JBMR Plus, 2024, **8**, ziae126
https://doi.org/10.1093/jbmrpl/ziae126
Advance access publication: October 17, 2024
Research Article

Evaluating the relationship between glycemic control and bone fragility within the UK Biobank: observational and one-sample Mendelian randomization analyses

Samuel Ghatan[1], Fjorda Koromani[1], Katerina Trajanoska[2], Evert F.S. van Velsen[1], Maryam Kavousi[3], M. Carola Zillikens[1], Carolina Medina-Gomez[1], Ling Oei[1], Fernando Rivadeneira[1,*]

[1]Department of Internal Medicine, Erasmus Medical Center, 3015 GD, Rotterdam, The Netherlands
[2]Canada Excellence Research Chair in Genomic Medicine, Victor Philip Dahdaleh Institute of Genomic Medicine, Department of Human Genetics, Faculty of Medicine and Health Sciences, McGill University, QC H3G 2M1, Montreal, QC, Canada
[3]Department of Epidemiology, Erasmus Medical Center, 3015 GD, Rotterdam, The Netherlands
*Corresponding author: Fernando Rivadeneira, Postal address: P.O box 20403000 CA Rotterdam, NL, Street address: Dr. Molewaterplein 40, 3015 GD, Rotterdam, NL, The Netherlands (f.rivadeneira@erasmusmc.nl)

Abstract

We aimed to: (1) examine the relationship between glycemic control, BMD estimated from heel ultrasound (eBMD) and fracture risk in individuals with type 1 (T1D) and type 2 diabetes (T2D) and (2) perform a one-sample Mendelian randomization (MR) study to explore potential causal associations between glycemic control, eBMD, and fractures. This study comprised 452 131 individuals from the UK Biobank with glycated hemoglobin A1C (HbA_{1c}) and eBMD levels. At baseline, 4078 participants were diagnosed with T1D and 23 682 with T2D. HbA_{1c} was used to classify patients into "adequately-" (ACD; $n = 17\,078$; $HbA_{1c} < 7.0\%/53$ mmol/mol) and "inadequately-" (ICD; $n = 10\,682$; $HbA_{1c} \geq 7.0\%/53$ mmol/mol) controlled diabetes. In individuals with T1D, a 1% unit (11 mmol/mol) increase in HbA_{1c} levels was associated with a 12% increase in fracture risk (HR: 1.12, 95% CI [1.05-1.19]). Fracture risk was highest in individuals with T1D and ICD (HR 2.84, 95%CI [2.53, 3.19]), followed by those with ACD (HR 2.26, 95%CI [1.91, 2.69]), as compared to subjects without diabetes. Evidence for a non-linear association between HbA_{1c} and fracture risk was observed (F-test ANOVA p-value = 0.002) in individuals with T2D, with risk being increased at both low and high levels of HbA_{1c}. Fracture risk between the T2D ACD and ICD groups was not significantly different (HR: 0.97, 95%CI [0.91-1.16]), despite increased BMD. In MR analyses genetically predicted higher HbA_{1c} levels were not significantly associated with fracture risk (causal risk ratio: 1.04, 95%CI [0.95-1.14]). We did observe evidence of a non-linear causal association with eBMD (quadratic test p-value = 0.0002), indicating U-shaped relationship between HbA_{1c} and eBMD. We obtained evidence that lower HbA_{1c} levels will reduce fracture risk in patients with T1D. In individuals with T2D, lowering HbA_{1c} levels can mitigate the risk of fractures up to a threshold, beyond which the risk may begin to rise again.

Keywords: type 2 diabetes, type 1 diabetes, fractures, BMD, glycemic control, Mendelian randomization, HbA1c

Introduction

Diabetes mellitus is a common metabolic disorder characterized by elevated blood glucose levels, affecting millions of individuals worldwide. Type 1 diabetes (T1D) is an autoimmune disease resulting in the destruction of pancreatic beta cells and a subsequent lack of insulin production, typically manifesting in childhood. Type 2 diabetes (T2D), in contrast, arises from insulin resistance and relative insulin deficiency, commonly linked to obesity and lifestyle factors, and usually occurs in adults. While T1D necessitates lifelong insulin therapy, T2D is often managed through lifestyle modifications, oral medications, and sometimes insulin. While the detrimental effects of poor glycemic control in diabetes on various organ systems have been extensively investigated,[1] its impact on skeletal health remains an area of ongoing research. Previous studies have established fracture risk as a complication of diabetes.[2–4] Fractures pose significant challenges, leading to considerable morbidity, mortality, and healthcare costs.[5,6] However, understanding the complex relation between glycemic control, measured by glycated hemoglobin (HbA_{1c}) levels, and fracture risk in individuals with both T1D and T2D is crucial for implementing preventive measures and improving patient outcomes.[7] Additionally, investigating the relationship between glycemic control and BMD, a key determinant of fracture susceptibility, can offer insights into potential therapeutic strategies for mitigating the skeletal complications associated with diabetes. Glycemic control has been shown to be a key determinant of other complications of diabetes including microvascular disease, myocardial infarction, and all-cause mortality.[1] To address these knowledge gaps comprehensively, we conducted a large-scale observational study utilizing data from the UK Biobank, a well-characterized population-based resource.[8] Our study aimed to examine the relationship between glycemic control, BMD, and fracture risk in individuals with T1D and T2D; and utilize linear and non-linear Mendelian randomization (MR) to explore potential causal associations.

Received: July 21, 2024. **Accepted:** September 2, 2024

Methods

Study population

The UK Biobank is a prospective cohort study that recruited up to 502 410 participants across 22 centers in the United Kingdom. Participants were aged between 40 and 59 yr at baseline.[8] HbA_{1c} levels were measured in 482 253 individuals across 2 center visits. The initial assessment visit took place between 2006 and 2010 and encompassed 95% of the measurements taken. The second assessment visit took place between 2012 and 2013. The date of HbA_{1c} measurement was established as baseline. Individuals with prevalent fractures at baseline were excluded (fracture definition provided below). The median follow-up time was 11.72 yr during which 20 414 incident fractures occurred. The follow-up time end date was determined as the date of the last recorded fracture. Individuals who did not fracture or died before the end date were censored. The date of an individual's death was obtained via death registry (data field 40 000). A subset of 452 131 individuals also had estimated BMD (eBMD) measurements available, obtained over 3 center visits. The initial visit took place between 2006 and 2010 encompassing 90% of the measurements taken. Subsequent visits took place between 2012-2014 and 2014-2016. The MR study was performed on a subset of 379 600 individuals of European ancestry who had genotype information available.[9] The UK Biobank was conducted in full accordance with the ethical principles outlined in the Declaration of Helsinki, as revised in 2013, including adherence to protocols approved by their respective institutional ethics review committees and all participants provided written informed consent.

Diabetes definition and glycemic measures

Individuals with T2D were identified either via self-reporting at baseline (data field 130709), obtaining an HbA_{1c} level $> 6.5\%$ (48 mmol/mol) at baseline, or via a recorded International Classification of Diseases, Tenth Revision (ICD-10) code E11 (non-insulin-dependent diabetes mellitus) (data field 130708). Similarly, individuals with T1D were defined using self-reported data, as well as, the ICD-10 code E10 (Insulin-dependent diabetes mellitus). Plasma HbA_{1c} levels were measured using variant II turbo Hemoglobin Testing System; from Bio-Rad. Individuals with diabetes and HbA_{1c} levels $>7\%$ (53 mmol/mol) were considered to have inadequate glycemic control, conversely those with $<7\%$ were considered as having adequate control, according to American Diabetes Association (ADA) guidelines.[10] The duration of diabetes was determined based on either the self-reported age of diabetes diagnosis (data field 2976) or the earliest date of the corresponding ICD-10 code. In cases where self-reported ages were unavailable or entered incorrectly, they were replaced with the first date of the ICD-10 code. Pre-diabetes was defined using ADA guidelines as HbA_{1c} levels of 5.7-6.4% (39-46 mmol/mol).[11]

Fractures and BMD measurements

Estimated BMD (eBMD) was calculated from measured quantitative ultrasound speed of sound and broadband ultrasound attenuation of the heel. Measurements were collected over 3 timepoints. The data collection and quality control of eBMD measurements were conducted in accordance with the procedure outlined by the GEFOS consortium.[12] Fractures were defined according to ICD-10 codes. A full list of the codes used can be located in Supplementary Table S1. Fractures of the skull, face, hands and feet, atypical femoral fractures, pathological fractures due to malignancy, periprosthetic, and healed fracture codes were excluded. Individuals who retracted their informed consent, as of May 4, 2023, were removed.

Complications

Cardiovascular disease was defined using the ICD-10 codes I20, angina pectoris; I21, acute myocardial infarction; 122 subsequent ST elevation (STEMI) and non-ST elevation (NSTEMI) myocardial infarction; I23, certain current complications following STEMI and NSTEMI; I24, other acute ischemic heart diseases; or I25, chronic ischemic heart disease. Chronic kidney disease was defined by ICD-10 code N18; Chronic kidney disease. Diabetic retinopathy was defined by the ICD-10 codes: E08.3 - diabetes mellitus due to underlying condition with ophthalmic complications, E09.3 - drug or chemical induced diabetes mellitus with ophthalmic complications, E10.3 - T1D mellitus with ophthalmic complications, E11.3 - T2D mellitus with ophthalmic complications, E13.3 - other specified diabetes mellitus with ophthalmic complications.

Statistical analyses

Observational associations

The reporting of epidemiological results was undertaken following the STROBE and STROBE-MR guidelines. The effect of HbA_{1C} on BMD and fracture risk was estimated from linear regression and Cox proportional hazards models, respectively. Estimates were also obtained for the association between glycemic groups (ie, ACD, ICD, pre-diabetes) and outcomes in comparison to diabetes-free individuals. Additionally, to compare the fracture risk between glycemic groups hazard ratios (HR) were obtained when setting ACD as the reference group. The continuous effect of HbA_{1c} levels on eBMD and fractures was assessed in T1D, T2D, and diabetes-free sub-group analyses. Non-linear effects were also evaluated using linear tail-restricted cubic spline models with 3 knots using the "rms" package in R. The number of knots was determined by visual inspection of plots and using Akaike information criterion, to compare models with different degrees of freedom. The significance of the non-linear spline terms was evaluated using an F-test ANOVA test. All models were adjusted for: age, age^2, sex, height, weight, smoking status (data field 20116), alcohol intake (data field 1558), creatinine (23478), c-reactive protein (data field 30710), menopause (data field 2724), genetic ethnicity (data field 22006), self-reported ethnicity (data field 21000), index of Multiple Deprivation (data field 26410), and duration of moderate activity (data field 894). In Cox models the proportionality of hazards assumption was evaluated using Schoenfeld residual plots. Variables with missing information (less than 20%) were imputed using the mice package in R.[13] All observational analyses described here were adjusted for multiple testing of 2 phenotypes using Bonferroni correction.

Sensitivity analyses

For sensitivity analyses, a second higher HbA_{1c} cut-off was defined at 9% (75 mmol/mol) with those above it being classified as having adequate control and vice versa. This cut-off was motivated by papers showing increased fracture risk at higher HbA_{1c} cut-offs.[14,15] Additionally, to investigate the association between low HbA_{1c} and fracture risk, we further

split those individuals with T2D and ACD by an HbA_{1c} level of below 5.4% (36 mmol/mol) (the mean of the sample) and labeled these individuals as hypoglycemic. The risk of fractures in individuals with pre-diabetes was also evaluated. The effect of diabetes duration of fracture risk was also assessed in individuals with T2D and available duration data ($n = 19\,263$) by including diabetes duration as a covariate in the Cox model. Additionally, disease duration was categorized into 3 groups: less than 5 yr, more than 5 yr less but than 10 yr, and 10 yr and greater. Effect estimates were obtained using Cox regression models with the group of less than 5 yr as the reference category. Lastly, to assess the potential effect diabetic complications and comorbidities on fracture risk and HbA1c levels we adjusted our statistical models for hemoglobin concentration (data field 30020), as a measure of anemia, chronic kidney disease, cardiovascular disease, and diabetic retinopathy, using the ICD codes as reported in the previous section of Complications.

Selection of genetic instrumental variables

We selected 88 conditionally independent genetic variants as instrumental variables for HbA_{1c} levels (Supplementary Table S2) based on their association with HbA_{1c} ($p < 5 \times 10^{-8}$) in a genome-wide association study of 191 362 individuals of European ancestry.[16] Palindromic SNPs were removed and replaced by linkage disequilibrium proxies ($r^2 > 0.80$). Standardized genetic risk scores (GRS) were constructed using the 88 genetic variants for individuals with available genetic data. To test the instrument strength, linear regression models, adjusted for age at baseline, sex, and 10 genetic principal components of ancestry, were constructed. The GRS explained 3% of the variance of HbA_{1c} within the UK Biobank population and displayed an F statistic of 1068.

Linear MR

For a genetic variant to act as a valid instrumental variable in an MR analysis it had to satisfy the following assumptions: (1) relevance: reliably associated with each of the exposures included in the model; (2) exclusion restriction: the variant needs to be associated with the outcome only through the exposure of interest; and (3) independence: the variant should be conditionally independent of the outcome given the exposure and confounding factors.[17] To obtain linear causal inferences, we applied a 2-stage least squares regression (2SLS) for continuous outcomes and structural mean models for binary outcomes.[18,19] Both regression stages were adjusted by age, sex, and the first 10 principal components of genetic ancestry. A sensitivity analysis was performed by removing related individuals (kingship coefficient < 0.0884) and recalculating regression estimates.[20] MR power calculations were derived using the mRnd web tool.[21]

Non-linear MR

To assess non-linear causal effects we sought to stratify individuals and calculate localized average causal effects (LACE) using the ratio method (instrumental variable outcome association divided by the instrumental variable exposure association).[22] To stratify individuals we adopted the doubly-ranked method,[22] which overcomes an important limitation of the residual method, namely that it assumes that the effect of the genetic instrument on the exposure is constant and linear. Briefly, the doubly-ranked method is a non-parametric approach for exposure stratification, such that, the stratification is not a function of the instrumental variables and each stratum has a different average level of the exposure. This involves the initial ranking of individuals based on their level of the instrumental variables, which are then divided into pre-strata. After this, the individuals within each pre-stratum are ranked based on their level of exposure and further divided into strata. Additional test statistics for non-linearity were calculated using the "SUMnlmr" R package.[23] Next, we obtained causal inferences on the shape of the exposure-outcome relationship using fractional polynomials.[24] These polynomials represent the stratification of individuals into either 10 strata or 100 strata. Non-linearity tests included: the fractional polynomial degree test, the fractional polynomial non-linearity test, the quadratic test and Cochran's Q-test. The fractional polynomial degree test was used to indicate the polynomial degree preference, with a low p-value indicating preference of a degree 2 polynomial. The fractional polynomial non-linearity tests the best-fitting fractional polynomial of a degree 1 against the linear model, with a low p-value indicating a preference of fractional polynomials of degree 1. The quadratic test meta regresses LACE estimates against the mean value of the exposure in each stratum, with a lower p-value indicating non-linearity. Lastly, the Cochran's Q-test, tests also whether the LACE estimates differ more than expected by chance, with a lower p-value indicating non-linearity.[23,24]

Results

Population characteristics

This study comprised 452 131 individuals from the UK Biobank cohort study. At baseline, 4078 (0.9%) individuals had T1D and 23 682 (5.2%) had T2D. A third group comprised of individuals without diabetes ($n = 424\,371$) with a median glycated hemoglobin levels (HbA_{1c}) level of 5.4%. A median of HbA_{1c} level of 7.4% was observed in individuals with T1D and of 6.6% in those with T2D. Individuals with each type of diabetes were stratified into 2 groups: $HbA_{1c} \geq 7.0\%/53$ mmol/mol defined as inadequately controlled diabetes (ICD; $n = 10\,682$); representing 62.9% of those with T1D ($n = 2565$) and 34.3% of those with T2D ($n = 8117$); and those with $HbA_{1c} < 7.0\%/53$ mmol/mol, defined as adequately controlled diabetes (ACD; $n = 17\,078$). The median follow-up time was 11.7 yr period during which 20 414 incident fractures occurred. Fractures consisted of 2376 (12%) of the hip, 1913 (10%) of the vertebra, 5254 of the forearms (26%), 2664 of the ankles (14%). Other fracture sites include the upper arm, pelvis, leg, patella, rib, scapula, sternum, and clavicle (Supplementary Table S1). The mean age of the participants was 58 yr old (SD = 8) with similar proportion of men and women (Table 1). In individuals with T2D the mean disease duration was 5.5 yr (SD = 5.2) before inclusion at baseline. Estimated BMD (eBMD), derived via heel ultrasound, was measured in all individuals.

Glycemic control and eBMD

In individuals with T1D, HbA_{1c} levels were not significantly associated with eBMD (beta = 0.01SD, 95%CI [−0.03, 0.02]) (Supplementary Table S5), although there was weak evidence suggesting a non-linear U-shaped relationship (F-test ANOVA p-value = 0.08) (Figure 1a). In T1D, the ACD (beta = −0.03

Table 1. Participant baseline characteristics.

		Women	Men
N		247 712	204 419
Age (yr)		58 (50-63)	58 (50-64)
Height (cm)		162 (158-167)	176 (171-180)
Weight (kg)		69 (61.6-78.4)	84 (76-93)
eBMD (g/cm^2)		0.51 (0.43-0.59)	0.56 (0.48-0.64)
HbA1c (%)		5.4 (5.2-5.6)	5.4 (5.2-5.6)
Type 2 diabetes, *n*(%)		9417 (3.8%)	14 265 (7.0%)
Type 1 diabetes, *n*(%)		1752 (0.7%)	2326 (1.1%)
ACD (<7%/53 mmol/mol), *n*(%)		7057 (2.8%)	10 021(5.0%)
ICD (>7%/53 mmol/mol), *n*(%)		4112 (1.7%)	6570 (3.2%)
Incident fractures, *n*(%)		13 636 (5.5%)	6778 (3.3%)
Moderate activity (min/d)		60 (30-66)	60 (30-66)
Creatinine (umol/L)		63.6 (57.3-71.2)	79.3 (72.2-87.8)
CRP (mg/L)		1.47 (0.67-2.83)	1.34 (0.68-2.56)
Deprivation[a]		15.2 (8.16-20.67)	15.59 (8.27-21.35)
Menopause	Yes	150 586	0
	No	58 105	0
	Not sure (had a hysterectomy)	28 215	0
	Not sure (other reason)	10 565	0
Smoking	Current	22 129	25 368
	Previous	78 245	78 755
	Never	147 338	100 296
Alcohol intake	Daily	40 165	52 273
	3-4 times a week	51 272	53 968
	1-2 a week	64 199	53 036
	1-3 a month	32 311	18 063
	Occasionally	36 740	14 595
	Never	23 025	12 484

Values depict medians and interquartile-ranges. Abbreviations: ACD, adequately controlled diabetes; CRP, C-reactive protein; eBMD, estimated BMD; ICD, inadequately controlled diabetes. [a]Deprivation refers to the English indices of deprivation. A composite measure of social economic status.

SD, 95%CI [−0.09, 0.03]) and ICD (beta = −0.08 SD, 95%CI [−0.12, −0.03]) groups displayed lower eBMD with respect to HbA_{1c} levels, in comparison to diabetes-free individuals (Figure 2a). In individuals with T2D, a 1% (11 mmol/mol) increase in HbA_{1c} levels was associated with a 0.01 SD eBMD increase (95%CI [0.00-0.02]) (Supplementary Table S5). No evidence of a non-linear association between HbA_{1c} and eBMD was observed for T2D in sub-group analyses (F-test ANOVA p-value = 0.72) (Figure 1b). In the T2D analysis, stratification by glycemic control yielded concordant results with a positive association between HbA_{1c} and eBMD in the ACD (beta = 0.05SD, 95%CI [0.03, 0.07]) and ICD (beta = 0.09SD, 95%CI [0.06, 0.11]) groups. Individuals with ICD had increased eBMD in comparison to those with ACD (beta = 0.04, 95%CI [0.01, 0.07]). In diabetes-free individuals, HbA_{1c} levels were negatively associated to eBMD (beta = −0.05SD, 95%CI [−0.06, −0.04]). In addition, evidence for a non-linear association between HbA_{1c} and eBMD was also observed (F-test ANOVA p-value = 0.02) (Figure 1c).

Glycemic control and fracture risk

A 1% (11 mmol/mol) increase in HbA_{1c} levels was associated with a 12% increase in fracture risk (HR: 1.12, 95%CI [1.04-1.19]) in individuals with T1D (Figure 1d, Supplementary Table S3). Risk estimates were similar between men and women (Supplementary Table S4). In the T1D sub-group analysis Cox regression Schoenfeld residual plots indicated a violation of the proportionality of hazards assumption for age as a covariate. However, upon conducting analyses stratified by age, with groups delineated as <38-50, <50-55, <55-60, <60-65, and <65-81 yr, we found that the HR adhered to the proportionality assumption (Supplementary Figure S1). Subsequently, associations between glycemic control groups and fracture risk were evaluated. As compared to individuals without diabetes, those with T1D had increased fracture risk in both the ACD (HR 2.26, 95%CI [1.86, 2.66]) and ICD (HR 2.84, 95%CI [2.53, 3.26]) groups (Figure 2b). Furthermore, individuals with ICD had a higher fracture risk than those with ACD (1.27, 95%CI [1.01, 1.53]), when tested using a linear regression with ACD as the reference group.

Fracture risk in individuals with T2D showed evidence for a U-shaped non-linear association with increased risk (F-test ANOVA p-value = 0.002) observed at both low and high levels of HbA_{1c} (Figure 2e). A similar non-linear association was observed in diabetes-free individuals (F-test ANOVA p-value <0.001), although attenuated within healthy HbA_{1c} levels (Figure 2f). Duration of diabetes was found to be associated to fracture risk (Supplementary Figure S2). However, when included in the T2D Cox model no modification of the effect of HbA_{1c} on fracture risk was observed. Fracture risk was increased in T2D ACD (1.19, 95%CI [1.10, 1.29]) and ICD (1.23, 95%CI [1.10, 1.36]) groups, in comparison to diabetes-free individuals (Figure 2b). However, individuals with ICD did not have an increased fracture risk in comparison to those with ACD (0.97, 95%CI [0.90, 1.04]). Dichotomizing on an HbA_{1c} level of 9% produced similar observations (Supplementary Figure S3, Supplementary Table S3). We further split individuals with T2D and ACD with an HbA_{1c} level of lower than 5.4% and labeled this group as hypoglycemic (n = 1401). Individuals within the hypoglycemic group had the greatest

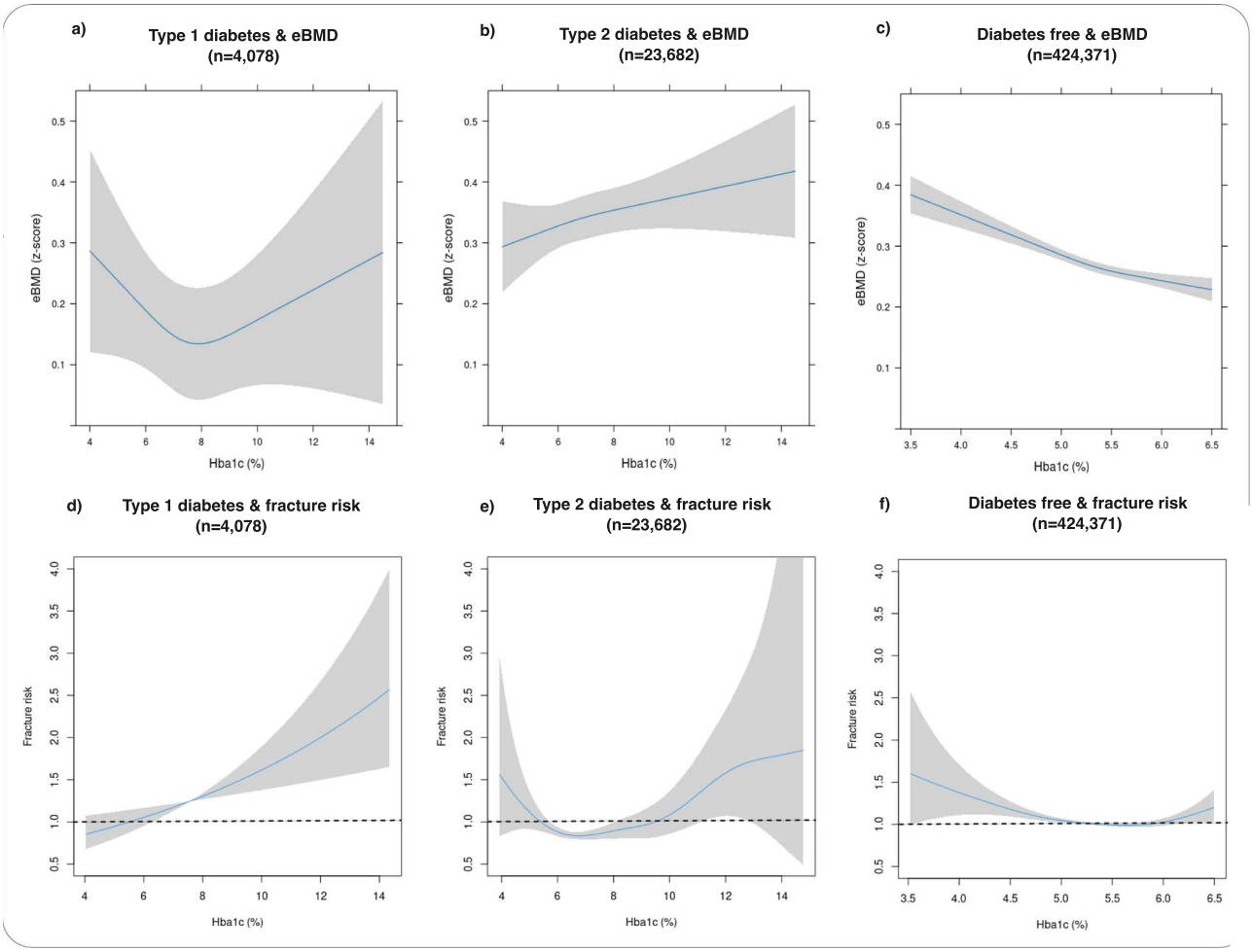

Figure 1. Associations between HbA1c and eBMD (a-c)/fracture risk (d-f) by diabetes status and type. Reference value was set to 5.4% (36 mmol/mol) for all analysis groups. The shaded area represents the 95% CI. For eBMD plots the covariates were set to the mean values. For example, for type 2 diabetes the covariates were: age = 61, sex = male, weight = 87.9, height = 169, alcohol intake = 1-2 per week. Creatinine = 72.18 CRP = 2.1, deprivation score = 17.42, ethnicity = white European, duration of moderate activity = 60, non-smoker. Abbreviations: CRP, C-reactive protein; eBMD, estimated BMD.

fracture risk (1.49, 95%CI [1.16, 1.83]) (Supplementary Figure S4). Additionally, individuals with pre-diabetes did not present higher fracture risk (HR: 0.99, 95%CI [0.95-1.04]; $n = 61\,951$) in comparison to controls.

One-sample MR

One-sample MR analyses revealed no significant evidence of a linear or a non-linear association between genetically predicted HbA_{1c} levels and fracture risk (power = 0.94) (Supplementary Figure S5, Supplementary Tables S6 and S7). With regards to eBMD, we observed significant evidence of a negative linear association between genetically predicted HbA_{1c} levels and eBMD, in diabetes-free (beta = −0.09, 95%CI [−0.12, −0.05]) individuals and in the total population (beta = −0.08, 95%CI [−0.11, −0.05]) (Supplementary Figure S6a, Supplementary Table S8). We observed no significant difference in effect estimates when related individuals were removed (Supplementary Table S8). Non-linear MR revealed strong non-linear relationship between HbA_{1c} and eBMD in the overall population. The quadratic test yielded a p-value of 0.0002. This was supported by evidence from the fractional polynomial test (p-value = 0.009). Additional, evidence suggested that best-fitting fractional polynomial of degree 2 fitted the data better than the best-fitting fractional polynomial of degree 1 (p-value = 0.02). The best-fitting fractional polynomial of degree 2 had powers 1 and -1. The Cochran Q test was also significant (p-value = 0.001). Graphs and strata estimates indicated a negative slope from HbA_{1c} levels between 4 and 6.5%, after which the slope becomes positive (Supplementary Figure S6b, Supplementary Tables S9–S11). We tested whether this relationship changed when controlling for various complications and found no evidence of a change (Supplementary Table S12). Yet, these analyses were underpowered (see Supplementary Tables S6 and S8 for power calculations).

Discussion

We confirm an increased fracture risk in individuals with T1D and T2D from a relatively healthy young cohort.[25] Furthermore, we observed a linear association between HbA_{1c} and an increased fracture risk in individuals with T1D and a non-linear association in individuals with T2D. Individuals with T1D and ICD (>7%/53 mmol/mol) had greater fracture risk, than those with ACD (<7%/53 mmol/mol). In T2D, fracture risk did not differ between ACD and ICD groups even after increasing the HbA_{1c} dichotomization cut-off to 9% (75 mmol/mol). This lack of change in fracture risk was

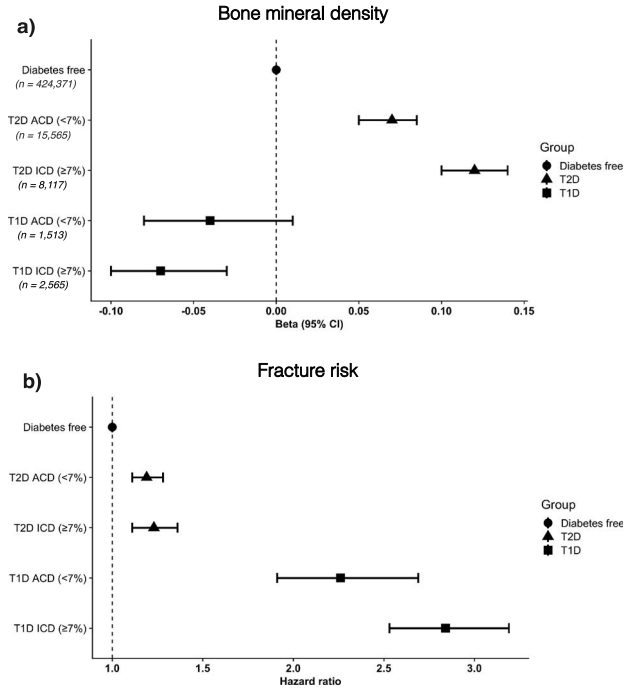

Figure 2. (a) Forest plot depicting associations between eBMD and glycemic control groups in comparison to controls. (b) Forest plot depicting fracture risk ratios of glycemic control groups in comparison to controls. Abbreviations: ACD, adequately controlled diabetes; eBMD, estimated BMD; ICD, inadequately controlled diabetes .

observed despite higher BMD levels in individuals with ICD. However, individuals with T2D and an HbA_{1c} level below 5.4% (36 mmol/mol) had the greatest fracture risk. In T1D, we observed evidence that lowering HbA_{1c} levels will reduce fracture risk. In T2D, we observed evidence that both higher and lower HbA_{1c} levels were associated with increased fracture risk. Our work brings to perspective the results of some previous studies[15,26–29] indicating that individuals with T2D and ACD present lower or similar fracture risk to that of diabetes-free individuals. Leveraging genetic information, we observed no association between genetically predicted higher HbA_{1c} levels and fracture risk. However, we did observe evidence of a non-linear association between genetically predicted HbA_{1c} levels and eBMD in the total population. We observed a negative association between HbA_{1c} and eBMD between 4.0 and 6.5% (20-48 mmol/mol), after which the association to eBMD became positive. These results are concordant with our findings in the observational analysis and provide evidence of a causal non-linear relationship.

Previous studies have suggested that the increased fracture risk in T2D is largely mediated by poor glycemic control.[15,26–29] While individuals with T2D and ICD showed a higher fracture risk compared to those without diabetes, the ACD group also had an increased fracture risk. No difference between ICD and ACD groups regarding fracture risk was observed, even after increasing the cut-off to 9% (75 mmol/mol). Paradoxically, ICD was associated with higher eBMD, but this did not beget an expected decrease in fracture risk, a well-established phenomenon observed in individuals with T2D.[2] Many of the studies reporting a significant relationship between poor glycemic control and fracture risk report varying cut-offs of HbA_{1c} levels for defining adequate/inadequate glycemic control. Clinicians often utilize cut-offs or threshold values to assist in the process of making decisions. However, their effectiveness is a subject of debate.[30] Cut-offs are problematic because they typically correspond to the specific population being studied and thus seldom produce consistent outcomes when applied to separate studies or datasets. Additionally, employing cut-offs to categorize a continuous predictor can potentially hinder accurate risk prediction.[31] Our results describe a U-shaped relationship between HbA_{1c} and fracture risk in individuals with T2D and diabetes-free individuals. Similarly, a large retrospective study of 652 901 elderly male veterans with T2D observed that fracture risk was not increased in individuals with $HbA_{1c} > 8.5\%$ (69 mmol/mol), but was increased in individuals with $HbA_{1c} < 6.5\%$ (48 mmol/mol).[32] Individuals with low levels of HbA_{1c} can be considered as hypoglycemic.[33] Evidence suggests that hypoglycemia can lead to a loss of balance and an increase in falls, which can subsequently increase their fracture risk.[34] While this explanation seems plausible, it is important to state that the ADA suggests that HbA_{1c} "does not provide a measure of glycemic variability or hypoglycemia".[31] Alternatively, the observed association with increased fracture risk in individuals with very low HbA_{1c} levels could be confounded by the use of glucose-lowering medication. However, this would not explain the increased risk in diabetes-free individuals. Individuals with anemia or other conditions shortening the lifespan of red blood cells (ie, glucose-6-phosphate dehydrogenase deficiency, sickle-cell disease, etc.), may have underestimated HbA_{1c} levels.[35] Considering the association between anemia and heightened risk of fractures,[36] we incorporated measured hemoglobin concentrations into our statistical models to evaluate their impact. The inclusion of hemoglobin markedly enhanced the model's explanatory power, as evidenced by a significant F-test ANOVA (p-value <2x10^-16). Nevertheless, the estimated effects of HbA_{1c} were not statistically different from those in the model that excluded hemoglobin concentrations (Supplementary Table S12). In T1D, poor glycemic control was linearly associated with increased fracture risk. No significant differences in eBMD were observed across glycemic control groups. This could be a consequence of non-linear effects (for which we observed weak evidence) and/or low statistical power. In line with our findings, a nested case–control study in the United Kingdom reported no differences in fracture risk in relation to glycemic control among individuals with T2D. However, similar to our study, they did observe a difference in fracture risk for those with T1D.[37] Other studies also report increased fracture risk in T1D individuals with poor glycemic control.[14,38] Nevertheless, these studies reported different magnitude of effects likely due to differences in underlying cohort populations. Differences in the relationship between HbA_{1c} and bone fragility across the different types of diabetes are expected. This can be a consequence of the distinct pathological mechanisms, age of disease onset and duration.[39,40] Diabetes disease duration has been shown to affect both fracture risk and glycemic control in T1D and T2D.[14] However, when including duration as a covariate in our analysis, we observed no modification of the association between HbA_{1c} and fracture risk. Emanuelsson et al. published a one-sample MR study that tested the association between glucose concentrations and fragility fractures in the UK Biobank and 2 studies from Copenhagen.[41] They reported a causal association between high non-fasting glucose concentrations and increased risk of arm fracture in the Copenhagen studies and UK Biobank combined (RR 1.41 [1.11, 1.79], p=0.004), with

similar results for fasting glucose and HbA_{1c} in 2-sample MR analyses (ORs 1.50 [1.03, 2.18], p=0.03; and 2.79 [1.12, 6.93], p=0.03, respectively). Mendelian randomization estimates for any fragility fracture did not reach statistical significance (UK Biobank OR 1.11 [0.91, 1.34], p=0.31). The results of the Emanuelsson study are consistent with the results we report here. Reporting no significant causal association between HbA_{1c} concentrations and all fracture types. However, the Emanuelsson study has increased power from the addition of the Copenhagen studies which combined have a higher prevalence of fractures (13.5%) than the UK Biobank (3.8%).

Our study has some limitations inherent to observational studies. Despite the large sample size, the low prevalence of incident fractures in the study population means statistical power is still limited for the one-sample MR analyses of fracture risk. Power calculations for diabetes-specific stratified MR analyses suggest that a sample size of 218 351 individuals with diabetes is needed to achieve 80% power (Supplementary Table S6). We were unable to account fully for the consequences of diabetes disease duration and inadequate control in this study. Yet, controlling for diverse diabetes complications like diabetic retinopathy, kidney disease, and microvascular disease showed consistent effect estimates (Supplementary Table S12). Furthermore, we employed HbA_{1c} which was only measured at baseline and therefore was not reflective of long-term glycemic control. Additionally, the UK Biobank has been reported to suffer from "healthy volunteer bias".[25] As such, longitudinal studies with repeated measurements of HbA_{1c}, a detailed assessment of diabetes duration, and evaluation of DXA-derived BMD change over time will provide a more comprehensive understanding of these associations. Additionally, prospective studies should consider examining other factors such as glycemic variability, hypoglycemic events, previous fractures and the effects of glucose-lowering medications on fracture risk. Lastly, the non-linear MR method has a number of limitations.[42]

We observed distinct relationships between HbA_{1c} and bone fragility in T1D and T2D and postulate that this is due to the underlying differences in disease pathology, including the anabolic effect of insulin on bone. We advise against the use of hard cut-offs for defining adequate/inadequate glycemic control as these can vary across the populations being studied. We obtained evidence that lower HbA_{1c} levels will reduce fracture risk in patients with T1D. In individuals with T2D, lowering HbA_{1c} levels can mitigate the risk of fractures up to a threshold, beyond which the risk may begin to rise once more. Our study contributes to the growing body of evidence on the relationship between glycemic control, fractures, and bone health, highlighting the need for individualized management strategies in individuals with diabetes to optimize skeletal outcomes and reduce fracture risk. Furthermore, longitudinal studies with repeated measurements are needed to identify the key determinants of fracture risk in T2D.

Acknowledgements

John Kemp for providing assistance in coding and quality control of the estimated bone mineral density data. This research has been conducted using the UK Biobank Resource (accession ID: 67864). We express our gratitude to the study participants, whose participation made this work possible, and to the numerous colleagues who contributed to the collection, phenotypic characterization of clinical samples, as well as genotyping and analysis of GWAS data. S.G. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Authors' contributions

Samuel Ghatan (Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Writing—original draft, Writing—review & editing), Fjorda Koromani (Investigation, Methodology, Supervision, Writing—review & editing), Katerina Trajanoska (Writing—review & editing), Evert van Velsen (Writing—review & editing), Maryam Kavousi (Writing—review & editing), M. Carola Zillikens (Writing—review & editing), Carolina Medina-Gomez (Writing—review & editing), Ling Oei (Conceptualization, Methodology, Project administration, Supervision, Writing—original draft, Writing—review & editing), and Fernando Rivadeneira (Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing—original draft, Writing—review & editing). S. Ghatan, L. Oei, F. Rivadeneira, designed the study. S. Ghatan performed the analysis. S. Ghatan, L.Oei, F. Rivadeneira, C. Medina-Gomez, F. Koromani, and K. Trajanoska drafted the manuscript. All authors contributed to the interpretation of data and critical revision of the manuscript. All authors read and approved the final version of the manuscript. Equal contribution: Ling Oei and Fernando Rivadeneira.

Supplementary material

Supplementary material is available at *JBMR Plus* online.

Funding

This project has received funding from the European Union's Horizon 2020 research and innovation program under the MARIE SKŁODOWSKA-CURIE grant agreement no. 860898.

Conflicts of interest

None declared.

Data availability

HbA1C summary statistics can be obtained from MAGIC consortium website (https://magicinvestigators.org/downloads/). All data generated or analyzed during this study are included in this published article [and its supplementary information files].

Originality and prior publication

Parts of the results from this manuscript have been presented previously at the European Calcified Tissue Society (ECTS) 2023 congress in Liverpool, England on 16th of April.

Ethics approval and consent to participate

All studies were conducted in full accordance with the ethical principles outlined in the Declaration of Helsinki, as revised in 2013, including adherence to protocols approved by their respective institutional ethics review committees and all participants provided written informed consent.

References

1. Holman RR, Paul SK, Bethel MA, Matthews DR, Neil HAW. 10-year follow-up of intensive glucose control in type 2 diabetes.

N Engl J Med. 2008;359(15):1577–1589. https://doi.org/10.1056/NEJMoa0806470
2. Hofbauer LC, Busse B, Eastell R, et al. Bone fragility in diabetes: novel concepts and clinical implications. *Lancet Diabetes Endocrinol*. 2022;10(3):207–220. https://doi.org/10.1016/S2213-8587(21)00347-8
3. Vilaca T, Schini M, Harnan S, et al. The risk of hip and non-vertebral fractures in type 1 and type 2 diabetes: a systematic review and meta-analysis update. *Bone*. 2020;137:115457. https://doi.org/10.1016/j.bone.2020.115457
4. Kvist AV, Nasser MI, Vestergaard P, Frost M, Burden AM. Site-specific fracture incidence rates among patients with type 1 diabetes, type 2 diabetes, or without diabetes in Denmark (1997–2017). *Diabetes Care*. 2023;46(3):633–642. https://doi.org/10.2337/dc22-1004
5. Johnell O. The socioeconomic burden of fractures: today and in the 21st century. *Am J Med*. 1997;103(2):S20–S26. https://doi.org/10.1016/S0002-9343(97)90023-1
6. Burge RT, Worley D, Johansen A, Bhattacharyya S, Bose U. The cost of osteoporotic fractures in the UK: projections for 2000–2020. *J Med Econ*. 2001;4(1-4):51–62. https://doi.org/10.3111/200104051062
7. Ferrari SL, Abrahamsen B, Napoli N, et al. Diagnosis and management of bone fragility in diabetes: an emerging challenge. *Osteoporos Int*. 2018;29(12):2585–2596. https://doi.org/10.1007/s00198-018-4650-2
8. Sudlow C, Gallacher J, Allen N, et al. UK Biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. *PLoS Med*. 2015;12(3):e1001779. https://doi.org/10.1371/journal.pmed.1001779
9. Bycroft C, Freeman C, Petkova D, et al. The UK Biobank resource with deep phenotyping and genomic data. *Nature*. 2018;562(7726):203–209. https://doi.org/10.1038/s41586-018-0579-z
10. Association, A.D. 6. Glycemic targets. *Diabetes Care*. 2014;38(Supplement_1):S33–S40. https://doi.org/10.2337/dc15-S009
11. American Diabetes, A. Diagnosis and classification of diabetes mellitus. *Diabetes Care*. 2013;37(Supplement_1):S81–S90. https://doi.org/10.2337/dc14-S081
12. Morris JA, Kemp JP, Youlten SE, et al. An atlas of genetic influences on osteoporosis in humans and mice. *Nat Genet*. 2019;51(2):258–266. https://doi.org/10.1038/s41588-018-0302-x
13. Zhang Z. Multiple imputation with multivariate imputation by chained equation (MICE) package. *Ann Transl Med*. 2016;4(2):30. https://doi.org/10.3978/j.issn.2305-5839.2015.12.63
14. Leanza G, Maddaloni E, Pitocco D, et al. Risk factors for fragility fractures in type 1 diabetes. *Bone*. 2019;125:194–199. https://doi.org/10.1016/j.bone.2019.04.017
15. Conway BN, Long DM, Figaro MK, May ME. Glycemic control and fracture risk in elderly patients with diabetes. *Diabetes Res Clin Pract*. 2016;115:47–53. https://doi.org/10.1016/j.diabres.2016.03.009
16. Chen J, Spracklen CN, Marenne G, et al. The trans-ancestral genomic architecture of glycemic traits. *Nat Genet*. 2021;53(6):840–860. https://doi.org/10.1038/s41588-021-00852-9
17. Burgess S, Davey Smith G, Davies NM, et al. Guidelines for performing Mendelian randomization investigations. *Wellcome Open Res*. 2019;4:186. https://doi.org/10.12688/wellcomeopenres.15555.1
18. Burgess S, Davey Smith G, Davies NM, et al. Guidelines for performing Mendelian randomization investigations [version 2; peer review: 2 approved]. *Wellcome Open Research*. 2020;4(186). https://doi.org/10.12688/wellcomeopenres.15555.2
19. Paul SC, Tom MP, Frank W. Estimating structural mean models with multiple instrumental variables using the generalised method of moments. *Stat Sci*. 2015;30(1):96–117
20. Manichaikul A, Mychaleckyj JC, Rich SS, Daly K, Sale M, Chen WM. Robust relationship inference in genome-wide association studies. *Bioinformatics*. 2010;26(22):2867–2873. https://doi.org/10.1093/bioinformatics/btq559
21. Brion M-JA, Shakhbazov K, Visscher PM. Calculating statistical power in Mendelian randomization studies. *Int J Epidemiol*. 2013;42(5):1497–1501. https://doi.org/10.1093/ije/dyt179
22. Tian H, Mason AM, Liu C, Burgess S. Relaxing parametric assumptions for non-linear Mendelian randomization using a doubly-ranked stratification method. *PLOS Genetics* 2023;19(6):e1010823. https://doi.org/10.1371/journal.pgen.1010823.
23. Amy M Mason, Stephen Burgess, Software Application Profile: SUMnlmr, an R package that facilitates flexible and reproducible non-linear Mendelian randomization analyses, *International Journal of Epidemiology*, Volume 51, Issue 6, December 2022, Pages 2014–2019. https://doi.org/10.1093/ije/dyac150.
24. Staley JR, Burgess S. Semiparametric methods for estimation of a nonlinear exposure-outcome relationship using instrumental variables with application to Mendelian randomization. *Genet Epidemiol*. 2017;41(4):341–352. https://doi.org/10.1002/gepi.22041
25. Fry A, Littlejohns TJ, Sudlow C, et al. Comparison of sociodemographic and health-related characteristics of UK Biobank participants with those of the general population. *Am J Epidemiol*. 2017;186(9):1026–1034. https://doi.org/10.1093/aje/kwx246
26. Oei L, Zillikens MC, Dehghan A, et al. High bone mineral density and fracture risk in type 2 diabetes as skeletal complications of inadequate glucose control: the Rotterdam study. *Diabetes Care*. 2013;36(6):1619–1628. https://doi.org/10.2337/dc12-1188
27. Wang B, Wang Z, Poundarik AA, et al. Unmasking fracture risk in type 2 diabetes: the association of longitudinal glycemic hemoglobin level and medications. *J Clin Endocrinol Metab*. 2022;107(4):e1390–e1401. https://doi.org/10.1210/clinem/dgab882
28. Li C-I, Liu CS, Lin WY, et al. Glycated hemoglobin level and risk of hip fracture in older people with type 2 diabetes: a competing risk analysis of Taiwan Diabetes Cohort Study. *J Bone Miner Res*. 2015;30(7):1338–1346. https://doi.org/10.1002/jbmr.2462
29. Hidayat K, Fang QL, Shi BM, Qin LQ. Influence of glycemic control and hypoglycemia on the risk of fracture in patients with diabetes mellitus: a systematic review and meta-analysis of observational studies. *Osteoporos Int*. 2021;32(9):1693–1704. https://doi.org/10.1007/s00198-021-05934-2
30. Altman DG, Lausen B, Sauerbrei W, Schumacher M. Dangers of using "optimal" cutpoints in the evaluation of prognostic factors. *J Natl Cancer Inst*. 1994;86(11):829–835. https://doi.org/10.1093/jnci/86.11.829
31. Douglas GA, Patrick R. The cost of dichotomising continuous variables. *BMJ*. 2006;332(7549):1080
32. Lee RH, Sloane R, Pieper C, et al. Glycemic control and insulin treatment alter fracture risk in older men with type 2 diabetes mellitus. *J Bone Miner Res*. 2019;34(11):2045–2051. https://doi.org/10.1002/jbmr.3826
33. Karges B, Rosenbauer J, Kapellen T, et al. Hemoglobin A1c levels and risk of severe hypoglycemia in children and young adults with type 1 diabetes from Germany and Austria: a trend analysis in a cohort of 37,539 patients between 1995 and 2012. *PLoS Med*. 2014;11(10):e1001742. https://doi.org/10.1371/journal.pmed.1001742
34. Lee AK, Juraschek SP, Windham BG, et al. Severe hypoglycemia and risk of falls in type 2 diabetes: the Atherosclerosis Risk in Communities (ARIC) study. *Diabetes Care*. 2020;43(9):2060–2065. https://doi.org/10.2337/dc20-0316
35. Ford ES, Cowie CC, Li C, Handelsman Y, Bloomgarden ZT. Iron-deficiency anemia, non-iron-deficiency anemia and HbA1c among adults in the US*. *J Diabetes*. 2011;3(1):67–73. https://doi.org/10.1111/j.1753-0407.2010.00100.x
36. English E, Idris I, Smith G, Dhatariya K, Kilpatrick ES, John WG. The effect of anaemia and abnormalities of erythrocyte indices on HbA1c analysis: a systematic review.

Diabetologia. 2015;58(7):1409–1421. https://doi.org/10.1007/s00125-015-3599-3
37. Vavanikunnel J, Charlier S, Becker C, et al. Association between glycemic control and risk of fracture in diabetic patients: a nested case-control study. *J Clin Endocrinol Metab*. 2019;104(5):1645–1654. https://doi.org/10.1210/jc.2018-01879
38. Schwartz AV, Backlund JC, de Boer IH, et al. Risk factors for lower bone mineral density in older adults with type 1 diabetes: a cross-sectional study. *Lancet Diabetes Endocrinol*. 2022;10(7):509–518. https://doi.org/10.1016/S2213-8587(22)00103-6
39. DeFronzo RA, Ferrannini E, Groop L, et al. Type 2 diabetes mellitus. *Nat Rev Dis Prim*. 2015;1(1):15019. https://doi.org/10.1038/nrdp.2015.19
40. Katsarou A, Gudbjörnsdottir S, Rawshani A, et al. Type 1 diabetes mellitus. *Nat Rev Dis Prim*. 2017;3(1):17016. https://doi.org/10.1038/nrdp.2017.16
41. Emanuelsson F, Afzal S, Jørgensen NR, Nordestgaard BG, Benn M. Hyperglycaemia, diabetes and risk of fragility fractures: observational and Mendelian randomisation studies. *Diabetologia*. 2024;67(2):301–311. https://doi.org/10.1007/s00125-023-06054-8
42. Wade KH, Hamilton FW, Carslake D, Sattar N, Davey Smith G, Timpson NJ. Challenges in undertaking nonlinear Mendelian randomization. *Obesity (Silver Spring)*. 2023;31(12):2887–2890. https://doi.org/10.1002/oby.23927