasing alleles in this locus increase serum DHEAS via enhanced conversion of DHEA to DHEAS, reflected by an increased ratio of DHEAS to DHEA (25). While DHEA is a biologically active steroid, DHEAS is the more abundant but inactive storage form of DHEA (5). Thus, the association between genetic markers for serum DHEAS in the SULT2A1 locus and any possible effects of DHEA on bone health parameters will most likely be weak or at worst in the opposite direction of the true effect of DHEA on bone health parameters. Thus, partly invalid genetic instruments were used as exposures in the only previous female specific MR analysis of DHEAS. This most likely contributed to the heterogeneity of the MR finding for the association between DHEAS and eBMD in the heel of women and most likely diluted a possible causal effect from valid genetic instruments. Second, the associations with the eBMD outcome in that study were derived from summary statistics from a preliminary high throughput GWAS pipeline in the Neale lab (26), investigating multiple phenotypes from the UK Biobank but lacking a thorough validation procedure of each individual phenotype investigated and including only about half of the women in the UK Biobank.

Thus, treatment with DHEA increases LS-BMD in postmenopausal women while the role of endogenous adrenal-derived DHEA for LS-BMD and fracture risk in women is unknown. Therefore, using 2-sample MR analyses, we set out to determine whether endogenous DHEAS is causally associated with LS-BMD and/or fracture risk in women. For this we used valid genetic predictors of serum DHEAS derived from the largest available female specific GWAS meta-analysis (12), as well as the largest available female-specific data sets for LS-BMD and fracture outcomes.

Materials and Methods

Study design

We performed a two-sample MR study to assess the association of serum DHEAS (exposure) with BMD and fracture risk (outcomes). From the largest available genome wide association study (GWAS) of circulating DHEAS in women, we derived instrumental variables for DHEAS (12). As outcome measures, we used female-specific DXA-derived BMD GWAS summary statistics available from the GEFOS consortium (27), while data on fractures as well as estimated BMD in the heel were derived from the UK Biobank (28) using individual-level data of women of white ancestry.
Exposure

**Genetic predictors of DHEAS:** We obtained genetic predictors of endogenous DHEAS in women from the largest available female specific GWAS meta-analysis of log-transformed serum DHEAS comprising a total of 8,565 women of European origin from 6 epidemiological cohorts, with a mean age across the cohorts between 50 to 74 years and an overall mean age of 55 years (12). The female specific GWAS meta-analysis revealed one major locus on chromosome 7 consisting of 41 single nucleotide polymorphisms (SNPs) with genome-wide significance (P value < 5×10^-8). These 41 SNPs were used as candidate SNPs to be included in two alternative MR methods in the present study. A less pronounced genome-wide significant signal was also identified in the SULT2A1 locus. However, SULT2A1 is involved in the conversion of biologically active DHEA to the inactive storage form DHEAS. Thus, a polymorphism increasing the levels of DHEAS may not translate to an increase in the levels of the effector steroid DHEA. To avoid dubious associations or bias, we therefore did not use genetic signals from the SULT2A1 locus as genetic instruments in the present study.

The identified 41 GWAS significant SNPs on chromosome 7 were checked for validity as instrumental variables using individual level data from the UK Biobank, according to the following exclusion criteria: imputation information score less than 0.6; departure from the Hardy-Weinberg equilibrium at Bonferroni corrected significance; or violation of the MR assumption that the genetic variant should be unrelated to factors potentially confounding any association with the outcomes, including baseline age, smoking and physical activity at Bonferroni corrected significance. For the locus based analyses which used inverse variance weighted MR (IVW) analysis that accounts for correlation among genetic variants (29,30), we selected the genetic variants with the lowest P values having a pairwise squared correlation (r^2) less than 0.4, resulting in two remaining genetic markers (rs11761528 and rs17277546, Supplementary Table S3 and S4) (31). For the locus-based MR principal components analyses (MR-PCA) (30,32) we included all 41 genome wide significant genetic variants. Population specific correlations between variants were estimated with LDlink (33) using the 1000 Genomes Project phase 3.

**Measurements of DHEAS:** The exposure was genetically predicted log transformed serum DHEAS (µmol/L) adjusted for age and acquired from the female-specific GWAS meta-analysis by Zhai et al (12). Standardized beta values and standard errors for all presented genome-wide significant DHEAS SNPs were estimated from given P-values, allele frequencies and number of women as described by Rietveld et al (34).

Outcomes

**Lumbar spine BMD and Femoral neck BMD:** Genetic associations with lumbar spine (LS-BMD; n= 22 177) and femoral neck (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) (27). These summary statistics originate from a meta-analysis of 17 cohorts of women with a mean age across the cohorts between 33 to 76 years and an overall mean age of 59 years. GWAS genotyping was performed by each individual study following standard protocols, and imputation was then carried out on ~2.5 million SNPs from HapMap Phase 2 release 22. BMD of lumbar spine
(L1-4) (LS) and femoral neck (FN) was measured by DXA (27). The effect of each gene variant on 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 (34).

**Estimated BMD:** For eBMD in the heel and fracture outcomes we used individual-level data from women in the UK Biobank. From 2006 to 2010, the UK Biobank recruited around 500,000 individuals aged 37 to 73 years from across the United Kingdom. Participants provided biological samples, completed questionnaires, underwent assessments, had nurse led interviews and provided blood for future analysis. Follow-up using record linkage to all health service encounters and mortality data is ongoing (35). Genotyping was undertaken with two very similar arrays from Affymetrix (Santa Clara, California): the UK BiLEVE array and the UK Biobank Axiom array. Genotype imputation to a reference set combining the UK10K haplotype and the Haplotype Reference Consortium reference panels was performed (36,37). To reduce confounding by population stratification, we restricted our analysis to participants of white ancestry and excluded participants for the following reasons: withdrawn consent; sex mismatch (derived by comparing genetic and reported sex); sex chromosome aneuploidy; poor quality genotyping (missing rate >1.5%); or relatedness (unrelated samples used in UK Biobank PCA calculations were selected). Individual participant-level data of DHEAS-associated SNPs, relevant covariates (age, height and weight) as well as eBMD were available for 196,614 women. eBMD (g/cm²) was derived as a linear combination of speed of sound and bone ultrasound attenuation (eBMD = 0.0025926 × (bone ultrasound attenuation + speed of sound) − 3.687) as analysed by Quantitative ultrasound of the heel (38). 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). The standardized additive effect of each genetic variant on eBMD was estimated in a linear regression model and adjusted for age, height, weight and principal components. The UK Biobank has received ethical approval from the Northwest Multicentre Research Ethics and informed consent was obtained from all participants. The present research was approved by the UK Biobank Research and Access Committee (application number 51784).

**Fractures:** Female forearm fracture cases in the UK Biobank were identified by International Classification of Diseases (ICD) codes (ICD10, S52; ICD9, 813) in registers in combination with self-reported data. Female hip fracture cases in the UK Biobank were identified by ICD codes (ICD10, S72.0, S72.1 and S72.2; ICD9, 820) in registers. Only hip fracture cases >30 years old were included. Logistic models were used to estimate the SNP association with fracture, adjusted for age (simple and quadratic terms), height, weight and principal components. Individual participant-level data of DHEAS-associated SNPs, relevant covariates and information on forearm fractures were available for 237,572 women including 11,564 forearm fracture cases. Corresponding values for hip fractures were in total 238,565 women including 2,604 hip fracture cases.
Statistical analysis

Causal effects

The primary causal effects estimates were obtained using random effects IVW that account for correlations among genetic variants (29). IVW analysis using correlated genetic variants can be viewed as a weighted generalized linear regression of the genetic associations with the outcome on the genetic associations with the exposure, with the correlations among genetic variants incorporated into the weighting matrix. As the IVW analysis included only two genetic markers, sensitivity analysis with Egger regression to identify potential presence of directional pleiotropy was not possible (39). As an alternative MR method we performed a locus based approach using MR-PCA (explaining 99% of the genetic variance of the weighted correlation matrix) utilizing all 41 GWAS-significant genetic markers on chromosome 7 (32). IVW analysis using correlated genetic variants analyses were performed using the MendelianRandomisation package in R (version 4.1.0) (40); MR-PCA was implemented using R code from Luo et al (30).

Power calculation

Power calculations were performed for MR analysis of eBMD and fracture using the lead genetic signal for DHEAS, explaining 1.2 % of the variance in serum DHEAS in women when evaluated in an independent data set, (24). With a sample size of 22 177 we had 67 % power at $\alpha = 0.05$ to detect an effect size of 0.15 change in a two-stage least squares analysis of standardized DHEAS vs standardized LS-BMD. With a sample size of 196 614 we had 100 % power at $\alpha = 0.05$ to detect an effect size of 0.15 change in a two-stage least squares analysis of standardized DHEAS vs standardized eBMD. Equivalently we had 84% power to detect an odds ratio of 0.75 per SD increase in log DHEAS in a two-stage least squares analysis of DHEAS vs forearm fracture with a sample size of 237 572 and 11 564 cases. Similar calculations for hip fracture using a sample size of 238 565 and 2 604 cases revealed a modest power of 29 % to detect an odds ratio of 0.75 per SD increase in log DHEAS. Calculations were done using an online tool (https://shiny.cnsgenomics.com/mRnd/) (41).

Results

To determine whether circulating DHEAS is causally associated with BMD and fracture risk in women, we performed a locus based IVW MR analysis. As an alternative method we also performed a locus based MR using MR-PCA including all 41 GWAS-significant genetic markers on chromosome 7 derived from the largest available female specific GWAS meta-analysis (32). In general, the locus based IVW and MR-PCA analyses yielded very similar effect estimates for the different bone traits evaluated (figure 1-3, Supplementary Tables S1 and S2) (31).
Higher levels of DHEAS were causally associated with increased LS-BMD in women.

Genetic associations with DXA-derived BMD were obtained from female specific GWAS summary statistics available from the GEFOS consortium (n= 22,900) (27). Using random effect IVW MR, we observed that a 1 SD genetically instrumented increase in log serum DHEAS was associated with a 0.21 SD increase in LS-BMD (P value: 0.01) and a similar effect estimate was observed using the MR-PCA method (0.22 SD increase per SD increase in DHEAS; P value: 0.006) (figure 1, Supplementary Tables S1 and S3) (31). For FN-BMD the corresponding IVW estimate was 0.14 and not statistically significant; whereas the MR-PCA estimate of 0.16 did show statistical significance (P value: 0.04). These analyses of LS-BMD and FN-BMD as outcomes showed no sign of heterogeneity (Supplementary Table S1) (31).

Because we observed the most pronounced causal association with LS-BMD, a bone site with high trabecular bone content, we also evaluated the causal association between serum DHEAS and eBMD in the calcaneus which is also a bone with high trabecular bone content (42). Using female specific individual level data from the well-powered UK Biobank, we observed that a 1 SD genetically instrumented increase in log serum DHEAS was associated with a 0.08 SD increase in eBMD (P value: <0.001) and a similar effect estimate was observed using the MR-PCA method (0.10 SD increase per SD increase in DHEAS; P value: <0.001) (figure 2, Supplementary Table S1) (31). Analyses stratified for menopausal status demonstrated that DHEAS was directly causally associated with eBMD in postmenopausal women (figure 2). Although the effect estimate was only marginally less pronounced, no statistically significant causal effect of DHEAS on eBMD was observed in less powered analyses of premenopausal women (figure 2, Supplementary Table S1) (31).

Increased levels of DHEAS were associated with decreased risk of forearm fracture in women.

To determine the causal association between DHEAS and osteoporotic fractures in women, we used female specific individual level data from the UK Biobank including 11,564 forearm fracture cases and 2,604 hip fractures cases. A genetically instrumented increase in DHEAS was associated with a decreased risk of forearm fracture with an odds ratio (OR) of 0.70 per 1 SD increase of DHEAS in the IVW analysis (95% CI: 0.55 – 0.88, P value: 0.002) and an OR of 0.69 per 1 SD increase in the MR-PCA (95% CI: 0.55 – 0.87, P value: 0.002; figure 3, Supplementary Tables S2 and S4) (31). In less powered analyses no statistically significant effect of DHEAS on hip fractures was observed (figure 3).
Discussion

Although DHEA treatment studies have demonstrated that exogenous DHEA treatment increases LS-BMD in postmenopausal women (11), the role of endogenous adrenal-derived DHEA(S) for LS-BMD and fracture risk in women has been unclear. We herein report that a genetically determined increase in circulating DHEAS is causally associated with increased LS-BMD and decreased forearm fracture risk in women. Together these studies support a biologically important beneficial effect of both exogenous and endogenous DHEA(S) on bone health in women.

In the present study, we observed a robust causal association of circulating DHEAS with LS-BMD but not FN-BMD. These findings are consistent with a recent well-performed pooled-analysis of four randomized clinical trials, demonstrating that treatment with DHEA for 12 months increases LS-BMD but not FN-BMD in postmenopausal women (11). The more robust effect of both exogenous and endogenous DHEAS on LS-BMD, which is mainly dependent on trabecular bone, compared with FN-BMD, which is mainly dependent on cortical bone, indicates that DHEA(S) preferentially regulates trabecular bone mass in women. A similar more pronounced effect on LS-BMD compared with FN-BMD is also seen for estradiol and testosterone (43), the sex steroids responsible for the main downstream effects of DHEA(S) (5).

As we observed the most pronounced causal association with LS-BMD, we also evaluated the causal association of serum DHEAS with eBMD in the calcaneus, which is a bone site with a high trabecular bone content, using well-powered individual level data from the UK Biobank. A clear direct causal association between circulating DHEAS and eBMD was observed in women. In a recent MR study, using partly invalid genetic instruments derived from a modestly powered GWAS meta-analysis (24) and a preliminary dataset that used only about 50% of the UK Biobank women for the eBMD outcome, the causal association between circulating DHEAS and eBMD in women did not reach statistical significance (23). In addition, the causal associations of circulating DHEAS with LS-BMD analysed by golden standard DXA and fracture risk in women were not evaluated in that study.

Importantly, higher genetically predicted circulating DHEAS substantially reduced the risk of forearm fractures of women in the present study when evaluated using individual level data in the UK Biobank. This finding is the first causal link between DHEA(S) and fractures in women. A majority of the forearm fractures occurs in the distal forearm which is a site of predominantly trabecular bone (44,45). In contrast, in a less powered analysis we observed no causal association between DHEAS and hip fracture risk in women. It is possible that the lack of DHEA effect on FN-BMD in the DHEA treatment studies in women (11) and the lack of a robust causal association between endogenous circulating DHEAS and FN-BMD in the present study may explain why hip fracture risk, to a large extent dependent on FN-BMD (46), was not reduced by genetically increased circulating DHEAS in the present study.

We hypothesized that endogenous DHEAS levels would be of special importance for BMD in postmenopausal women, when sex steroid precursors supplied by the adrenal glands are the major source of circulating sex steroids (5,6). There was no explicit information on menopausal status in the data sets on LS-BMD and FN-BMD from the GEFOS consortium but given the mean age of 59
years, the majority of included individuals must have been postmenopausal. As we had access to individual level data and information on postmenopausal status available in the UK Biobank, we could perform analyses stratified for menopausal status, demonstrating that DHEAS was directly causally associated with eBMD in postmenopausal women. No statistically significant causal effect of DHEAS on eBMD was observed in the less powered analyses of premenopausal women. We propose that adrenal-derived DHEA(S) contributes to bone health in postmenopausal women that are lacking ovary-derived sex steroids.

This study has several strengths. We obtained genetic instruments of circulating DHEAS in women from the largest available female specific GWAS meta-analysis, ensuring solid genetic instruments. In addition, our MR analyses of the different BMD parameters and the forearm fracture outcomes were well powered to detect moderate effect sizes. We only used female-specific data sets for the selection of genetic instruments and for the outcome analyses in the MRs. Finally, we obtained very similar effect estimates for the different bone traits evaluated using the IVW and the MR-PCA methods.

Some limitations of the present study should also be emphasized. Our analyses were restricted to participants of white ancestry, and additional analyses are necessary to investigate whether our results also apply to those of other ethnicities. As the public available sex stratified GEFOS data of LS-BMD and FN-BMD do not include sub-analyses by menopausal status or age, we could not test our hypothesis regarding the importance of menopausal status on this data set. The analyses of hip fractures included relatively few fracture cases, resulting in limited statistical power in the MR. It should also be mentioned that the current analyses are based on circulating DHEAS levels, a steroid mainly believed to serve as a storage of the effector steroid DHEA. MR analysis using instrumental variables of DHEA instead of DHEAS could be informative, but no female specific GWAS of circulating DHEA is yet available. DHEA levels are however subject to diurnal variations (47), which could potentially weaken any associations, especially when relying on single measurements. Finally, valid genetic instruments for females were only available from one locus. Using the major strong genetic signals in this locus, we performed powerful locus-based IVW and principal component MR analyses that take into account all available DHEAS-related genetic information from that locus as suggested by Burgess et al (30,32,48). However, the use of a locus-based methodology makes it difficult to completely rule out horizontal pleiotropy that may cause potential bias in our MR estimates. Nevertheless, a similar pattern with a causal effect of DHEA(S) on LS-BMD but not FN-BMD, that we saw in the present study of genetically predicted circulating DHEAS, was seen in the previous clinical trials of DHEA treatment in women (11) and supports the validity of the MR findings in the present study.

In conclusion, genetically predicted serum DHEAS increases LS-BMD and decreases forearm fracture risk in women. Based on the results of the present study and previous RCTs of DHEA treatment, we propose that both endogenous adrenal-derived DHEA(S) and pharmacological DHEA treatment improve bone health in women.
Data Availability

Restrictions apply to the availability of some or all data generated or analyzed during this study to preserve patient confidentiality. Individual-level data used to derive results for eBMD and fracture associations can be obtained with an approved application to the UK Biobank. All other data used is publicly available and accessible online.
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Legends to figures

Figure 1 Estimated causal effects of DHEAS on lumbar spine BMD (LS-BMD) and femoral neck BMD (FN-BMD) in women.

Beta estimates and 95 % confidence intervals (CI) are given in SD BMD per SD increase of log DHEAS. N = number of women with BMD data. IVW = inverse variance weighted. MR-PCA = the locus-based MR method using principal component analysis.

Figure 2 Estimated causal effects of DHEAS on estimated BMD (eBMD) by ultrasound in the heel in women.

Beta estimates and 95 % confidence intervals (CI) are given in SD eBMD per SD increase of log DHEAS. N = number of women with BMD data. IVW = inverse variance weighted. MR-PCA = the locus-based MR method using principal component analysis.

Figure 3 Estimated causal effects of DHEAS on fracture risk in women.

Odds ratios (ORs) and 95 % confidence intervals are given per SD increase of log DHEAS. N total / N cases = the total number of women and the number of women with fractures, respectively. IVW = inverse variance weighted. MR-PCA = the locus-based MR method using principal component analysis.
| Group            | N   |
|------------------|-----|
| **Lumbar spine BMD** |     |
| MR-PCA           | 22,177 |
| IVW              | 22,177 |
| **Femoral neck BMD** |     |
| MR-PCA           | 22,900 |
| IVW              | 22,900 |

Change in SD of BMD per one SD increase in log DHEAS
**Figure 2**

| Group               | N     |
|---------------------|-------|
| **All women**       |       |
| IVW                 | 196,614 |
| MR-PCA              | 196,614 |
| **Premenopausal**   |       |
| IVW                 | 45,252 |
| MR-PCA              | 45,252 |
| **Postmenopausal**  |       |
| IVW                 | 120,389 |
| MR-PCA              | 120,389 |

Change in SD of eBMD per one SD increase in log DHEAS
Figure 3

| Group            | N total / N cases | OR per 1 SD increase in log DHEAS |
|------------------|-------------------|----------------------------------|
| **Forearm fractures** |                   |                                  |
| IVW              | 237 572 / 11 564  |                                  |
| MR-PCA           | 237 572 / 11 564  |                                  |
| **Hip fractures** |                   |                                  |
| IVW              | 238 565 / 2 604   |                                  |
| MR-PCA           | 238 565 / 2 604   |                                  |

0.6  0.8  1  1.2  1.4