ORIGINAL ARTICLE

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# A Mendelian Randomization Study of the Effect of Type-2 Diabetes and Glycemic Traits on Bone Mineral Density

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## ABSTRACT

Type-2 diabetes (T2D) is associated in observational studies with both higher bone mineral density (BMD) and higher fracture risk for given BMD. These relationships may however be confounded by factors such as body mass index (BMI). Here we used Mendelian randomization (MR) to obtain non-confounded estimates of the effect of T2D and glycemic traits on BMD. We identified genetic variants strongly associated with T2D risk (34,840 T2D cases and 114,981 controls) and fasting glucose (133,010 nondiabetic individuals), but not associated with BMI, and determined the effects of these variants on BMD (up to 83,894 individuals). Using these variants as instrumental variables, we found that a genetically-increased risk of T2D increased femoral neck BMD (+0.034 SD in BMD per unit increase in log-odds of T2D [95% CI, 0.001 to 0.067; $p = 0.044$]). Genetically-increased fasting glucose also increased femoral neck BMD (+0.13 SD in BMD per mmol/L increase in fasting glucose [95% CI, 0.01 to 0.25; $p = 0.034$]). Similar nonsignificant trends were observed for the effects of T2D and fasting glucose on lumbar spine BMD. Our results indicate that both genetically-increased T2D risk and genetically-increased fasting glucose have weak positive effects on BMD. © 2016 American Society for Bone and Mineral Research.

**KEY WORDS:** OSTEOPOROSIS; HUMAN ASSOCIATION STUDES; BONE MINERAL DENSITY; DIABETES

## Introduction

Osteoporosis is a common, chronic condition that is associated with high morbidity and cost.[1] Many factors are associated with osteoporosis and its most clinically important risk factor, bone mineral density (BMD), including thyroid disease, diabetes, and steroid exposure. Numerous observational studies indicate that risk factors for fracture in individuals with diabetes are distinct from those in nondiabetic individuals. Individuals with type-2 diabetes (T2D) have a higher fracture risk than those without the disease,[2] and observational studies provide evidence for both poorer bone quality and higher fracture risk for a given BMD in individuals with T2D compared to those without this condition.[3] Despite this higher fracture risk, individuals with T2D tend to have a higher BMD than nondiabetic individuals.[4]

Does T2D have a direct effect on BMD? Because of confounding in observational studies between T2D and other metabolic factors known to influence bone homeostasis,[5] the precise effects of T2D and other glycemic traits on BMD remain unclear. Addressing the problem of confounding in conventional epidemiologic study designs is challenging. The effects of a disease condition are difficult to evaluate experimentally because randomization to the disease condition is not possible. Moreover, subgroup analyses of clinical trials in which a disease exposure is modified through treatment can be difficult to interpret because of potential off-target effects of treatment. Finally, causal inference based on statistical adjustment of observational estimates to account for potential confounders has numerous inherent limitations,[6] such as remaining limited to known and properly measured confounders.

To assess for a causal relationship between T2D and BMD, we used Mendelian randomization (MR), an application of the method of instrumental variables to the analysis of genetic data.[7] MR is a study design in which genetic variants are employed as instrumental variables for estimating the non-confounded effect of an exposure (such as T2D) on an outcome (such as BMD).

In the method of MR, genetic variants are used as instruments for inferring the specific effect of an exposure (eg, T2D risk) on an outcome (eg, BMD) in the presence of confounders. In

Received in original form September 9, 2016; revised form December 2, 2016; accepted December 15, 2016. Accepted manuscript online December 16, 2016.
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Journal of Bone and Mineral Research, Vol. 32, No. 5, May 2017, pp 1072–1081
DOI: 10.1002/jbmr.3063
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two-sample MR using summary-level data,[7,8] which we undertake here, findings from separate genomewide association study (GWAS) studies for exposure, outcome, and possible confounders are assessed to identify independent genetic variants that satisfy the three key assumptions underlying the MR method[7,9]: namely, that (1) the variants are associated with the exposure; (2) they have no pleiotropic associations with confounders of the exposure-outcome relationship; and (3) they have no association with the outcome except possibly through their association with the exposure. For each variant that satisfies these conditions, the summary-level data on the effect-size and standard-error estimates are then analyzed to obtain an independent estimate of the effect of the exposure on the outcome. Finally, these estimates are then pooled using methods from meta-analysis to obtain a more reliable estimate of the effect of the exposure on the outcome.

Although common genetic variants typically have only small effects on complex diseases, the combined use of multiple variants as instruments increases the statistical power to detect associations between exposure and outcome.[8,10–12] Because MR studies make use of the random assortment of alleles at conception, their estimates are much less vulnerable to confounding than observational epidemiologic studies. Furthermore, because allele assignment at meiosis precedes the onset of disease, MR studies are not prone to reverse causation. Last, MR studies describe the effect of lifetime exposure to an allele, whereas randomized controlled trials assess the effect of an intervention applied typically for less than a decade. For these reasons, when suitable genetic variants are available, MR studies provide evidence supporting, or contradicting, a causal association between exposure and outcome.

Here, we analyzed GWAS data using MR to obtain quantitative estimates of the causal effect of T2D and related glycemic traits on femoral neck BMD (FN-BMD) and lumbar spine BMD (LS-BMD) (Fig. 1). In this approach, we analyzed GWAS data from the largest available studies to date of the genetic determinants of T2D[13] ($n = 34{,}840$ cases, 114,981 controls), fasting glucose[14] ($n = 133{,}010$ nondiabetic individuals), 2-hour glucose[14] ($n = 133{,}010$ nondiabetic individuals), and age- and weight-adjusted BMD[15] ($n = 83{,}894$ individuals). We drew from these studies a set of independent, genome-wide-significant ($p < 5 \times 10^{-8}$) genetic variants for T2D, fasting glucose (FG), and 2-hour glucose (2hGlu) to serve as instrumental variables (Fig. 2). We then analyzed summary-level GWAS data from the GIANT consortium[16] to identify and exclude variants with pleiotropic associations with BMI, a trait known to be associated with BMD. Finally, we used statistical methods to pool estimates from individual genetic variants to assess the effect of T2D, FG, and 2hGlu upon FN-BMD and LS-BMD.

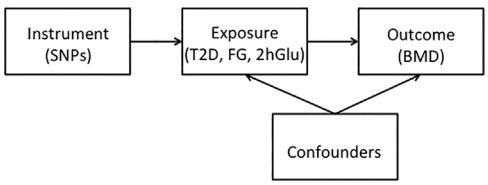


**Fig. 1.** Graph depicting the instrumental-variables model of the effect of T2D, FG, and 2hGlu on BMD. T2D = type-2 diabetes; FG = fasting glucose; 2hGlu = 2-hour glucose.

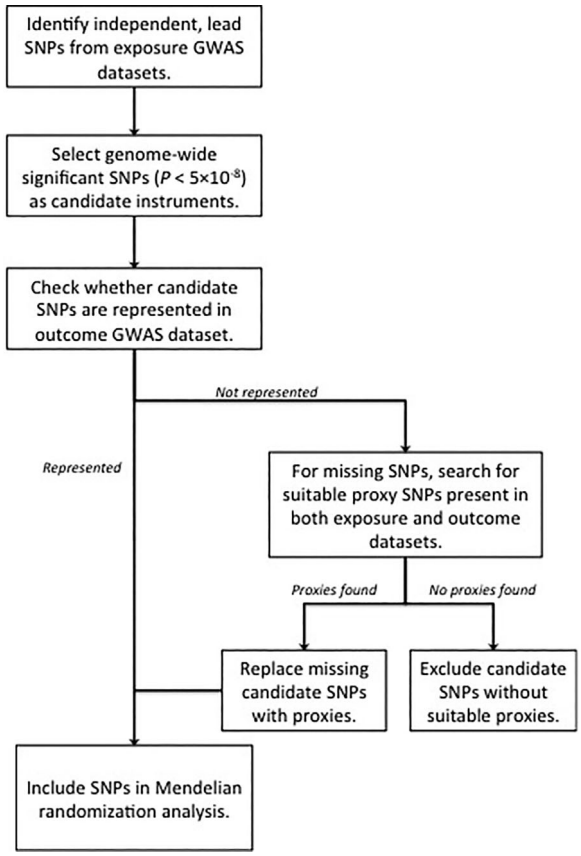


**Fig. 2.** Procedure for selecting instruments for assessing the effects of the exposures (T2D, FG, and 2hGlu) on the outcome (BMD). T2D = type-2 diabetes; FG = fasting glucose; 2hGlu = 2-hour glucose.

## Materials and Methods

### Candidate instrument selection

Our approach relies upon summary-level GWAS data to obtain MR estimates.[8,17–19] As described in the Introduction, we gathered data from large meta-analyses of GWAS examining the exposures (T2D, FG, 2hGlu) and outcomes (FN-BMD and LS-BMD) from the largest GWAS studies to date for these traits. In particular, we used the 2012 Estrada and colleagues[15] GEFOS study rather than the more recent GEFOS-Seq Study[20] of BMD. The GEFOS-Seq Study, whose goal was to identify rare genetic variants of large effect, used a comparable sample size to the previous 2012 GEFOS study but a different imputation panel (UK10K/1000GP). We chose to use the 2012 GEFOS study in our MR analysis because of its slightly larger discovery and combined sample size, as well as for ease of comparability with GWAS studies for glycemic traits, given the concordant imputation panels (HapMap). The GWAS BMD data are standardized through a regression model that adjusted for age and weight.[15] The GWAS data for T2D and glycemic traits (FG and 2hGlu) are publicly available for download, respectively, at http://diagram-consortium.org/downloads.html and at https://www.magicinvestigators.org/downloads/. The GWAS data for BMD are available at http://www.gefos.org/?q=content/data-release-2012.



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For BMI, summary-level results were drawn from the largest GWAS conducted to date for this trait.[16] Allele frequencies for variants used in our analysis were drawn from the 1000 Genomes dataset,[21] and linkage disequilibrium (LD) was calculated using the CEU LD ("Utah residents with Northern and Western European ancestry from the Centre d'Etude du Polymorphisme Humain [CEPH] collection linkage disequilibrium") structure. Linkage equilibrium of all variants was assessed using SNAP[22] applied to HapMap European samples ($R^2 \leq 0.05$). The BMI GWAS data are available for download at the GIANT consortium data files site (http://portals.broadinstitute.org/collaboration/giant/index.php/GIANT_consortium_data_files).

### Candidate instrument validation

To address the possibility that a pleiotropic effect of T2D and glycemic-trait variants on BMI could distort estimates of the effect of T2D on BMD, we identified variants with evidence of pleiotropic associations with BMI, and carried out MR analyses in which these pleiotropic variants were excluded. We considered variants with $p < 1 \times 10^{-5}$ in the confounder dataset (in our case, the BMI GWAS dataset) as significantly associated with the confounder. This choice of $p$ value threshold corresponds to the most lenient cutoff by the National Human Genome Research Institute's cutoff for archiving putative associations in its catalogue.[23,24] Variants with detectable association with BMI were removed from the analysis, and the remaining non-pleiotropic variants were taken as instruments for MR analysis. To assess the robustness of our causal estimates, we also computed the causal estimates using the much more stringent $p$ value cutoff $p < 0.05$ for pleiotropic association in the confounder dataset.

For each set of instruments, we obtained an inverse-variance weighted, pooled MR estimate ($\alpha_{pooled}$) of the effect of the exposure on the outcome. To compute this estimate, we use standard methods from meta-analysis for combining summary-level GWAS data from multiple genetic instruments.[8,12,17,18,25,26] This pooled MR estimate quantifies the effect of genetically mediated increases in the exposure (T2D and glycemic traits) on the outcome (adjusted BMD).

Assessing for heterogeneity in MR studies with multiple genetic instruments provides an approach for checking for the presence pleiotropic effects.[19] In particular, large heterogeneity suggests a failure of the instrumental-variable assumptions, for example due to pleiotropy. We quantified heterogeneity in the pooled MR estimates using the parameter $I^2$, whose point estimate and confidence intervals we obtained using standard methods.[27]

As an additional test for the presence of pleiotropy, we used the MR-Egger test,[28] a method analogous to the Egger test[29] for detecting small-study bias in meta-analyses. The MR-Egger test assesses for the presence of directional pleiotropy, a situation in which the pleiotropic effects of genetic variants on the outcome are preponderantly in one direction, rather than being balanced about the null. The method quantifies the effect of directional pleiotropy through a parameter λ, which is the constant term in a linear regression model that generalizes the standard MR analysis.[28] A value for λ significantly different from zero indicates that the genetic variants used as instruments have an effect on the outcome (FN-BMD or LS-BMD) through pathways other than the exposure (T2D or glycemic traits).

## Results

### T2D

For T2D, we used, as our initial set of instrumental variables, single nucleotide polymorphisms (SNPs) selected from the 38 genomewide significant ($p < 5 \times 10^{-8}$) SNPs associated with increased T2D risk identified in the DIAGRAM consortium, the largest meta-analysis to date of T2D GWAS studies.[13] The DIAGRAM meta-analysis included data from 34,840 T2D cases and 114,981 controls of predominantly European descent. For each of the susceptibility variants for T2D, we sought summary-level data for BMD from the GEFOS study,[15] because this is among the largest GWAS meta-analyses for BMD to date. This study profiled variants associated with FN-BMD and LS-BMD in 32,961 individuals of predominantly European descent in the discovery dataset, and 83,894 individuals including replication samples. The summary-level effect sizes reported by the GEFOS consortium were adjusted for sex, weight, and age.

In all, 37 of 38 significant T2D variants were represented in both the DIAGRAM and the GEFOS datasets (Table 1). One of the 38 T2D variants, rs11651052, was absent from the GEFOS dataset. This variant maps to the HNF1B locus, has a risk allele frequency of 0.44, and its effect size is a 0.095 increase in log-odds of T2D per allele ($p < 2 \times 10^{-11}$).[13] Moreover, no variant in close LD ($R^2 \geq 0.9$) with rs11651052 could serve as a proxy for it in our analysis. For this reason, we excluded it from further analyses. The remaining set of 37 candidate variants provided the basis for our analysis of the effect of T2D on BMD. We confirmed that these variants were not in LD ($R^2 < 0.05$). Of these 37 T2D variants, five had evidence of association with BMI ($p < 1 \times 10^{-5}$), and therefore we carried out analyses both with and without these pleiotropic variants.

Our results show a weak but positive effect of T2D on FN-BMD. In our analysis using the full set of 37 SNPs, we found that a genetically-increased risk of T2D raised FN-BMD; the estimated effect was a +0.033 SD increase in BMD per unit increase in log-odds of T2D (95% CI, 0.01 to 0.061; $p = 0.017$) (Table 2*A*). Moreover, the heterogeneity in the individual causal estimates was modest ($I^2 = 16\%$; 95% CI, 0% to 44%). In the analysis using the instrument set of 32 non-pleiotropic variants, which excluded the five variants associated with BMI, the estimated effect-size and heterogeneity were similar (+0.034 SD increase in BMD per unit increase in log-odds of T2D [95% CI, 0.001 to 0.067; $p = 0.044$]; $I^2 = 16\%$ [95% CI, 0% to 46%]) (Fig. 3, Table 2*B*).

For the effect of T2D on LS-BMD, the point estimates were similar to those for FN-BMD (+0.022 SD increase in BMD per unit increase in log-odds of T2D [95% CI, –0.01 to 0.051; $p = 0.133$], using all SNPs; and +0.026 SD increase in BMD per unit increase in log-odds of T2D [95% CI, –0.01 to 0.061; $p = 0.148$] with pleiotropic SNPs removed). Although the 95% CIs encompassed zero, the CIs largely overlapped those of FN-BMD and were consistent with the hypothesis that the effect of T2D on BMD is not strong.

We performed sensitivity analyses in which all T2D variants with $p < 0.05$ in the BMI dataset were excluded from analysis (Table 2*C*). Although CIs for the effect of T2D on FN-BMD and LS-BMD included the null, these CIs were narrow. For FN-BMD, the estimated effect was +0.01 SD increase in BMD per unit increase in log-odds of T2D (95% CI, –0.03 to 0.06; $p = 0.564$). For LS-BMD, the estimated effect was +0.003 SD increase in BMD per unit increase in log-odds of T2D (95% CI, –0.05 to



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**Table 1.** Characteristics of SNPs Considered for Use in Mendelian Randomization Analysis of the Effect of T2D on BMD risk

| SNP | Locus | Effect allele | Other allele | T2D effect (beta) | T2D *p* value | FN-BMD effect (SE) | LS-BMD effect (SE) |
|---|---|---|---|---|---|---|---|
| rs10203174 | THADA | C | T | 0.13 | 9.50E–12 | –0.022 (0.014) | –0.0164 (0.015) |
| rs10401969 | CILP2 | C | T | 0.12 | 7.00E–09 | 0.011 (0.018) | 1.00E–04 (0.019) |
| rs10811661 | CDKN2A/B | T | C | 0.17 | 3.70E–27 | –0.002 (0.010) | 0.0019 (0.011) |
| rs10830963 | MTNR1B | G | C | 0.095 | 5.30E–13 | 0.012 (0.011) | 0.0045 (0.0115) |
| rs10842994 | KLHDC5 | C | T | 0.095 | 6.10E–10 | –0.0035 (0.01) | –0.0018 (0.0107) |
| rs1111875 | HHEX/IDE | C | T | 0.10 | 2.00E–19 | –0.0061 (0.0082) | –0.0143 (0.0087) |
| rs11717195 | ADCY5 | T | C | 0.10 | 6.50E–14 | 0.030 (0.0098) | 0.0178 (0.0104) |
| rs12571751 | ZMIZ1 | A | G | 0.077 | 1.00E–10 | 0.0099 (0.0081) | 0.0061 (0.0086) |
| rs12899811 | PRC1 | G | A | 0.077 | 6.30E–09 | –0.0035 (0.009) | 0.0119 (0.0096) |
| rs12970134 | MC4R | A | G | 0.077 | 1.20E–08 | 0.0065 (0.0091) | –0.0041 (0.0097) |
| rs13389219 | GRB14 | C | T | 0.068 | 1.00E–08 | 0.013 (0.0083) | –0.0035 (0.0088) |
| rs1359790 | SPRY2 | G | A | 0.077 | 1.40E–08 | 0.016 (0.0091) | 0.0117 (0.0096) |
| rs1496653 | UBE2E2 | A | G | 0.086 | 3.60E–09 | 0.012 (0.01) | 0.0126 (0.0105) |
| rs1552224 | ARAP1 | A | C | 0.10 | 1.80E–10 | –0.0034 (0.011) | –0.0038 (0.0118) |
| rs163184 | KCNQ1 | G | T | 0.086 | 1.20E–11 | –0.0068 (0.0083) | –0.0017 (0.0088) |
| rs17168486 | DGKB | T | C | 0.10 | 5.90E–11 | 0.0037 (0.011) | 0.0059 (0.0111) |
| rs1801282 | PPARG | C | G | 0.12 | 1.10E–12 | 0.0026 (0.013) | –0.0056 (0.0136) |
| rs2075423 | PROX1 | G | T | 0.068 | 8.10E–09 | 0.0096 (0.0088) | 0.0112 (0.0092) |
| rs2261181 | HMGA2 | T | C | 0.12 | 1.20E–09 | 0.019 (0.014) | 0.004 (0.0143) |
| rs243088 | BCL11A | T | A | 0.068 | 1.80E–08 | 0.0074 (0.0083) | 5.00E–04 (0.0089) |
| rs2796441 | TLE1 | G | A | 0.068 | 5.40E–09 | 0.0049 (0.0092) | 0.0104 (0.0099) |
| rs2943640 | IRS1 | C | A | 0.095 | 2.70E–14 | –0.0095 (0.0085) | 0.0085 (0.009) |
| rs3802177 | SLC30A8 | G | A | 0.13 | 1.30E–21 | 0.0043 (0.0091) | –0.0033 (0.0097) |
| rs4402960 | IGF2BP2 | T | G | 0.12 | 2.40E–23 | 0.013 (0.0088) | 0.018 (0.0092) |
| rs4458523 | WFS1 | G | T | 0.095 | 2.00E–15 | –8.0E–04 (0.0083) | 0 (0.0087) |
| rs459193 | ANKRD55 | G | A | 0.077 | 6.00E–09 | –0.015 (0.0095) | –0.0041 (0.0101) |
| rs516946 | ANK1 | C | T | 0.086 | 2.50E–10 | 8.0E–04 (0.0095) | –0.0096 (0.0101) |
| rs5215 | KCNJ11 | C | T | 0.068 | 8.50E–10 | 0.0023 (0.0084) | –0.0012 (0.0089) |
| rs6795735 | ADAMTS9 | C | T | 0.077 | 7.40E–11 | 0.0045 (0.0082) | –0.0015 (0.0087) |
| rs6878122 | ZBED3 | G | A | 0.095 | 5.00E–11 | 0.0073 (0.009) | 0.0092 (0.0097) |
| rs7177055 | HMG20A | A | G | 0.077 | 4.60E–09 | –0.017 (0.009) | –0.006 (0.0096) |
| rs7202877 | BCAR1 | T | G | 0.11 | 3.50E–08 | –0.0029 (0.014) | 0.0036 (0.0145) |
| rs7756992 | CDKAL1 | G | A | 0.16 | 7.00E–35 | 0.0061 (0.0092) | –0.0013 (0.0097) |
| rs7903146 | TCF7L2 | T | C | 0.33 | 1.20E–139 | 0.019 (0.0091) | 0.0145 (0.0097) |
| rs7955901 | TSPAN8 | C | T | 0.068 | 6.50E–09 | 0.0057 (0.0081) | –0.0077 (0.0086) |
| rs849135 | JAZF1 | G | A | 0.10 | 3.10E–17 | 0.008 (0.0083) | 0.0137 (0.0088) |
| rs9936385 | FTO | C | T | 0.12 | 2.60E–23 | –0.0144 (0.0085) | –0.0126 (0.0091) |

FN-BMD = femoral-neck BMD; LS-BMD = lumbar-spine BMD.

0.05; $p = 0.899$), again supporting the hypothesis that the effect of T2D has only a weak effect on adjusted BMD.

## Glycemic traits in nondiabetic patients

We gathered summary data on FG and 2hGlu from the largest meta-analysis to date (carried out by the MAGIC consortium) of GWA studies ($n = 133{,}010$ nondiabetic individuals) examining the genetic architecture of glycemic traits in nondiabetic individuals.[14] For FG, 33 independent genomewide significant SNPs were selected (Table 3*A*). For 2hGlu, six independent genomewide significant SNPs were selected (Table 3*B*). All significant FG and 2hGu variants were present in the GEFOS GWAS reporting FN-BMD and LS-BMD. Thus, all were further evaluated for inclusion in our MR analysis. Of the 33 FG variants, three were also associated with BMI ($p < 1 \times 10^{-5}$). Of the six 2hGlu variants, two were also significantly associated with BMI ($p < 1 \times 10^{-5}$). Just as for T2D, we carried out analyses both including and excluding these pleiotropic variants.

Using all 33 FG variants, we found that genetically-increased FG increased FN-BMD in nondiabetic individuals (+0.13 SD increase in BMD per 1 mmol/L increase in fasting glucose [95% CI, 0.02 to 0.25; $p = 0.027$], Table 2*A*). The set of individual estimates based on FG variants had low heterogeneity ($I^2 = 24\%$; 95% CI, 0% to 51%). Using the 30 non-pleiotropic FG variants as instruments yielded similar effect and heterogeneity estimates (+0.13 SD increase in BMD per 1 mmol/L increase in fasting glucose [95% CI, 0.01 to 0.25; $p = 0.034$], $I^2 = 21\%$ [95% CI, 0% to 50%]; Fig. 4, Table 2*B*). For the effect of FG on LS-BMD, the point estimates were similar to those for FN-BMD, although the confidence intervals again included the null (+0.081 SD increase in BMD per 1 mmol/L increase in fasting glucose [95% CI, –0.044 to 0.21; $p = 0.206$], $I^2 = 31\%$ [95% CI, 0% to 55%], using all SNPs; +0.082 SD increase in BMD per 1 mmol/L increase in fasting glucose [95% CI, –0.045 to 0.21; $p = 0.211$], $I^2 = 31\%$ [95% CI, 0% to 56%], with pleiotropic SNPs removed).

For 2hGlu, although the estimates for the full set of SNPs were statistically significant, and the estimates for the non-pleiotropic



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**Table 2.** Mendelian Randomization Estimates of the Effect of T2D Risk, FG, and 2hGlu on Femoral Neck BMD

| Exposure trait | Outcome trait | Effect $\alpha_{pooled}$ (95% CI) | SE $s(\alpha_{pooled})$ | $p$ | $I^2$ % (95% CI) | SNPs ($n$) |
|---|---|---|---|---|---|---|
| (*A*) Pooled effect estimates, based on all SNPs | | | | | | |
| T2D | FN-BMD | 0.033 (0.01 to 0.061) | 0.014 | 0.017 | 16 (0–44) | 37 |
| | LS-BMD | 0.022 (–0.01 to 0.051) | 0.015 | 0.133 | 0 (0–37) | 37 |
| FG | FN-BMD | 0.13 (0.02 to 0.25) | 0.060 | 0.027 | 24 (0–51) | 33 |
| | LS-BMD | 0.081 (–0.044 to 0.21) | 0.064 | 0.206 | 31 (0–55) | 33 |
| 2hGlu | FN-BMD | 0.10 (0.02 to 0.19) | 0.043 | 0.017 | 51 (0–80) | 6 |
| | LS-BMD | 0.10 (0.01 to 0.19) | 0.046 | 0.028 | 11 (0–77) | 6 |
| (*B*) Pooled effect estimates, pleiotropic SNPs excluded ($p < 1 \times 10^{-5}$ in confounder dataset) | | | | | | |
| T2D | FN-BMD | 0.034 (0.001 to 0.067) | 0.017 | 0.044 | 16 (0–46) | 32 |
| | LS-BMD | 0.026 (–0.01– 0.061) | 0.018 | 0.148 | 0 (0–40) | 32 |
| FG | FN-BMD | 0.13 (0.01 to 0.25) | 0.062 | 0.034 | 21 (0–50) | 30 |
| | LS-BMD | 0.082 (–0.045 to 0.21) | 0.066 | 0.211 | 31 (0–56) | 30 |
| 2hGlu | FN-BMD | 0.089 (–0.027 to 0.20) | 0.059 | 0.134 | 66 (1–89) | 4 |
| | LS-BMD | 0.06 (–0.06 to 0.18) | 0.062 | 0.354 | 30 (0–75) | 4 |
| (*C*) Pooled effect estimates, nominally associated SNPs excluded ($p < 0.05$ in confounder dataset) | | | | | | |
| T2D | FN-BMD | 0.01 (–0.03 to 0.06) | 0.024 | 0.564 | 12 (0–48) | 18 |
| | LS-BMD | 0.003 (–0.05 to 0.05) | 0.025 | 0.899 | 0 (0–50) | 18 |
| FG | FN-BMD | 0.10 (–0.02 to 0.23) | 0.064 | 0.102 | 13 (0–46) | 21 |
| | LS-BMD | 0.068 (–0.07 to 0.21) | 0.072 | 0.346 | 21 (0–53) | 21 |
| 2hGlu | FN-BMD | –0.015 (–0.15 to 0.12) | 0.071 | 0.828 | 0 (0–90) | 3 |
| | LS-BMD | 0.0001 (–0.15 to 0.15) | 0.074 | 0.999 | 11 (0–91) | 3 |

The inverse-variance weighted pooled effect estimate $\alpha_{pooled}$ and its standard error $s(\alpha_{pooled})$ describe the effect of genetically mediated increases in T2D and glycemic traits on adjusted BMD. Effect sizes for T2D are reported as SD increase in BMD increase per unit increase in log-odds of T2D. Effect sizes for FG and 2hGlu are reported as SD increase in BMD per 1 mmol/L increase in glucose. We report estimates based on the full set of significant variants (*A*); the subset of variants excluding those significantly associated ($p < 1 \times 10^{-5}$) with BMI, a putative confounder (*B*); and the subset of variants excluding those nominally associated ($p < 0.05$) with BMI (*C*).

T2D = type-2 diabetes; FG = fasting glucose; 2hGlu = 2-hour glucose.

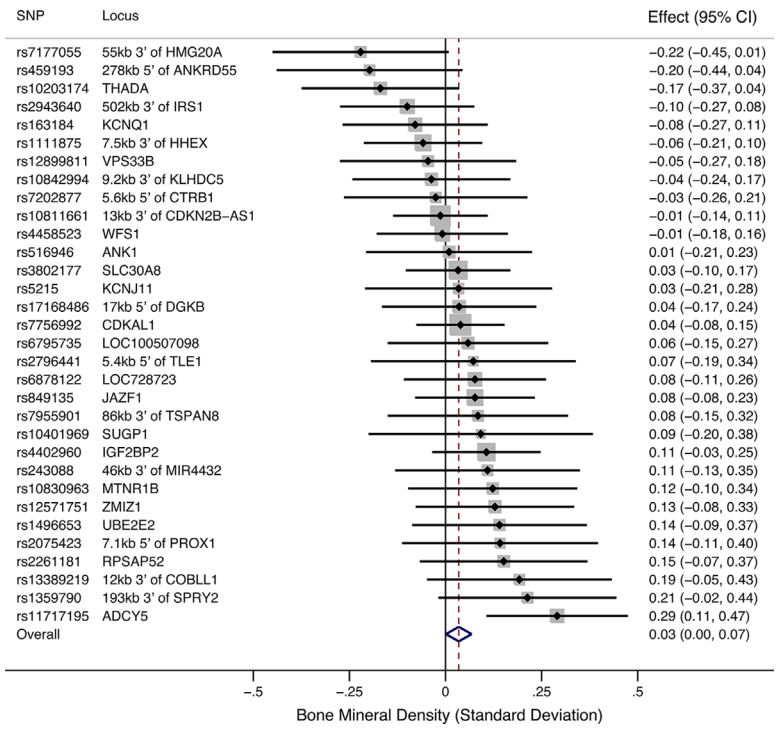


**Fig. 3.** The Mendelian randomization estimate of the effect of T2D on BMD. For each of the 32 significant non-pleiotropic T2D SNPs, the forest plot shows the estimate of the effect of genetically-increased T2D risk on femoral-neck BMD, as assessed for each SNP, the 95% confidence intervals (indicated with black lines), and the inverse-variance weights (proportional to the size of the gray squares). T2D = type-2 diabetes.



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**Table 3.** Characteristics of SNPs Considered for Use in Mendelian Randomization Analysis of the Effect of Glycemic Traits in Nondiabetic Patients on BMD

(*A*) Characteristics of FG Variants

| SNP | Lous | Effect allele | Other allele | FG effect (beta) | FG *p* value | FN-BMD effect (SE) | LS-BMD effcect (SE) |
|---|---|---|---|---|---|---|---|
| rs10747083 | P2RX2 | A | G | 0.013 | 7.57E–09 | 0.0023 (0.0092) | 0.004 (0.0096) |
| rs10811661 | CDKN2B | T | C | 0.024 | 5.65E–18 | –0.0022 (0.0104) | 0.0019 (0.011) |
| rs10814916 | GLIS3 | C | A | 0.016 | 2.26E–13 | 9.0E–04 (0.0081) | 0.0058 (0.0085) |
| rs10830963 | MTNR1B | C | G | –0.078 | 1.07E–215 | –0.0117 (0.0107) | –0.0045 (0.0115) |
| rs11039182 | MADD | T | C | 0.023 | 4.82E–22 | 0.0166 (0.0095) | –0.0079 (0.0101) |
| rs11195502 | ADRA2A | C | T | 0.032 | 1.97E–18 | 0.0406 (0.0144) | 0.0444 (0.0154) |
| rs11558471 | SLC30A8 | A | G | 0.029 | 7.80E–37 | 0.0041 (0.0091) | –0.0031 (0.0097) |
| rs11603334 | ARAP1 | G | A | 0.019 | 1.12E–11 | –0.0033 (0.0112) | –0.0032 (0.0118) |
| rs11607883 | CRY2 | G | A | 0.021 | 6.32E–24 | –0.0152 (0.0081) | –0.0023 (0.0087) |
| rs11619319 | PDX1 | A | G | –0.02 | 1.33E–15 | 0.0095 (0.0098) | 0.0064 (0.0104) |
| rs11708067 | ADCY5 | A | G | 0.023 | 1.30E–18 | 0.0265 (0.0101) | 0.0127 (0.0106) |
| rs11715915 | AMT | C | T | 0.012 | 4.90E–08 | 0.0086 (0.0095) | 0.021 (0.0101) |
| rs1280 | SLC2A2 | T | C | 0.026 | 8.56E–18 | 0.0022 (0.0126) | 0.013 (0.0133) |
| rs16913693 | IKBKAP | T | G | 0.043 | 3.51E–11 | –0.0188 (0.0256) | –0.0626 (0.0271) |
| rs174576 | FADS1 | C | A | 0.02 | 1.18E–18 | –0.0026 (0.0086) | –0.007 (0.0091) |
| rs2191349 | DGKB-TMEM195 | G | T | –0.029 | 1.28E–42 | 0.0079 (0.0081) | 0.0018 (0.0086) |
| rs2302593 | GIPR | C | G | 0.014 | 9.26E–10 | –0.011 (0.0084) | –0.0132 (0.0089) |
| rs2908289 | GCK | G | A | –0.057 | 3.32E–88 | –0.0027 (0.0108) | –0.0082 (0.0114) |
| rs340874 | PROX1 | C | T | 0.013 | 4.08E–10 | 0.0105 (0.0082) | 0.0046 (0.0087) |
| rs3783347 | WARS | G | T | 0.017 | 1.32E–10 | –0.0109 (0.0111) | –2.0E–04 (0.012) |
| rs3829109 | DNLZ | G | A | 0.017 | 1.13E–10 | 0.0073 (0.0117) | 0.0164 (0.0131) |
| rs4502156 | VPS13C-C2CD4A/B | T | C | 0.022 | 1.38E–25 | 0.0117 (0.0083) | 0.0089 (0.0089) |
| rs4869272 | PCSK1 | C | T | –0.018 | 1.02E–15 | 0.003 (0.0088) | 0.0061 (0.0092) |
| rs560887 | G6PC2 | C | T | 0.071 | 1.40E–178 | 0.0037 (0.0091) | –0.0067 (0.0097) |
| rs576674 | KL | G | A | 0.017 | 2.26E–08 | 0.0033 (0.0114) | 0.0026 (0.012) |
| rs6072275 | TOP1 | G | A | –0.016 | 1.66E–08 | –0.0143 (0.0117) | 0.0071 (0.0123) |
| rs6113722 | FOXA2 | G | A | 0.035 | 2.49E–11 | 0.0092 (0.0215) | –0.0374 (0.0225) |
| rs6943153 | GRB10 | C | T | –0.015 | 1.63E–12 | –0.0124 (0.0088) | –0.011 (0.0093) |
| rs7651090 | IGF2BP2 | A | G | –0.013 | 1.75E–08 | –0.0142 (0.0089) | –0.0188 (0.0092) |
| rs780094 | GCKR | C | T | 0.027 | 2.58E–37 | 0.0094 (0.0083) | 0.024 (0.0088) |
| rs7903146 | TCF7L2 | C | T | –0.022 | 2.71E–20 | –0.0186 (0.0091) | –0.0145 (0.0097) |
| rs9368222 | CDKAL1 | C | A | –0.014 | 1.00E–09 | –0.0073 (0.0092) | –1.0E–04 (0.0098) |
| rs983309 | PPP1R3B | G | T | –0.026 | 6.29E–15 | 0.0148 (0.0136) | 0.0034 (0.0145) |

(*B*) Characteristics of 2hGlu Variants

| SNP | Locus | Effect allele | Other allele | 2hGlu effect | 2hGlu *p* value | FN-BMD effect (SE) | LS-BMD effect (SE) |
|---|---|---|---|---|---|---|---|
| rs1019503 | ERAP2 | A | G | 0.063 | 8.87E–09 | –0.0094 (0.0081) | 0.0028 (0.0086) |
| rs11672660 | GIPR | T | C | 0.12 | 2.40E–16 | 0.0079 (0.0101) | 0.0217 (0.0107) |
| rs11717195 | ADCY5 | T | C | 0.09 | 1.87E–11 | 0.0303 (0.0098) | 0.0178 (0.0104) |
| rs11782386 | PPP1R3B | T | C | –0.099 | 2.15E–09 | –0.0052 (0.013) | 0.0178 (0.0141) |
| rs12255372 | TCF7L2 | T | G | 0.092 | 2.88E–12 | 0.0184 (0.0091) | 0.010 (0.0097) |
| rs6975024 | GCK | T | C | –0.1 | 5.25E–11 | –0.0036 (0.011) | –0.0083 (0.011) |

FG = fasting glucose; FN-BMD = femoral-neck BMD; LS-BMD = lumbar-spine BMD; 2hGlu = 2-hour postprandial glucose.

SNPs were similar to those for the full set of variants, the results for the subset of non-pleiotropic SNPs did not reach statistical significance. Using all six SNPs, we found that a genetic increase in 2hGlu increased FN-BMD in nondiabetic individuals (+0.10 SD increase in BMD per 1 mmol/L increase in 2hGlu [95% CI, 0.02 to 0.19; $p = 0.017$], Table 2*A*). The set of individual estimates based on 2hGlu variants had moderate heterogeneity ($I^2 = 51\%$; 95% CI, 0% to 80%]). Using four non-pleiotropic SNPs, we found similar results (+0.089 SD increase in BMD per 1 mmol/L increase in 2hGlu [95% CI, –0.027 to 0.20; $p = 0.134$], $I^2 = 66\%$ [95% CI, 1% to 89%]; Fig. 4, Table 2*B*).

For the effect of 2hGlu on LS-BMD, the point estimates were similar to those for FN-BMD (+0.10 SD increase in BMD per 1 mmol/L increase in 2hGlu [95% CI, 0.01 to 0.19; $p = 0.028$], using all SNPs, $I^2 = 11\%$ [95% CI, 0% to 77%]; and +0.06 SD increase in BMD per 1 mmol/L increase in 2hGlu [95% CI, –0.06 to



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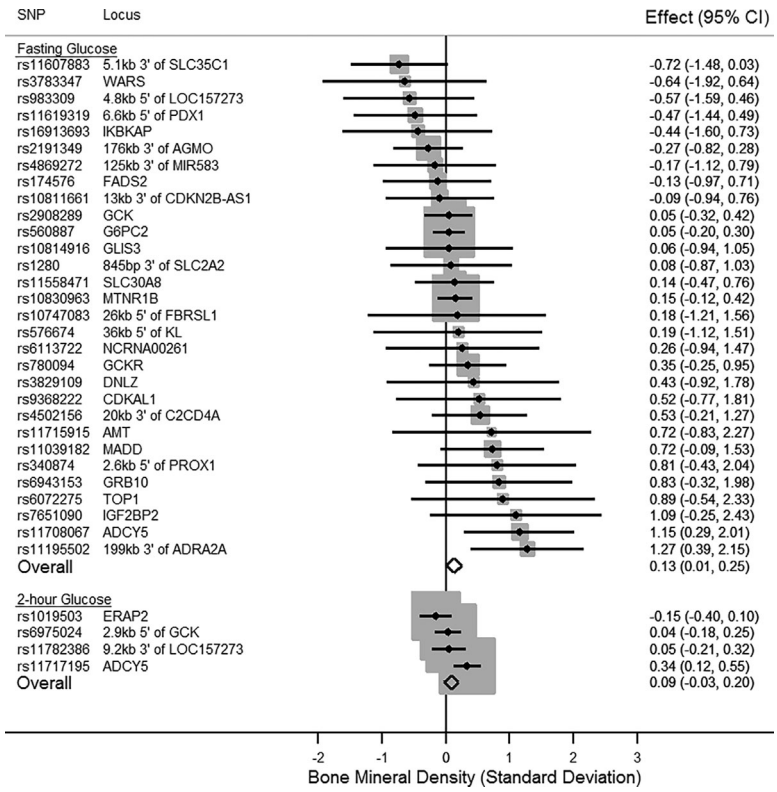


**Fig. 4.** The Mendelian randomization estimates of the effects of glycemic traits (FG and 2hGlu) on BMD. For each of the 30 significant non-pleiotropic FG SNPs and for each of the four significant non-pleiotropic 2hGlu SNPs, the forest plots show estimates of the effects of genetically-increased FG and 2hGlu on femoral neck BMD, as assessed for each SNP, the 95% confidence intervals (indicated with black lines), and the inverse-variance weights (proportional to the size of the gray squares). T2D = type-2 diabetes; FG = fasting glucose; 2hGlu = 2-hour glucose.

0.18; $p = 0.354$], $I^2 = 30\%$ [95% CI, 0% to 75%], with pleiotropic SNPs removed). Although the 2hGlu estimates for both FN-BMD and LS-BMD were not statistically significant, these estimates were based on data from only four variants.

We carried out sensitivity analyses in which all FG and 2hGlu variants with $p < 0.05$ in the BMI dataset were excluded (Table 2*C*). The direction of effect of FG on FN-BMD and LS-BMD was consistent, although the confidence intervals were wide and encompassed the null. For FN-BMD, the effect size was +0.10 SD increase in BMD per 1 mmol/L increase in FG (95%CI, –0.02 to 0.23; $p = 0.102$). For LS-BMD, the effect size was +0.068 SD increase in BMD per 1 mmol/L increase in FG (95% CI, –0.07 to 0.21; $p = 0.346$). For 2hGlu, the sensitivity analysis yielded little additional information; using $p < 0.05$ as the cutoff for pleiotropic association yielded estimates based on three variants with wide CIs that included the null (Table 2C).

### BMI and BMD

To examine the role of BMI as a possible confounder, we used standard MR to assess the effect of BMI on BMD, and used MR-Egger tests to assess for directional pleiotropy. Of the 97 genomewide significant BMI variants reported by the GIANT consortium[16] ($n = 322{,}154$ individuals), 77 were assessed in European-descent individuals, permitting comparison with the GWAS results from the DIAGRAM, MAGIC, and GEFOS. For FN-BMD, the effect estimate was +0.072 SD per unit increase in BMI (95% CI, –0.002 to 0.145; $p = 0.058$), $I^2 = 0\%$ (95% CI, 0% to 27%). For LS-BMD, the effect estimate was +0.035 SD per unit increase in BMI (95% CI, –0.044 to 0.11; $p = 0.385$), $I^2 = 23\%$ (95% CI, 0% to 43%) (Table 4). These nonsignificant results indicate that the effects of BMI on BMD have largely been removed through adjustment.

We carried out additional tests for detecting pleiotropy using the MR-Egger test,[28] a method that can provide evidence for confounding that would distort the MR results. None of the MR-Egger analyses provided evidence for statistically significant directional pleiotropy for any of the tested associations (Table 5). Although the regression analysis that underlies the MR-Egger test can also potentially provide estimates of the effect of exposure on outcome, the statistical power of this method is reduced compared to standard MR,[28] and none of these estimates were statistically significant for any of the tested associations. Overall, the findings of the MR-Egger analysis were consistent with our results suggesting that pleiotropic effects of BMI on BMD have been removed through adjustment.

## Discussion

Using multiple genetic variants for T2D, glycemic traits, and BMD from large European samples, our study found evidence that genetically-increased T2D risk and fasting glucose were associated with a small increase in FN-BMD adjusted for weight and age. We found low heterogeneity in the causal estimates obtained from multiple variants, indicating that



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**Table 4.** Mendelian Randomization Estimates of the Effect of BMI on BMD

| Exposure trait | Outcome trait | Effect $\alpha_{pooled}$ (95% CI) | SE s($\alpha_{pooled}$) | *p* | $I^2$ (95% CI) | SNPs (*n*) |
|---|---|---|---|---|---|---|
| BMI | FN-BMD | 0.072 (–0.002 to 0.145) | 0.038 | 0.058 | 0 (0–27) | 77 |
| | LS-BMD | 0.035 (–0.044 to 0.11) | 0.040 | 0.385 | 23 (0–43) | 77 |

The inverse-variance weighted pooled effect estimate $\alpha_{pooled}$ and its standard error s($\alpha_{pooled}$) describe the effect of genetically mediated increases in BMI on adjusted BMD, reported as standard deviations increase in BMD per unit increase in BMI.

**Table 5.** MR-Egger Tests for the Presence of Pleiotropy Affecting the Assessment of the Effects of the Exposures T2D, FG, and 2hGlu on the Outcomes FN-BMD and LS-BMD

| Exposure trait | Outcome trait | Effect λ (95% CI) | SE *s*(λ) | *p* | SNPs (*n*) |
|---|---|---|---|---|---|
| T2D | FN-BMD | –0.0005 (–0.008 to 0.007) | 0.0037 | 0.886 | 37 |
| | LS-BMD | –0.0013 (–0.0093 to 0.0067) | 0.0039 | 0.739 | 37 |
| FG | FN-BMD | 0.003 (–0.003 to 0.010) | 0.003 | 0.343 | 33 |
| | LS-BMD | 0.0054 (–0.0016 to 0.012) | 0.0034 | 0.126 | 33 |
| 2hGlu | FN-BMD | –0.021 (–0.076 to 0.035) | 0.020 | 0.364 | 6 |
| | LS-BMD | –0.012 (–0.07 to 0.047) | 0.021 | 0.596 | 6 |

The parameter λ (measured in units of standard deviation of BMD per allele) quantifies the effect of directional pleiotropy on BMD. A value for λ significantly different from zero indicates that the genetic variants used as instrumental variables affect the outcome through pathways other than the exposure. For each of the tested associations, the 95% CIs for λ included the null. Thus, there was no evidence of directional pleiotropy for any of the tested associations.

these associations are the direct effects of T2D and glycemic traits rather than the result of pleiotropic associations with other traits such as BMI. Furthermore, statistical tests for pleiotropy (MR-Egger tests) showed no evidence for directional pleiotropy for any of the associations we examined, providing further support that the MR analysis we have presented reliably estimates the effect of T2D and glycemic traits on adjusted FN-BMD. Similar nonsignificant trends were observed for the effects of T2D and FG on LS-BMD. Overall, the effects of T2D and FG on BMD were found to be weak.

In contrast to the weak association detected between T2D and FN-BMD, the association between T2D and LS-BMD did not reach statistical significance, raising the possibility that the effect of T2D on BMD is site-specific. A possible mechanism for site-specific effects of T2D on BMD could relate to the known disparate effects of T2D on cortical and trabecular bone[30–32] and the significant regional variation in bone microstructure throughout the skeleton.[33,34] Alternatively, measurement error for LS-BMD due to non-osteoporotic degenerative changes in the spine (such as osteophytes and degenerative disc disease)[35] or technical issues (such as positioning)[36] may have biased associations toward the null. However, it is unclear why measurement issues would disproportionately affect lumbar spine data compared to femoral neck data. Further research on mechanisms that might underlie site-specific effects of T2D on BMD and other bone properties is necessary to clarify these issues.

Although the association between 2hGlu and BMD was not statistically significant after excluding variants associated with BMI, the trend for 2hGlu suggests an effect on BMD similar to that observed for T2D and FG. Because only four of six SNPs associated with 2hGlu were available for use as instruments, power limitations may have precluded detecting an effect.

Previous studies have identified genetic variants affecting both BMD and fasting glucose.[37] For example, one such variant is found at the *ITGA1* locus, a gene previously found to influence bone healing[38] and insulin resistance.[39] Several mechanisms have been proposed to connect T2D with BMD and bone quality. Because insulin provides an anabolic signal to osteoblasts in bone,[40] insulin deficiency in type-1 diabetes leads to low bone mass, whereas elevated insulin levels in patients with T2DM might lead to higher BMD.[41] However, insulin resistance can occur in T2D, potentially impairing the physiological effects of insulin on bone. Moreover, the hyperglycemia that characterizes T2D has been suggested to impair bone quality,[2,42] perhaps by increased collagen cross-linking and increased concentrations of advanced glycation end products, which have been tied to increased fracture risk.[43]

To our knowledge, no MR study on the effect of T2D or other glycemic traits on BMD has yet been reported. Our use of multiple variants in the MR analysis increases the statistical power to detect causal associations. A key strength of our approach is that, because data on associations between exposure (T2D), outcome (BMD), and confounder (BMI) traits were generally gathered in different population samples, our approach reduces the possibility of overfitting effect-size estimates. Moreover, because we draw effect-size data from separate large-scale GWA studies for exposure and outcome traits, effect sizes are more precisely assessed than would be possible by the analysis of individual-level data from a smaller study.

Several factors could lead to bias in our estimate of the effect of genetically elevated risk of T2D and fasting glucose on BMD, including pleiotropy, population stratification, nonlinearity, and weak instrument bias. Pleiotropic effects of variants on both known and unmeasured confounders could have affected our results. To address the problem of possible pleiotropic associations between T2D, BMI, and BMD, we carried out analyses in which we excluded T2D and glycemic SNPs with significant associations with BMI. We also carried out MR-Egger tests, which yielded no evidence of directional pleiotropy. Although our MR estimates for the effects of T2D and FG on



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FN-BMD remained statistically significant after excluding BMI-associated variants, it is nevertheless possible that residual pleiotropy affected our results. Finally, because BMI may lie on the same causal pathway as T2D and glycemic traits, it is also possible that excluding BMI-associated variants may lead to less reliable estimates than including more variants, in particular if BMI acts upstream of T2D and glycemic traits.[44]

Although population stratification bias in our MR analysis is possible, this is unlikely because either genomic inflation factors for the different GWAS efforts were within acceptable limits or the reported summary statistics were corrected for genomic inflation.[13–15] Standard methods for assessing for population stratification require individual-level data.[45] However, because population stratification was indirectly measured with $I^2$, the low $I^2$ estimates indicated that both pleiotropic and stratification effects were limited.

An assumption of our instrumental-variables analysis was the linearity of the relationship between exposure and outcome. Nevertheless, this assumption might not hold because nonlinear processes, such as those due to feedback mechanisms, influence the relationship between the exposure and outcome. For example, many developmental processes display canalization,[46] in which feedback attenuates the phenotypic consequences of genetic variation. Because canalization tends to bias MR results toward the null,[46] the presence of a canalization process here was unlikely to alter the statistical significance or direction of the effects we detected through MR. Furthermore, simulation studies have shown that MR analyses generally yield population-averaged causal effect estimates even in the presence of nonlinear exposure-outcome relationships.[47]

MR estimates using multiple variants might be subject to weak instrument bias.[8,12] However, in two-sample MR studies, such as ours, such bias is toward the null.[17] Furthermore, all of our instruments were strongly associated with the exposures, greatly limiting weak instrument bias. Because our analysis showed a significant positive relationship between glycemic traits and BMD, weak instrument bias is unlikely to have distorted the direction or significance of our results.

Because genetic instruments generally represent lifelong exposures, the genetic associations tested here likely reflect the longstanding effect of hyperglycemia on BMD. Although limitations in available observational data[48] preclude a reliable epidemiologic assessment of the specific contribution of disease duration to the effect of T2D on BMD, our MR results are consistent with observational studies showing that individuals with poorly-controlled T2D, compared to individuals with well-controlled T2D or no T2D, had higher BMD.[48]

Our study does not directly address the effect of T2D and glycemic traits on fracture risk. In observational studies, individuals with poorly controlled T2D had higher fracture risk, as well as thicker femoral cortices in narrower bones.[49] Further research is required to quantify the effects of these traits on fracture risk, and to determine whether clinical prediction tools for fracture, such as Fracture Risk Assessment Tool (FRAX), should be refined to include T2D as a predictor.[50] Possible mechanisms implicated in the relationship between T2D and fracture risk include poorer bone quality in patients with T2D, resulting from decreased bone turnover, altered bone material properties, and changes to the bone microstructure in the setting of T2D-related microvascular disease.[32] Additionally, patients with T2D may have increased bone fragility due to changes to the function of osteocytes due to hyperglycemia, increased oxidative stress, accumulation of advanced glycation end-products, increased marrow adiposity, and increased circulating adipokines and inflammatory markers.[32] T2D-related complications, such as neuropathy and retinopathy, may also increase fall and fracture risk,[32] suggesting that potentially modifiable factors besides BMD may be targets for effective interventions for fracture prevention in patients with T2D.

In summary, our MR study provides evidence that genetic increases in T2D risk and fasting glucose have weak positive effects on femoral neck BMD. The effects observed in our study appear consistent with observational evidence. Our results highlight the importance of further research on the mechanisms and clinical significance of the effect of T2D on BMD.

### Disclosures

All authors state that they have no conflicts of interest.

## Acknowledgments

JBR is funded by CIHR, FRQS, CFI, and the Lady Davis Institute. AL is funded by a Canadian Diabetes Association postdoctoral research fellowship.

Authors' roles: Study design: OSA and JBR. Study conduct, data analysis, and data interpretation: OSA, AL, JA Miller, JA Morris, VF, MM, and JBR. Drafting manuscript: OSA, AL, and JBR. Revising manuscript content: OSA, AL, and JBR. Approving final version of manuscript: OSA, AL, JA Miller, JA Morris, VF, MM, and JBR. OSA takes responsibility for the integrity of the data analysis.

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