Electronic supplementary material (ESM)

Hyperglycaemia, diabetes and risk of fragility fractures: observational and Mendelian randomisation studies

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Supplementary Table 1.

Overview of genes, their involvement in glucose metabolism and associated tag single nucleotide polymorphisms (SNPs) used as instrument for glycaemia in the current study.

Gene	Genomic coordinates (GRCh38)	Encoded gene product	Function/involvement in glucose metabolism	References for function	Tag SNP (Rsid)	Tag SNP position (GRCh38)	Type of variant
G6PC2	2:168901291-168910000	Islet-specific glucose-6-phosphatase catalytic subunit-related protein	Regulation of beta-cell glucose-stimulated insulin secretion	DOI:10.2337/db12-1067[1] DOI: 10.1007/s40618-020-01483-3[2] DOI: 10.1016/j.jbc.2021.101534[3]	rs560887	2:168906638	Intron variant
GCK	7:44143213-44189439	Glucokinase	Beta-cell glucose metabolism, regulation of glucose-stimulated insulin secretion	DOI: 10.1186/s13059-023-02935-8[4] DOI: 10.1016/j.tem.2022.12.007[5] DOI: 10.2337/diab.39.6.647[6]	rs4607517	7:44196069	Intron variant
DGKB	7:14145049-14974858, 7: 15200317-15,562015	Diacylglycerol kinase beta	Cell signalling, regulation of glucose-stimulated insulin secretion	DOI: 10.1007/s00125-010-1753-5[7] DOI: 10.1038/s41467-020-18581-8[8] DOI: 10.1210/en.2013-1356[9]	rs2191349	7:15024684	Intergenic variant
ADCY5	3:123282296-123449090	Adenylate cyclase 5	Glucose signalling and regulation of insulin secretion, proinsulin-to-insulin conversion	DOI:10.2337/db13-1607[10] DOI:10.1371/journal.pone.0023639[11] DOI:10.2337/db17-0464[12]	rs11708067	3:123346931	Intron variant
CDKN2A/B	9:21967752-21995324 (A), 9:22002903-22009313 (B)	Cyclin-dependent kinase inhibitor 2A (p16) Cyclin-dependent kinase inhibitor 2B (p15)	Beta-cell mass and function, first phase glucose-induced insulin secretion	DOI:10.1016/j.tem.2015.01.008[13] DOI:10.1007/s00125-010-2038-8[14] DOI:10.2337/db09-0736[15] DOI:10.1152/ajpendo.00496.2010[16]	rs10811661	9:22134095	Upstream intergenic variant
					rs2383206	9:22115027	Intron variant
TCF7L2	10:112950247-113167678	Transcription factor 7-like 2	Transcription factor, regulatory function of GLP-1 induced insulin response, beta-cell function	DOI: 10.1172/JCI30706[17] DOI: 10.2337/db20-0573[18] DOI: 10.1007/s00125-007-0753-6[19]	rs7903146	10:112998590	Intron variant

Supplementary Table 2

Diagnose codes of fragility fracture used in the Copenhagen City Heart Study and the Copenhagen General Population Study (ICD8 and ICD10) and the UK Biobank (ICD9 and ICD10) as defined by the World Health Organization's codes of International Classification of Diseases, Eight, Ninth, and Tenth Revision (ICD8, ICD9, ICD10).

Endpoint	ICD8 codes	ICD9 codes	ICD10 codes
Hip fracture	820	8200, 8202, 8208	S720-S722
Spine fracture	805.10, 805.11, 805.19	8052, 8054, 8058	S220, S221, S320, S327a, S328a, M484, M485, M495
Arm fracture (proximal humerus and wrist)	812.00, 812.01, 812.02, 812.08, 812.09, 812.19, 813.21, 813.20, 813.28, 813.29	8120, 8122, 8134, 8138	S422-S423, S525, S526, S528

Supplementary Table 3

Genetic variants used as instruments for glucose and their association with non-fasting plasma glucose in the Copenhagen studies and in the UK Biobank. β-coefficients, standard error (SE) of the β-coefficients, and p values are derived by age and sex adjusted linear regression and are shown per additional risk allele of the genetic variant.

Gene	**Variant**	**Copenhagen studies**		**UK Biobank**	
		$\beta_{glucose}$ (SE)	**P $\beta_{glucose}$**	**$\beta_{glucose}$ (SE)**	**P $\beta_{glucose}$**
G6PC2	rs560887	0.08 (0.006)	1.6×10^{-44}	0.09 (0.003)	3.4×10^{-183}
GCK	rs4607517	0.06 (0.007)	3.0×10^{-17}	0.07 (0.004)	6.5×10^{-82}
DGKB	rs2191349	0.03 (0.005)	3.3×10^{-7}	0.04 (0.003)	5.4×10^{-35}
ADCY5	rs11708067	0.04 (0.006)	2.8×10^{-11}	0.04 (0.003)	3.0×10^{-39}
TCF7L2	rs7903146	0.05 (0.006)	5.7×10^{-20}	0.07 (0.003)	3.5×10^{-98}
CDKN2A/B	rs10811661	0.03 (0.007)	7.4×10^{-7}	0.04 (0.004)	3.3×10^{-25}
CDKN2A/B	rs2383206	0.02 (0.005)	3.3×10^{-3}	0.01 (0.003)	4.4×10^{-5}
Weighted allele score		0.05 (0.002)	1.8×10^{-101}	0.05 (0.001)	1.0×10^{-300}

Supplementary Table 4

Association of the weighted allele scores (WAS) with glucose and the potential confounders body mass index, alcohol intake, smoking (in pack-years) and level of physical activity. β-coefficients, standard error (SE) of the β-coefficients, and *p* values are derived by linear regression adjusted for age and sex.

Glucose and potential confounders	**Copenhagen studies Glucose WAS**		**UK Biobank Glucose WAS**	
	β (SE)	***p* value$_\beta$**	**β (SE)**	***p* value$_\beta$**
Glucose (mmol/L)	0.05 (0.003)	2.6×10^{-95}	0.05 (0.001)	1.0×10^{-300}
Body mass index (kg/m^2)	-0.03 (0.01)	0.002	-0.02 (0.01)	0.0001
Alcohol intake (units/week)	0.00 (0.02)	0.94	-0.00 (0.00)	0.72
Pack-years, smokers	0.07 (0.04)	0.06	0.04 (0.04)	0.24
Physical activity	0.00 (0.00)	0.40	0.00 (0.00)	0.36

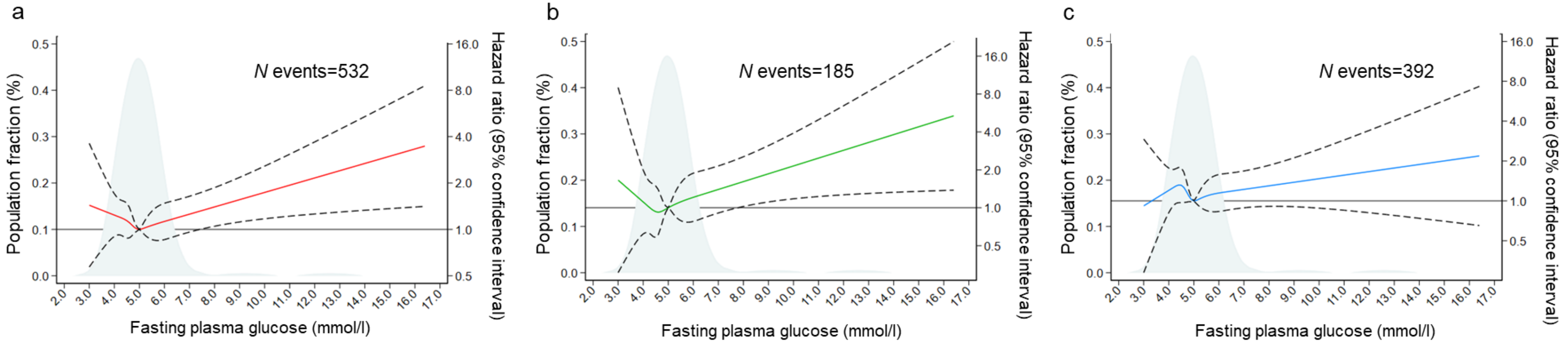
Supplementary Table 5.

Inverse-variance weighted regression, Mendelian randomisation (MR) Egger regression, and weighted median regression for the two-sample MR analyses using summary-level data from the Meta-Analyses of Glucose and Insulin-related traits Consortium (MAGIC) for the association between the seven genetic variants and fasting glucose and HbA_{1c}, respectively, and data on any fracture, hip fracture, and arm fracture from the Copenhagen cohorts. IVW = inverse variance weighted.

Analysis	**OR**	**95% CI**	***p* value**	***p* value MR Egger intercept**
Exposure: Fasting plasma glucose (MAGIC)				
Outcome: Any fragility fracture (Copenhagen studies):				
MR Egger IVW	1.36	0.89, 2.09	0.15	
MR Egger	2.10	0.85, 5.21	0.29	0.35
MR Median	1.00	0.65, 1.53	0.99	
Outcome: Hip fracture (Copenhagen studies):				
MR Egger IVW	0.90	0.53, 1.53	0.69	
MR Egger	1.04	0.32, 3.33	0.95	0.78
MR Median	0.62	0.28, 1.39	0.25	
Outcome: Arm fracture (Copenhagen studies):				
MR Egger IVW	1.50	1.03, 2.18	0.03	
MR Egger	2.28	1.05, 4.95	0.04	0.23
MR Median	2.28	1.05, 4.95	0.16	
Exposure: HbA_{1c} (MAGIC)				
Outcome: Any fragility fracture (Copenhagen studies):				
MR Egger IVW	2.47	0.95, 6.43	0.06	
MR Egger	7.63	0.99, 59.1	0.05	0.23
MR Median	2.96	1.44, 6.09	0.003	
Outcome: Hip fracture (Copenhagen studies):				
MR Egger IVW	0.90	0.53, 1.53	0.69	
MR Egger	1.97	0.08, 46.6	0.68	0.63
MR Median	0.62	0.28, 1.39	0.25	
Outcome: Arm fracture (Copenhagen studies):				
MR Egger IVW	2.79	1.12, 6.93	0.03	
MR Egger	11.19	2.26, 55.5	0.04	0.06
MR Median	3.68	1.61, 8.43	0.002	

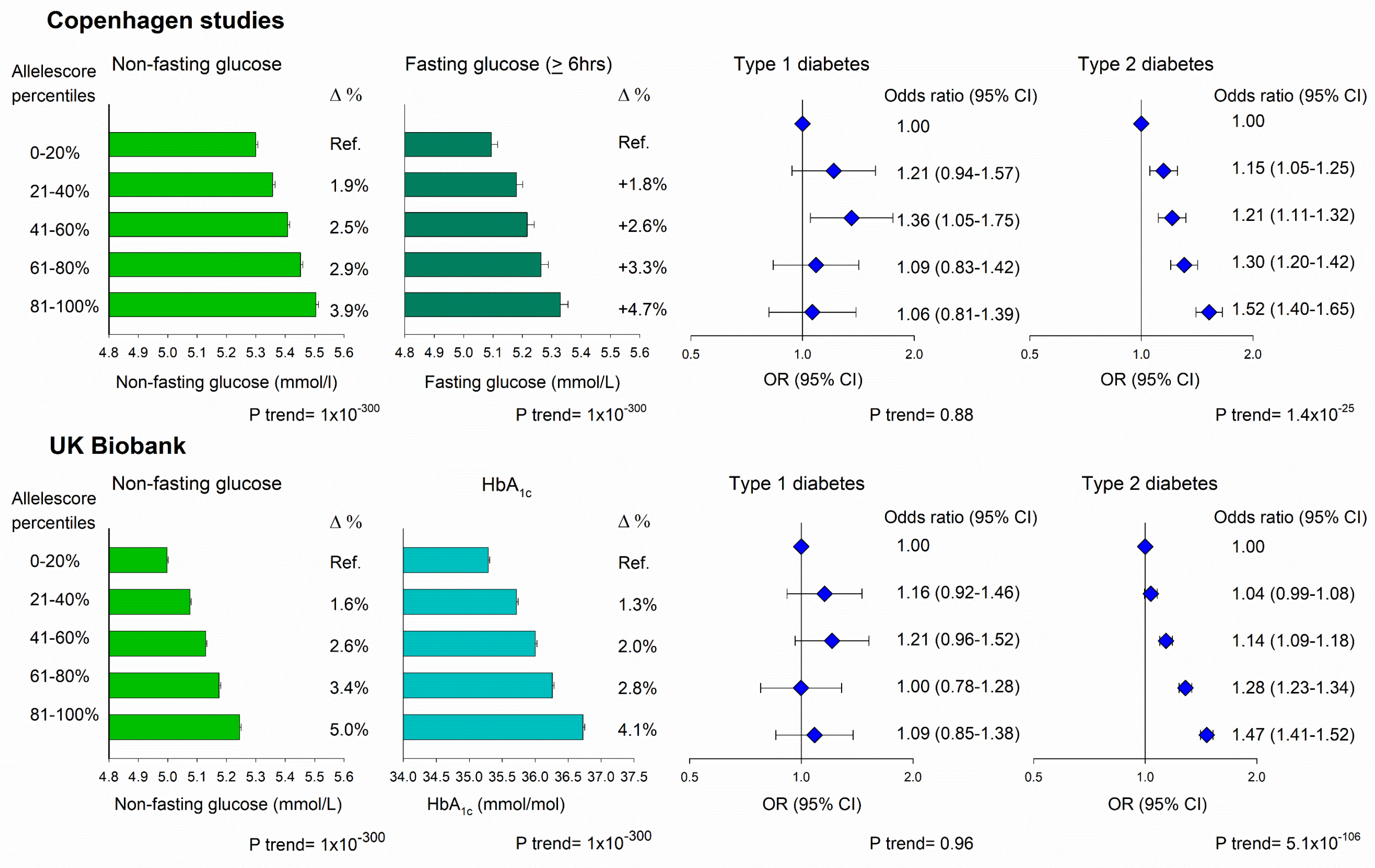
Supplementary Figure 1.

Risk of any fragility fracture (a), hip fracture (b), and arm fracture (c) (proximal humerus and wrist) in the Copenhagen studies as a function of fasting glucose concentrations by restricted cubic spline analyses incorporated into a Cox proportional hazards model and adjusted for sex, birth year, body mass index, current smoking, physical activity level, units of alcohol consumed per week, and menopausal status for women. Fasting was defined as ≥ 6 hours since last meal and was available in 7952 individuals. Solid lines denote hazard ratios and broken lines 95% confidence intervals. The reference was set to the population median (5.2 mmol/L). Light blue area shows the distribution of glucose concentrations in the population. To convert from mmol/L to mg/dL, multiply by 18. N=number.



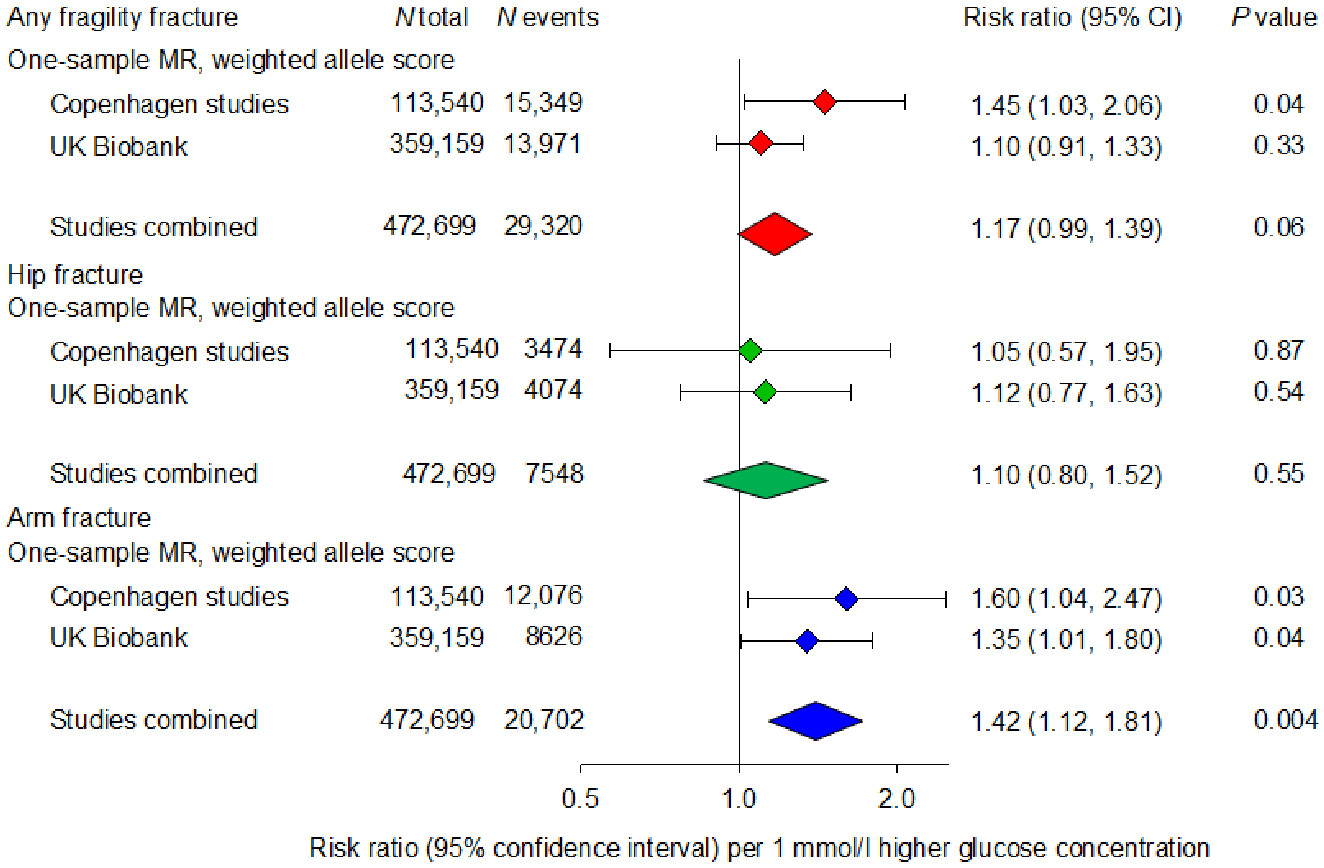
Supplementary Figure 2.

Mean concentrations of plasma non-fasting glucose (Copenhagen studies and the UK Biobank), fasting glucose (Copenhagen studies), glycated haemoglobin A1c (HbA_{1c}) (UK Biobank), and risk of type 1 and type 2 diabetes (Copenhagen studies and the UK Biobank) as a function of the glucose weighted allele scores in quintiles. Error bars denotes the standard error of the mean. Δ% denotes the percent higher concentration in non-fasting glucose, fasting glucose, and HbA_{1c} compared to the lowest quintile group. Fasting was defined ≥6 hours self-reported time since last meal. Risk of type 1 and type 2 diabetes was estimated by logistic regression adjusted for age and sex. CI= confidence interval.



Supplementary Figure 3

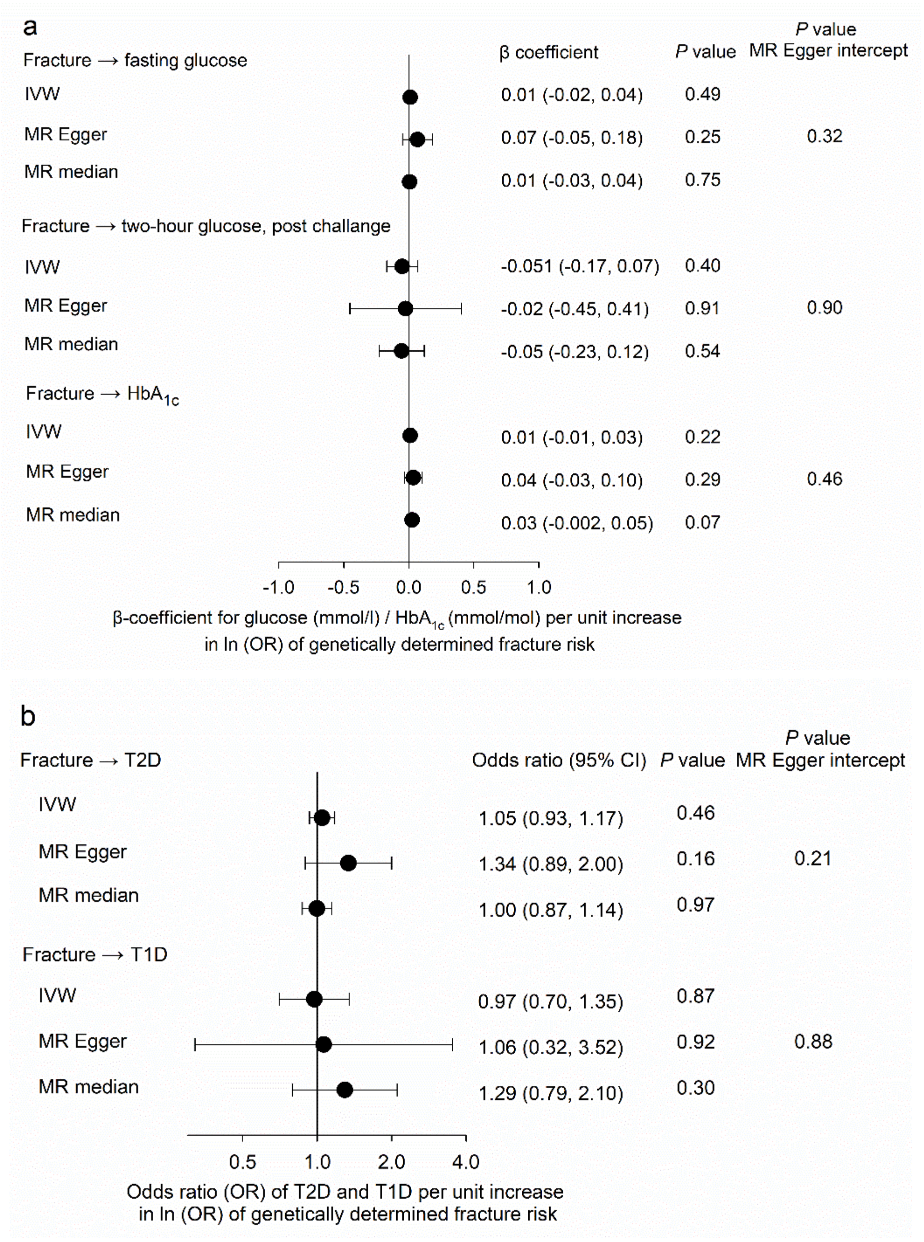
Mendelian randomisation analysis of risk of any fragility fracture, hip fracture, and arm fracture (proximal humerus and wrist) in the Copenhagen studies, in UK Biobank, and in the studies combined for a 1 mmol/l higher glucose level. Estimates were derived by instrumental variable analysis adjusted for age, sex, and body mass index. *N*=number, CI=confidence interval.



Any fragility fracture	*N* total	*N* events	Risk ratio (95% CI)	*P* value
One-sample MR, weighted allele score				
Copenhagen studies	113,540	15,349	1.45 (1.03, 2.06)	0.04
UK Biobank	359,159	13,971	1.10 (0.91, 1.33)	0.33
Studies combined	472,699	29,320	1.17 (0.99, 1.39)	0.06
Hip fracture				
One-sample MR, weighted allele score				
Copenhagen studies	113,540	3474	1.05 (0.57, 1.95)	0.87
UK Biobank	359,159	4074	1.12 (0.77, 1.63)	0.54
Studies combined	472,699	7548	1.10 (0.80, 1.52)	0.55
Arm fracture				
One-sample MR, weighted allele score				
Copenhagen studies	113,540	12,076	1.60 (1.04, 2.47)	0.03
UK Biobank	359,159	8626	1.35 (1.01, 1.80)	0.04
Studies combined	472,699	20,702	1.42 (1.12, 1.81)	0.004

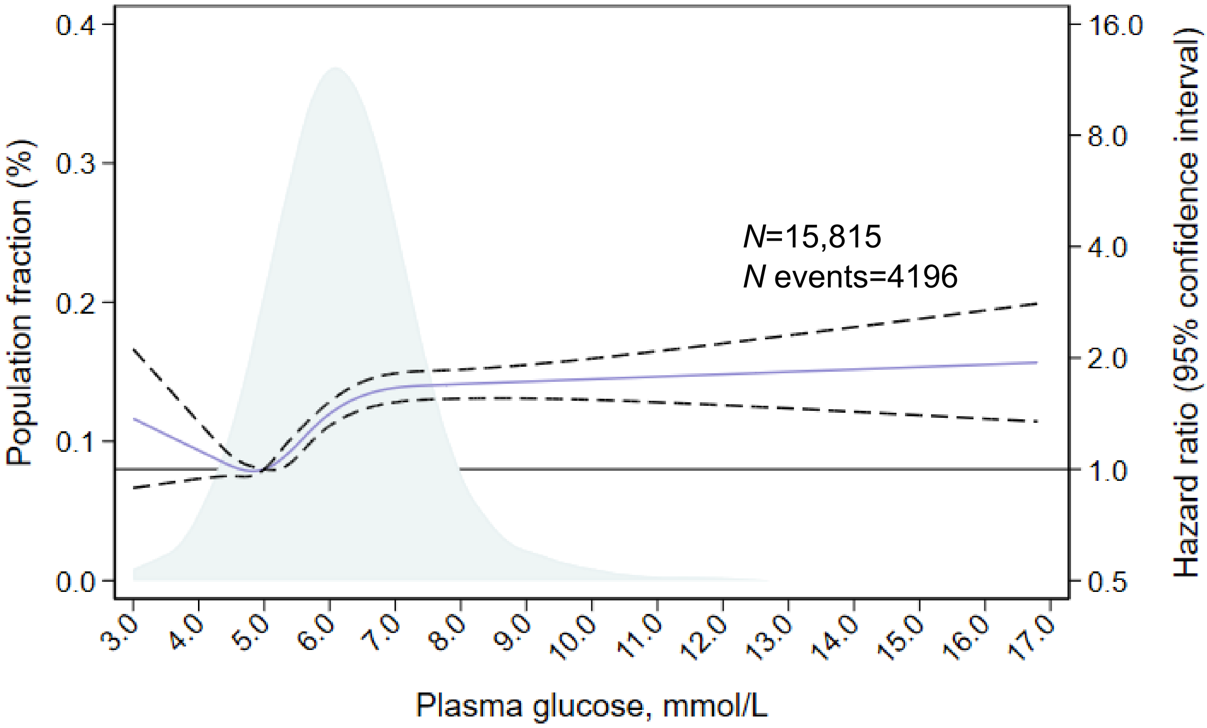
Supplementary Figure 4.

Two-sample MR analyses investigating the effect of fracture risk on fasting glucose, 2-hours post challenge glucose, HbA_{1c}, type 1, and type 2 diabetes. Publicly available data for 14 genetic variants associated with risk of fracture in the UK Biobank and replicated in the 23andMe cohorts was used as instrument and was combined with publicly available data on fasting glucose, 2-hours post-challenge glucose, and HbA1c concentrations from the Meta-Analyses of Glucose and Insulin-related traits Consortium (MAGIC), data for type 2 diabetes from the DIAMANTE consortium and data on type 1 diabetes from a GWAS by Forgetta et al. IVW = Inverse-variance weighted regression. MR = Mendelian randomization.



Supplementary Figure 5.

Risk of death in individuals with previous any fragility fracture in the Copenhagen studies as a function of non-fasting glucose concentrations on a continuous scale by restricted cubic spline analyses incorporated into a Cox proportional hazards model and adjusted for sex, birth year, body mass index, current smoking, physical activity level, units of alcohol consumed per week, and menopausal status for women. Solid lines denote hazard ratios and broken lines 95% confidence intervals. The reference was set to the population median (5.2 mmol/l). Light blue area shows the distribution of non-fasting glucose concentrations in the population. *N*=number.



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