

Online Appendix

Low plasma adiponectin in risk of type 2 diabetes: observational analysis and one- and two-sample Mendelian randomization analyses in 756,219 individuals

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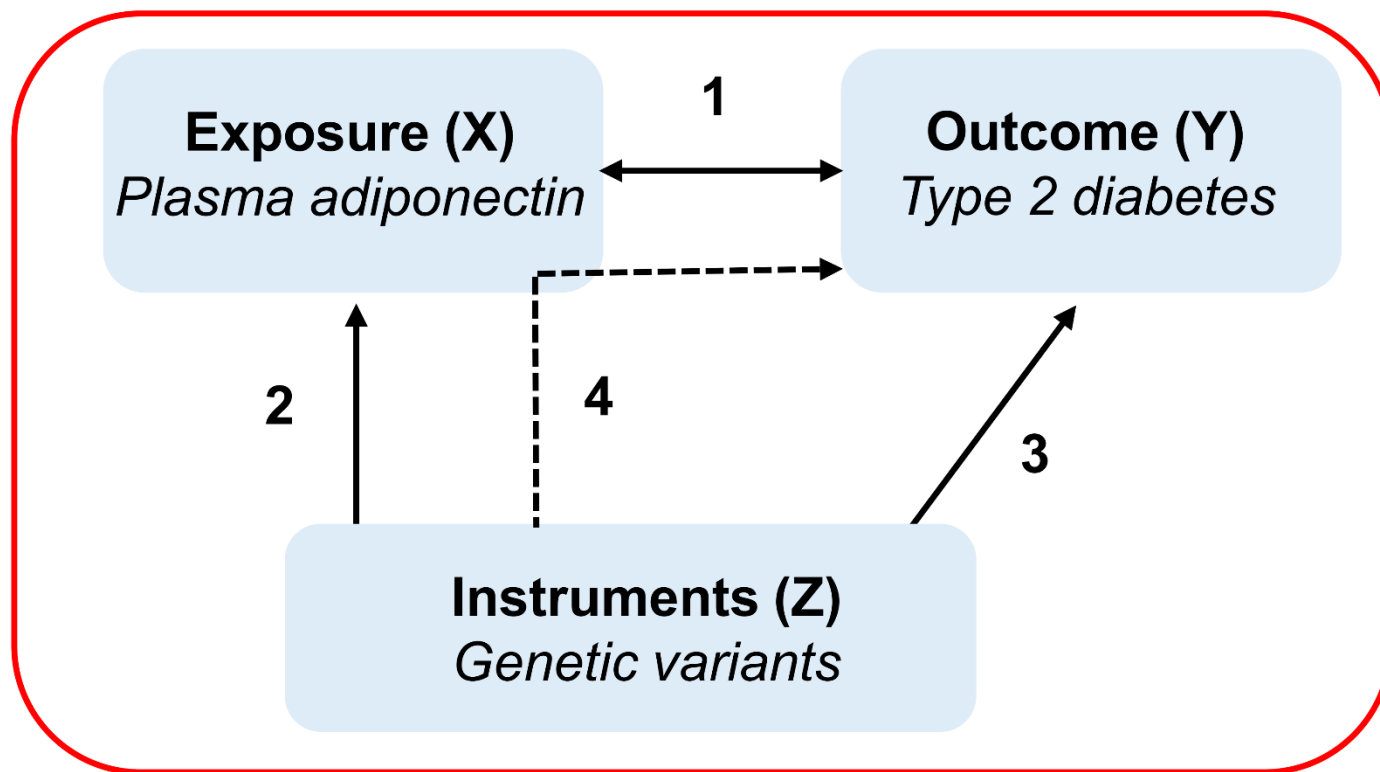


Figure S1. Testing the observational and causal relationship between plasma adiponectin and type 2 diabetes in a Mendelian randomization approach. *Step 1:* The association between plasma adiponectin as the exposure (X) and type 2 diabetes as the outcome (Y) is investigated (the observational association which may be prone to confounding and reverse causation). *Step 2:* The association between specific genetic variants (in this case rs2062632, rs266717, rs6810075, rs17366568, and rs2925979) as the instruments (Z) and plasma adiponectin as the exposure (X) is investigated (confirming the validity of the genetic variants as instruments for plasma adiponectin). *Step 3:* The direct association between genetic variants as the instruments (Z) and type 2 diabetes as the outcome (Y) is investigated (the genetic association which cannot be prone to confounding or reverse causation). *Step 4:* The association between genetically determined plasma adiponectin as the exposure-instrument (X+Z) and type 2 diabetes as the outcome (Y) is investigated (instrumental variable analysis to determine the causal effect of plasma adiponectin on type 2 diabetes).

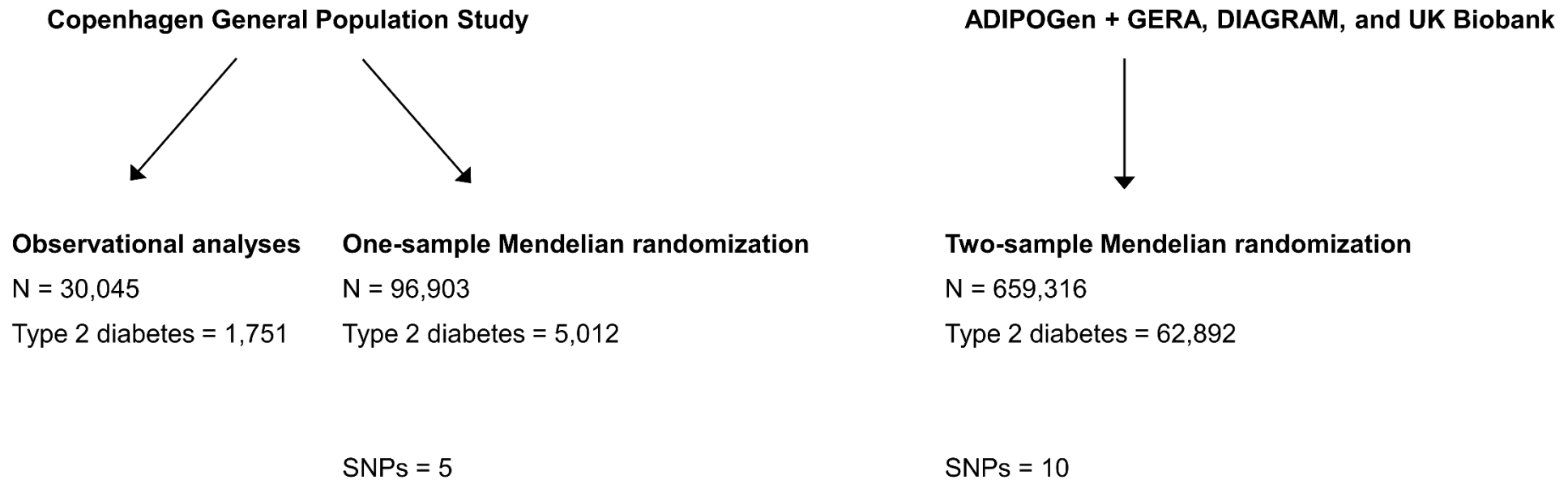


Figure S2. Flowchart for observational analyses and Mendelian randomization. In two-sample Mendelian randomization, ADIPOGen consortium(1) was used as the exposure cohort and GERA, DIAGRAM and UK Biobank, as the outcome cohort(2). GERA=Genetic Epidemiology Research on Aging cohort. DIAGRAM=Diabetes Genetics Replication and Meta-analysis. SNPs=single nucleotide polymorphisms.

Table S1. Information on genetic variants used in one-sample Mendelian randomization analysis in the Copenhagen General Population Study.

SNP	Gene	Chr	Effect allele	Other allele	EAF in European	Traits	Association with traits in previous studies, P-value	Association with traits in CGPS, P for trend
rs2062632	<i>KNG1</i>	3	T	C	0.75	Plasma adiponectin Plasma APTT	5×10^{-15} 1×10^{-7}	5×10^{-19} 6×10^{-71}
rs266717	<i>LINC02043</i>	3	T	C	0.48	Plasma adiponectin Plasma APTT	9×10^{-19} 5×10^{-14}	1×10^{-42} 4×10^{-43}
rs6810075	<i>ADIPOQ</i>	3	T	C	0.60	Plasma adiponectin	2×10^{-43}	3×10^{-37}
rs17366568	<i>ADIPOQ</i>	3	G	A	0.89	Plasma adiponectin	1×10^{-41}	1×10^{-96}
rs2925979	<i>CMIP</i> *	16	T	C	0.29	Plasma adiponectin HDL-Cholesterol BMI adjusted WHR	1×10^{-20} 9×10^{-22} 7×10^{-13}	4×10^{-25} 1×10^{-14} 9×10^{-6}

Information on single nucleotide polymorphisms (SNPs) was retrieved from <http://www.phenoscaner.medschl.cam.ac.uk>(3,4), <https://www.ensembl.org>, and previous adiponectin genome wide association studies(1,5,6). CGPS=Copenhagen General Population Study. Chr=chromosome. *In the *CDH13* locus.

Supplemental Table S2. Pairwise Linkage Disequilibrium Coefficients D' and R^2 in a European population of SNPs in the *ADIPOQ* locus used in one-sample Mendelian randomization analyses.

SNP		D'			
		rs2062632	rs266717	rs6810075	
R^2	rs2062632		0.108	0.063	0.015
	rs266717	0.004		0.615	0.212
	rs6810075	0.001	0.236		0.099
	rs17366568	0.000	0.005	0.002	

R^2 and D'	0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1
Correlation	Low-----High										

Information on linkage disequilibrium was retrieved from a web-based LD-link application(7). D prime (D') - an indicator of allelic segregation for two genetic variants. D' values range from 0 to 1 with higher values indicating tight linkage of alleles. A D' value of 0 indicates no linkage of alleles. D' is shown above diagonal of empty cells. R squared (R^2) - a measure of correlation of alleles for two genetic variants. R^2 values range from 0 to 1 with higher values indicating a higher degree of correlation. R^2 is shown below diagonal of empty cells. SNP=Single nucleotide polymorphism.

Table S3. Information on genetic variants used in two-sample Mendelian randomization analysis.

SNP	Gene	Chr	Effect allele	Other allele	EAF in European	Association with plasma adiponectin in GWAS, P-value	Other traits with potential effect on type 2 diabetes risk
rs2062632	<i>KNG1</i>	3	T	C	0.75	3 x 10 ⁻¹⁹	
rs6810075	<i>ADIPOQ</i>	3	T	C	0.60	4 x 10 ⁻⁴¹	
rs17366568	<i>ADIPOQ</i>	3	G	A	0.89	3 x 10 ⁻⁶⁶	
rs7964945	<i>CCDC92</i>	12	T	A	0.85	3 x 10 ⁻⁸	Trunk predicted mass
rs7955516	<i>RP11-284H19.1</i>	12	C	A	0.38	2 x 10 ⁻⁸	
rs1108842	<i>GNL3</i>	3	C	A	0.51	4 x 10 ⁻¹¹	BMI and WC
rs1597466	<i>SIAH2</i>	3	T	G	0.10	2 x 10 ⁻⁸	Hypertension
rs2927324	<i>CMIP</i>	16	T	C	0.55	1 x 10 ⁻¹¹	
rs601339	<i>RP11-324E6.6</i>	12	G	A	0.18	4 x 10 ⁻¹¹	HDL
rs731839	<i>PEPD</i>	19	G	A	0.33	2 x 10 ⁻¹³	HDL, triglycerides, fat percentages

Information on SNPs retrieved from [http://www.phenoscaner.medschl.cam.ac.uk\(3,4\)](http://www.phenoscaner.medschl.cam.ac.uk(3,4)) and <https://www.ensembl.org>. P-values for association with plasma adiponectin from ADIPOGen consortium (Table S2)(1).

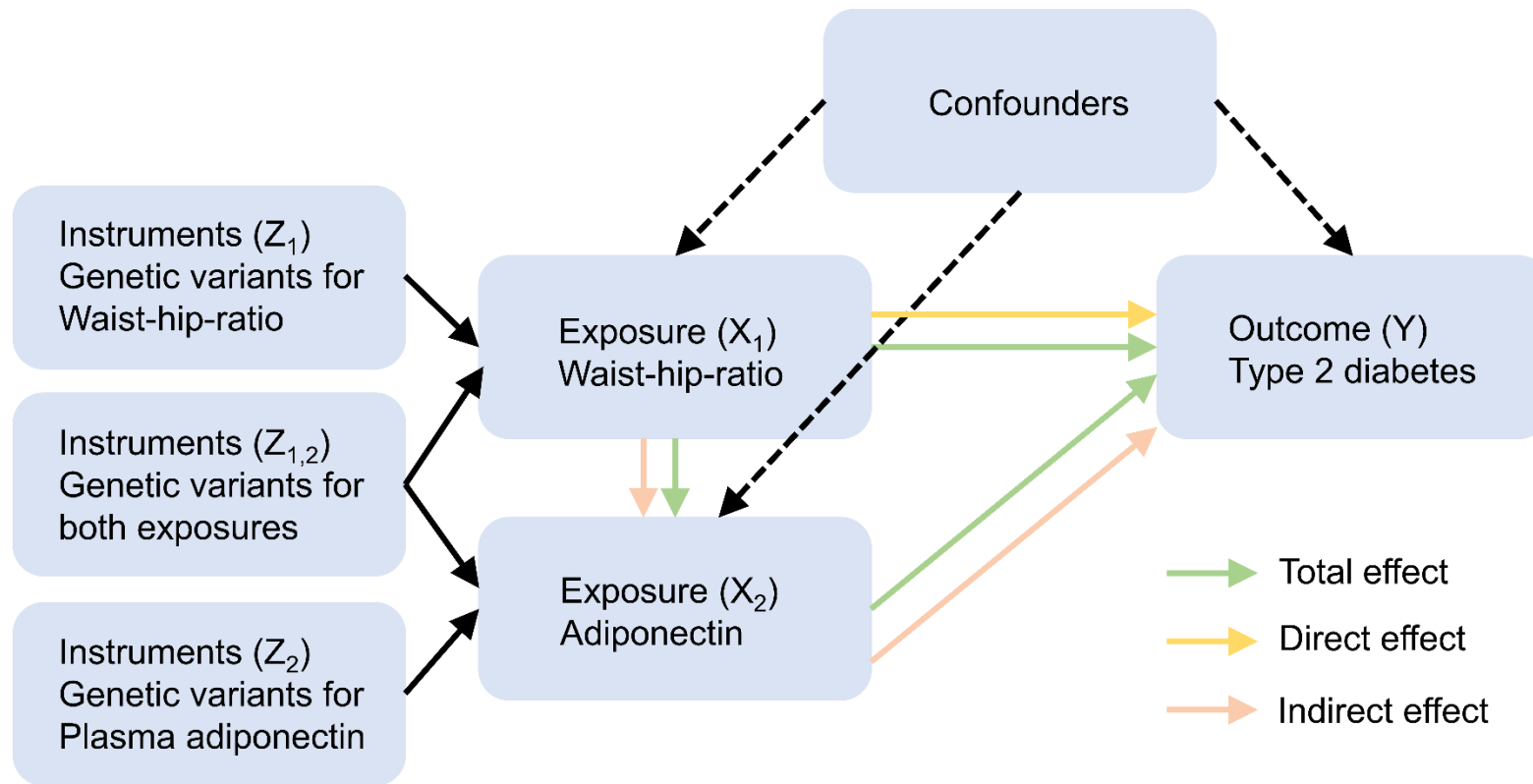


Figure S3. Direct acyclic graph testing the causal relationship between waist-hip-ratio, plasma adiponectin, and type 2 diabetes in a Multivariable Mendelian randomization. Solid arrows indicate the associations/validity investigated. Green arrows indicate the total effect of exposure X_1 on the outcome. Orange arrow indicate the direct effect of exposure X_1 on the outcome. Pink arrows indicate the indirect effect of exposure X_1 on the outcome. Indirect effect = Total effect – direct effect. The mediated effect of exposure X_1 through exposure X_2 = Indirect effect / Total effect. The Multivariable Mendelian randomization method for mediation estimation has previously been described(8). Information on exposures from waist-hip-ratio=GIANT consortium(9) and plasma adiponectin=ADIPOGen consortium(1). Information on outcome from type 2 diabetes GWAS=GERA, DIAGRAM, and UK Biobank(2). GERA=Genetic Epidemiology Research on Aging cohort. DIAGRAM=Diabetes Genetics Replication and Meta-analysis. GIANT= The Genetic Investigation of ANthropometric Traits. Information was retrieved using the MR-Base platform and MR_Practicals R package (including MRInstruments and TwoSampleMR)(10).

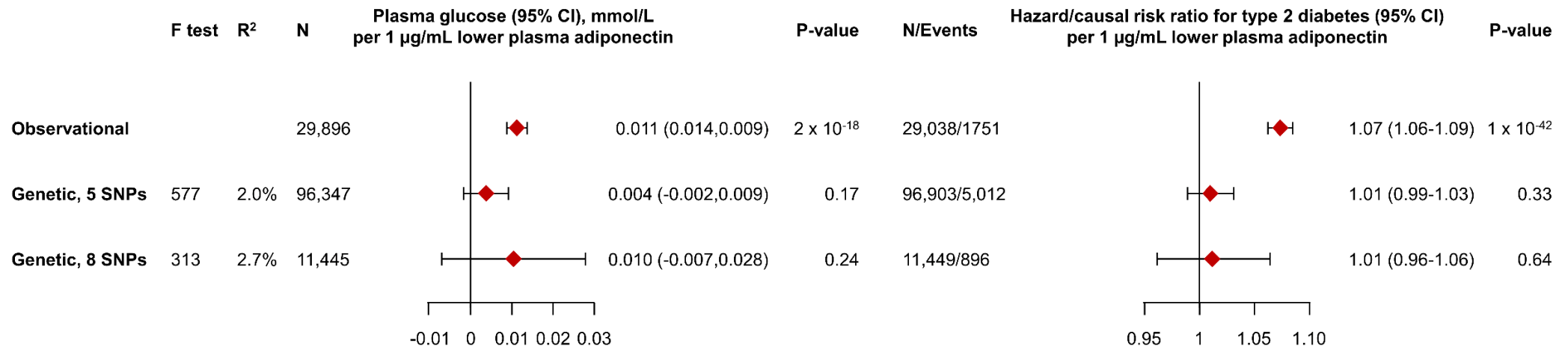


Figure S4. Observational and genetic association of plasma adiponectin with plasma glucose and risk of type 2 diabetes based on the Copenhagen General Population Study. Observational analyses used multiple linear regression and Cox regression multivariable adjusted for age, sex, body mass index, smoking status, tobacco consumption in pack-years, alcohol consumption, education, income, and leisure-time physical activity, and time since last meal (latter only for plasma glucose). Individuals with type 2 diabetes at baseline examination were excluded in observational analyses (corresponding to N=1,005). Mendelian randomization analyses used instrumental variable analyses with two-stage least-squares with internal weighted genetic adiponectin scores adjusted for age and sex. First, we used a targeted approach with five genetic variants in *ADIPOQ* locus (rs2062632, rs266717, rs6810075, and rs17366568) and *CDH13* (rs2925979) in the Copenhagen General Population. Second, we used a broad approach with eight genetic variants found in the ADIPOGen consortium to be associated with plasma adiponectin reaching genome wide significance level ($P < 5 \times 10^{-8}$) and with no linkage disequilibrium ($R^2 < 0.0001$) in a subpopulation from the Copenhagen General Population with available chip data (rs2062632, rs1108842, rs17366568, rs731839, rs6810075, rs7955516, rs7615090, and rs12051272).

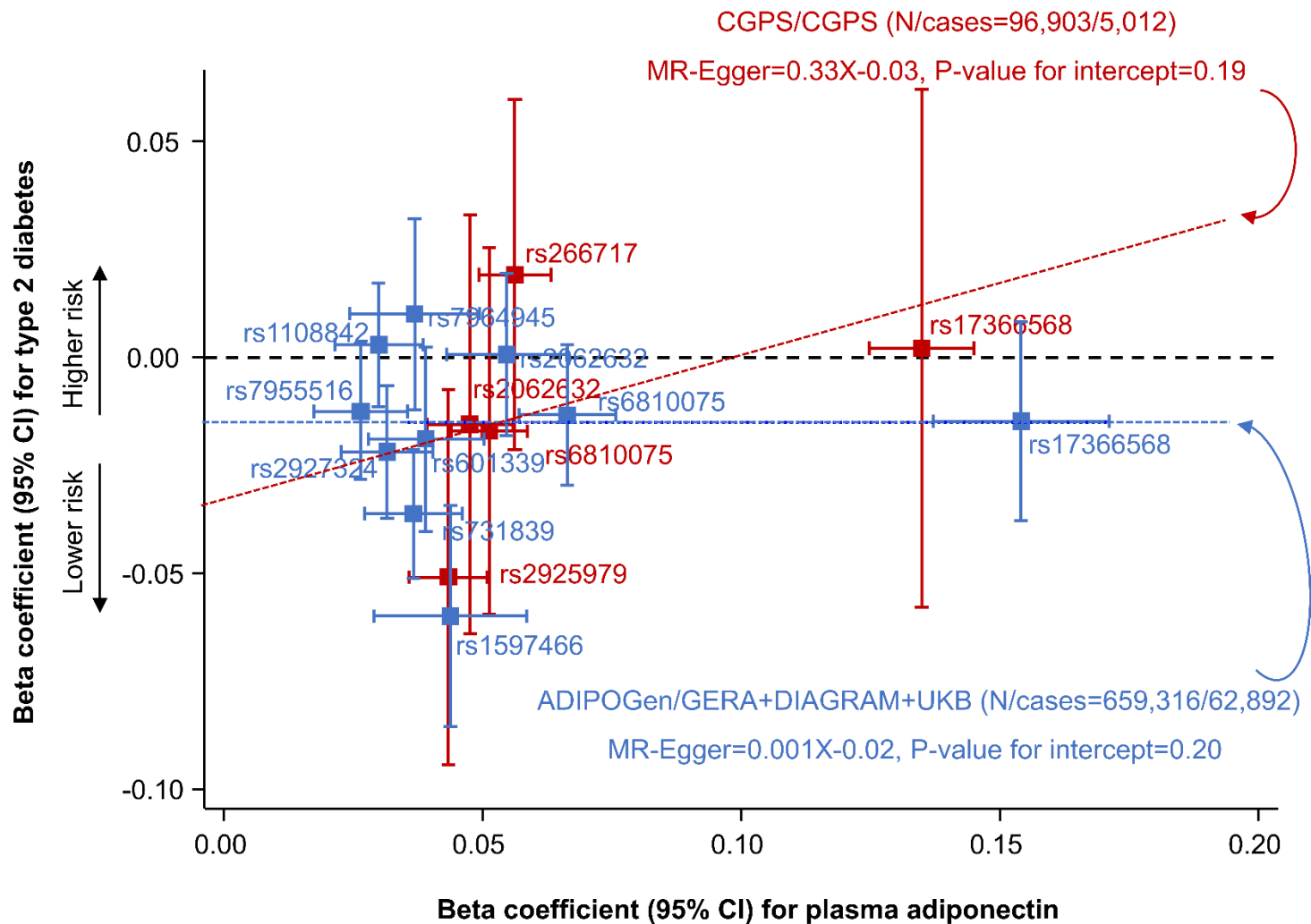


Figure S5. Individual single nucleotide polymorphism (SNP) associations with plasma adiponectin and type 2 diabetes in the Copenhagen General Population Study and in GERA, DIAGRAM and UK Biobank. Beta-values with 95% confidence interval indicated with red squares and whiskers for SNP associations in the Copenhagen General Population Study (CGPS). Beta-values with 95% confidence interval with blue squares and whiskers for SNP associations in GERA, DIAGRAM, and UK Biobank(2). Genetic information on plasma adiponectin was obtained from ADIPOGen(1). Dashed lines represent predicted values for type 2 diabetes using MR-Egger regression. GERA=Genetic Epidemiology Research on Aging cohort. DIAGRAM=Diabetes Genetics Replication and Meta-analysis. UKB=UK Biobank.

Supplemental Table S4. Power calculation for Mendelian randomization.

Exposure cohort	Outcome cohort	Sample size/cases	Type-I error rate	SNPs	Variance in exposure explained by the SNPs (R^2)	Causal risk ratio with 80% power to detect*
<i>Two-sample</i>						
ADIPOGen	GERA, DIAGRAM, UKB	659,316/62,892	0.05	10	0.08	1.05
<i>One-sample</i>						
CGPS	CGPS	96,903/5,012	0.05	5	0.02	1.35

Power calculations were performed using Burgess' online power calculator (<https://sb452.shinyapps.io/power/>)(11). *Causal effect (odds ratio, $\exp(\beta_1)$) per SD change in plasma adiponectin. SNP=Single nucleotide polymorphism. CGPS=Copenhagen General Population Study. GERA=Genetic Epidemiology Research on Aging cohort. DIAGRAM=Diabetes Genetics Replication and Meta-analysis. UKB=UK Biobank.

Supplemental Table S5. Mediation setup for proportion of waist-hip-ratio (WHR) effect on type 2 diabetes mediated through plasma adiponectin.

Method of estimation	SNPs	Beta coefficient WHR	Std. Error WHR	Beta coefficient Adiponectin	Std. Error Adiponectin	Intercept	P-value for intercept
Univariate MR – all SNPs	31	0.963	0.264	-0.345	0.244	NA	NA
MVMR (direct effect)	31	0.911	0.273	-0.178	0.217	NA	NA
MVMR, MR-Egger (direct effect)	31	0.898	0.282	-0.179	0.220	0.0018	0.78
Univariate - subset of SNPs (total effect)	23 (WHR) 8 (adiponectin)	0.958	0.305	-0.194	0.105	NA	NA

Multivariable Mendelian randomization (MVMR) estimates the direct effect of each exposure using the complete number of SNPs (=31). Three SNPs (rs731839, rs1316952, and rs17451107) from the WHR consortium (GIANT) which were in LD with SNPs from the plasma adiponectin consortium (ADIPOGen) were excluded. A subset of SNPs only associated with the exposure of interest was used for the univariate MR to estimate the total effect. The total effect of WHR=0.958, direct effect of WHR=0.911, total effect of plasma adiponectin=-0.194, direct effect of plasma adiponectin=-0.178. Information on exposures from WHR=GIANT consortium(9) and plasma adiponectin=ADIPOGen consortium(1). Information on outcome from type 2 diabetes GWAS=GERA, DIAGRAM, and UK Biobank(2). GERA=Genetic Epidemiology Research on Aging cohort. DIAGRAM=Diabetes Genetics Replication and Meta-analysis. SNPs=single nucleotide polymorphisms. WHR=waist-hip-ratio. MR=Mendelian randomisation. MVMR=Multivariable Mendelian randomization.

Supplemental Table S6. Two-sample Multivariable Mendelian randomization estimation.

MVMR (direct effect)	SNPs	Effect	Std. Error	95% Confidence Interval	P-value
<i>Type 2 diabetes</i>					
Waist-hip-ratio, standard deviation (SD)	31	0.911	0.273	0.353, 1.470	0.002
Plasma adiponectin, log ($\mu\text{g/mL}$)	31	-0.178	0.217	-0.656, 0.265	0.417

Multivariable Mendelian randomization estimates the direct effect of each exposure using the complete number of SNPs. Three SNPs (rs731839, rs1316952, and rs17451107) from the WHR consortium (GIANT) which were in LD with SNPs from the plasma adiponectin consortium (ADIPOGen) were excluded. Information on exposures from WHR=GIANT consortium(9) and plasma adiponectin=ADIPOGen consortium(1). Information on outcome from type 2 diabetes GWAS=GERA, DIAGRAM, and UK Biobank(2). GERA=Genetic Epidemiology Research on Aging cohort. DIAGRAM=Diabetes Genetics Replication and Meta-analysis. SNPs=single nucleotide polymorphisms.

Supplemental Table S7. Two-sample Multivariable Mendelian randomization for mediation estimation.

Mediation estimation	Effect	Std. Error	95% Confidence Interval	P-value
<i>Type 2 diabetes</i>				
Total effect of WHR	0.958	0.305	0.325, 1.590	0.005
Direct effect of WHR	0.911	0.273	0.353, 1.470	0.002
Indirect effect	0.046	0.038	-0.029, 0.121	0.227
Proportion mediated	0.048	0.037	-0.023, 0.120	0.187

Indirect effect = total effect – direct effect. Proportion mediated through adiponectin = indirect effect/total effect = 4.8% (95% CI: -2.3,12.0%).

References

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