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Supplemental Data

Causal Effects of Body Mass Index on Cardiometabolic Traits and Events: A Mendelian Randomization Analysis

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ARIC: The Atherosclerosis Risk in Communities Study is carried out as a collaborative study supported by National Heart, Lung, and Blood Institute contracts (HHSN268201100005C, HHSN268201100006C, HHSN268201100007C, HHSN268201100008C, HHSN268201100009C, HHSN268201100010C, HHSN268201100011C, and HHSN268201100012C), R01HL087641, R01HL59367 and R01HL086694; National Human Genome Research Institute contract U01HG004402; and National Institutes of Health contract HHSN268200625226C. The authors thank the staff and participants of the ARIC study for their important contributions. Infrastructure was partly supported by Grant Number UL1RR025005, a component of the National Institutes of Health and NIH Roadmap for Medical Research; **CARDIA**: Coronary Artery Risk in Young Adults: University of Alabama at Birmingham (N01-HC-48047, N01-HC-95095), University of Minnesota (N01-HC-48048), Northwestern University (N01-HC-48049), Kaiser Foundation Research Institute (N01-HC-48050), Tufts-New England Medical Center (N01-HC-45204), Wake Forest University (N01-HC-45205), Harbor-UCLA Research and Education Institute (N01-HC-05187), University of California, Irvine (N01-HC-45134, N01-HC-95100); **CFS**: The Cleveland Family Study (CFS) participants consists of first or selected second-degree relatives of a proband with either laboratory diagnosed obstructive sleep apnea or neighborhood control of an affected proband. Families were selected for genotyping on the basis of genetic informativity, including multigenerational data or individuals from the extremes of the distribution of apnea phenotype. This cohort was genotyped as part of the National Heart Lung and Blood Institute's (NHLBI) Candidate Gene Association Resource (CARe) (Musunuru, K., Lettre, G., Young, T., Farlow, D.N., Pirruccello, J.P., Ejebe, K.G., Keating, B.J., Yang, Q., Chen, M.H., Lapchyk, N. et al. Candidate gene association resource (CARe): design, methods, and proof of concept. Circ. Cardiovasc. Genet, 3, 267-275.); **CHS**: This research was supported by contracts HHSN268201200036C, N01HC85239, N01 HC55222, N01HC85079, N01HC85080, N01HC85081, N01HC85082, N01HC85083, N01HC85086, and grant HL080295 from the National Heart, Lung, and Blood Institute (NHLBI), with additional contribution from the National Institute of Neurological Disorders and Stroke (NINDS). Additional support was provided by AG023629 from the National Institute on Aging (NIA). A full list of principal CHS investigators and institutions can be found at http://www.chs-nhlbi.org/pubs/PubAcknowGuidelines.htm; **FHS**: The Framingham Heart Study began in 1948 with the recruitment of an original cohort of 5,209 men and women (mean age 44 years; 55 percent women). In 1971 a second generation of study participants was enrolled; this cohort consisted of 5,124 children and spouses of children of the original cohort. The

mean age of the offspring cohort was 37 years; 52 percent were women. A third generation cohort of 4,095 children of offspring cohort participants (mean age 40 years; 53 percent women) was enrolled beginning in 2002. At each clinic visit, a medical history was obtained with a focus on cardiovascular content, and participants underwent a physical examination including measurement of height and weight from which BMI was calculated; **MESA**: The Multi-Ethnic Study of Atherosclerosis Study (MESA) is a multicenter prospective cohort study initiated to study the development of subclinical cardiovascular disease. A total of 6814 women and men between the age of 45 and 84 year were recruited for the first examination between 2000 and 2002. Participants were recruited in six US cities (Baltimore, MD; Chicago, IL; Forsyth County, NC; Los Angeles County, CA; Northern Manhattan, NY; and St. Paul, MN). This study was approved by the institutional review boards of each study site, and written informed consent was obtained from all participants. This cohort was genotyped as part of the National Heart Lung and Blood Institute's (NHLBI) Candidate Gene Association Resource (CARe) (Musunuru, K., Lettre, G., Young, T., Farlow, D.N., Pirruccello, J.P., Ejebe, K.G., Keating, B.J., Yang, Q., Chen, M.H., Lapchyk, N. et al. Candidate gene association resource (CARe): design, methods, and proof of concept. Circ. Cardiovasc. Genet, 3, 267-275.); **WHI**: The WHI program is funded by the National Heart, Lung, and Blood Institute, National Institutes of Health, U.S. Department of Health and Human Services through contracts HHSN268201100046C, HHSN268201100001C, HHSN268201100002C, HHSN268201100003C, HHSN268201100004C, and HHSN271201100004C. A listing of WHI investigators can be found at https://cleo.whi.org/researchers/Documents%20%20Write%20a%20Paper/WHI%20Investigator%20Short%20List.pdf.

Figure S1. Identification of previous Mendelian randomization studies investigating the causal role of BMI for cardiometabolic traits

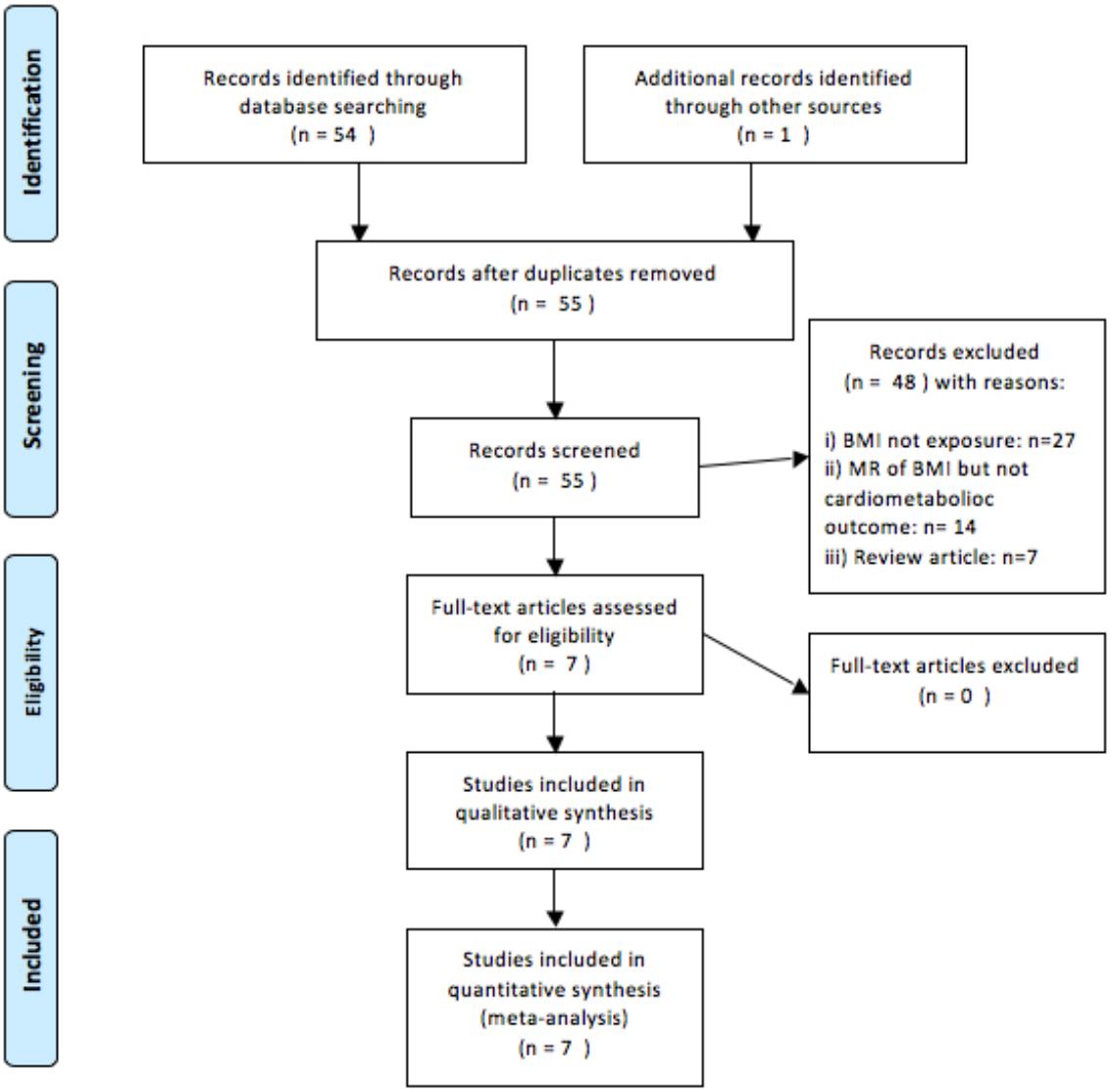


Figure S2. Allele frequencies of the 14 SNPs comprising the BMI genetic score in the collaborating studies

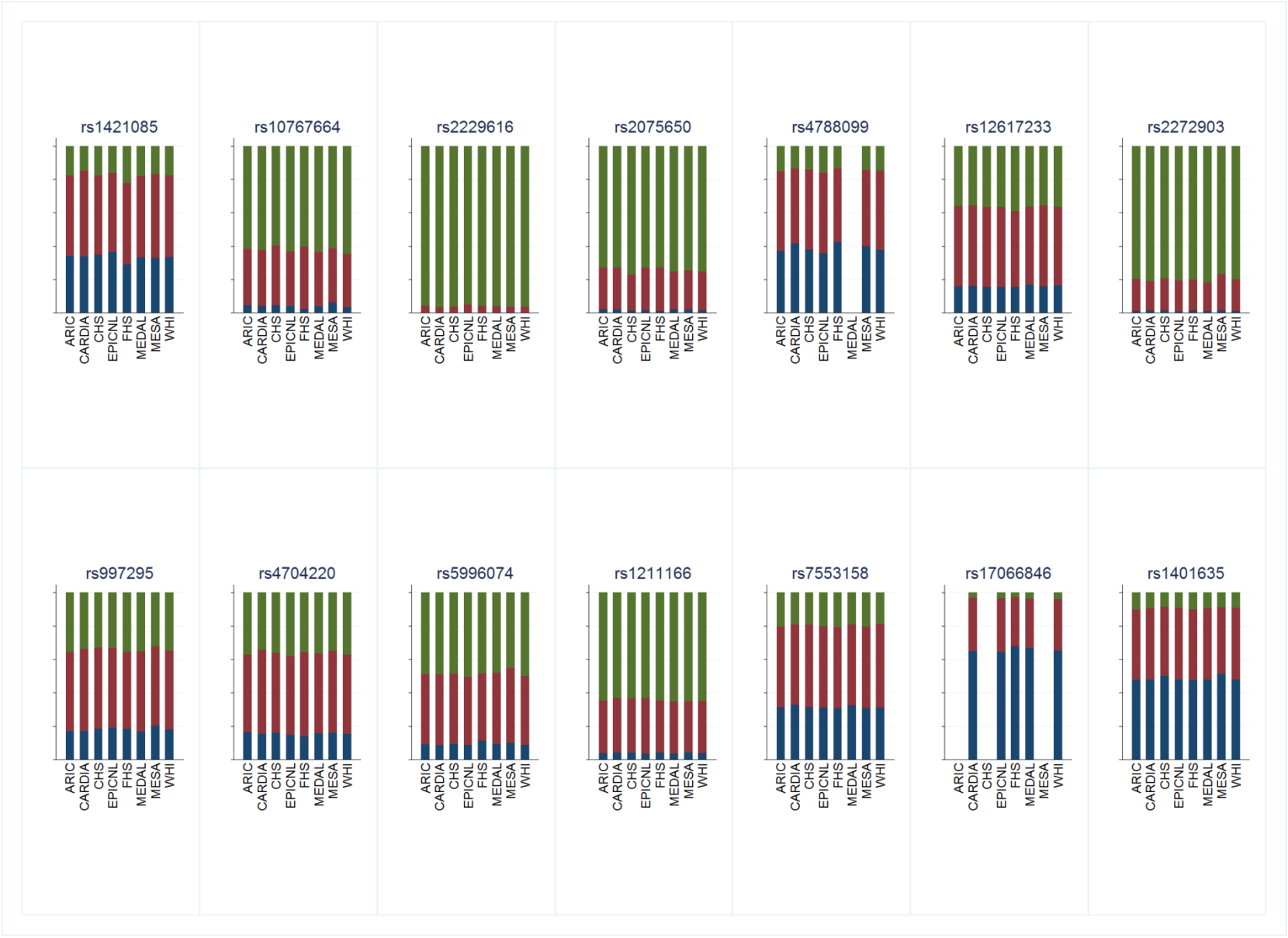


Figure S3. Histogram of weighted BMI genetic score in each study.

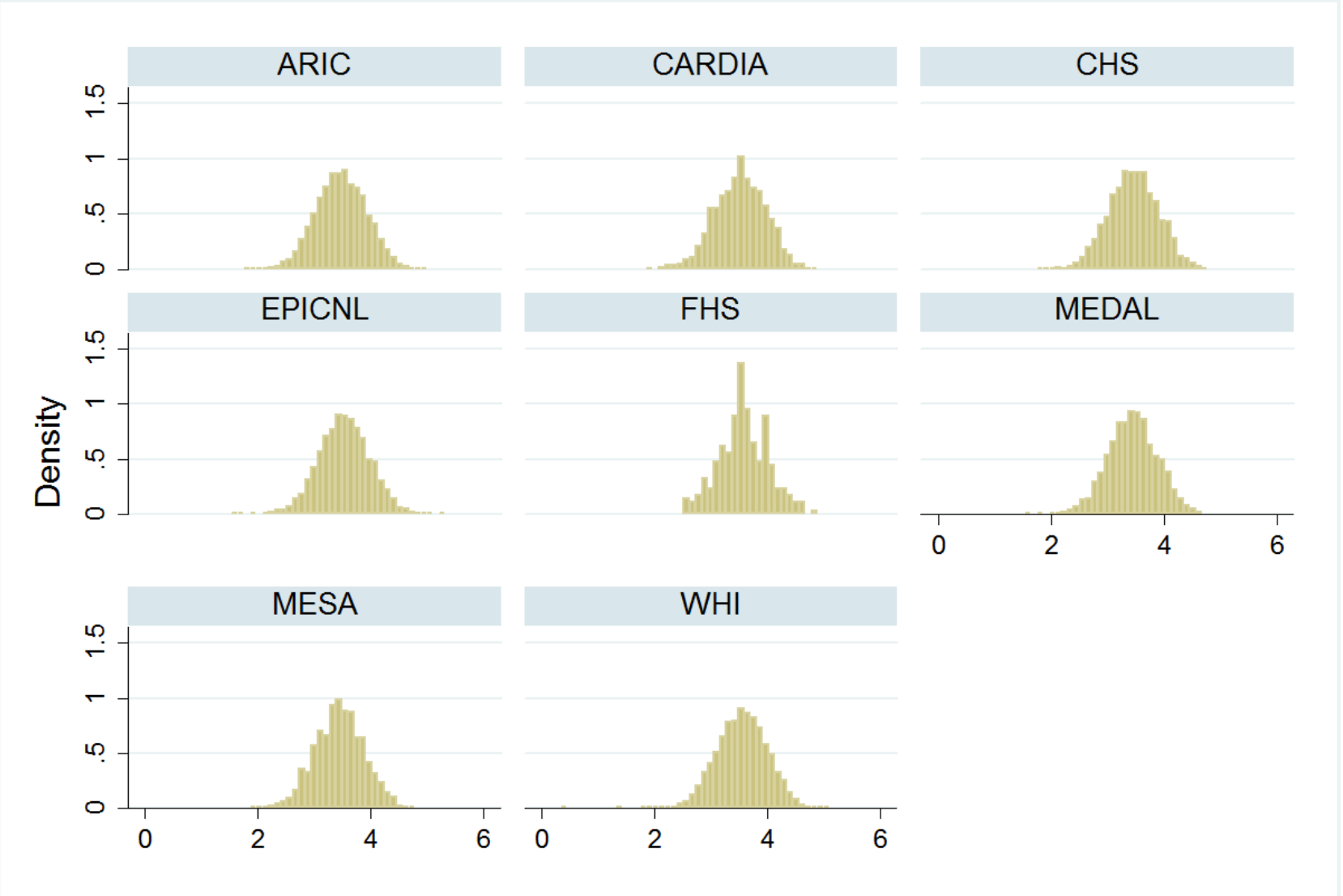
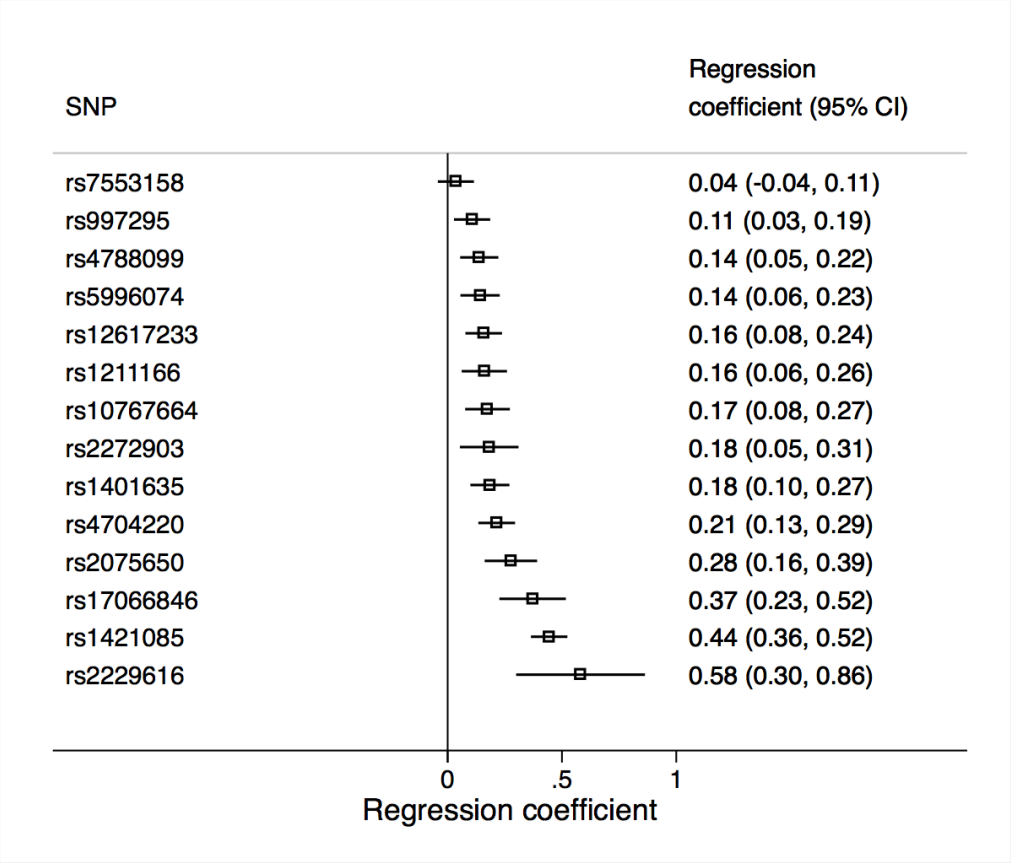
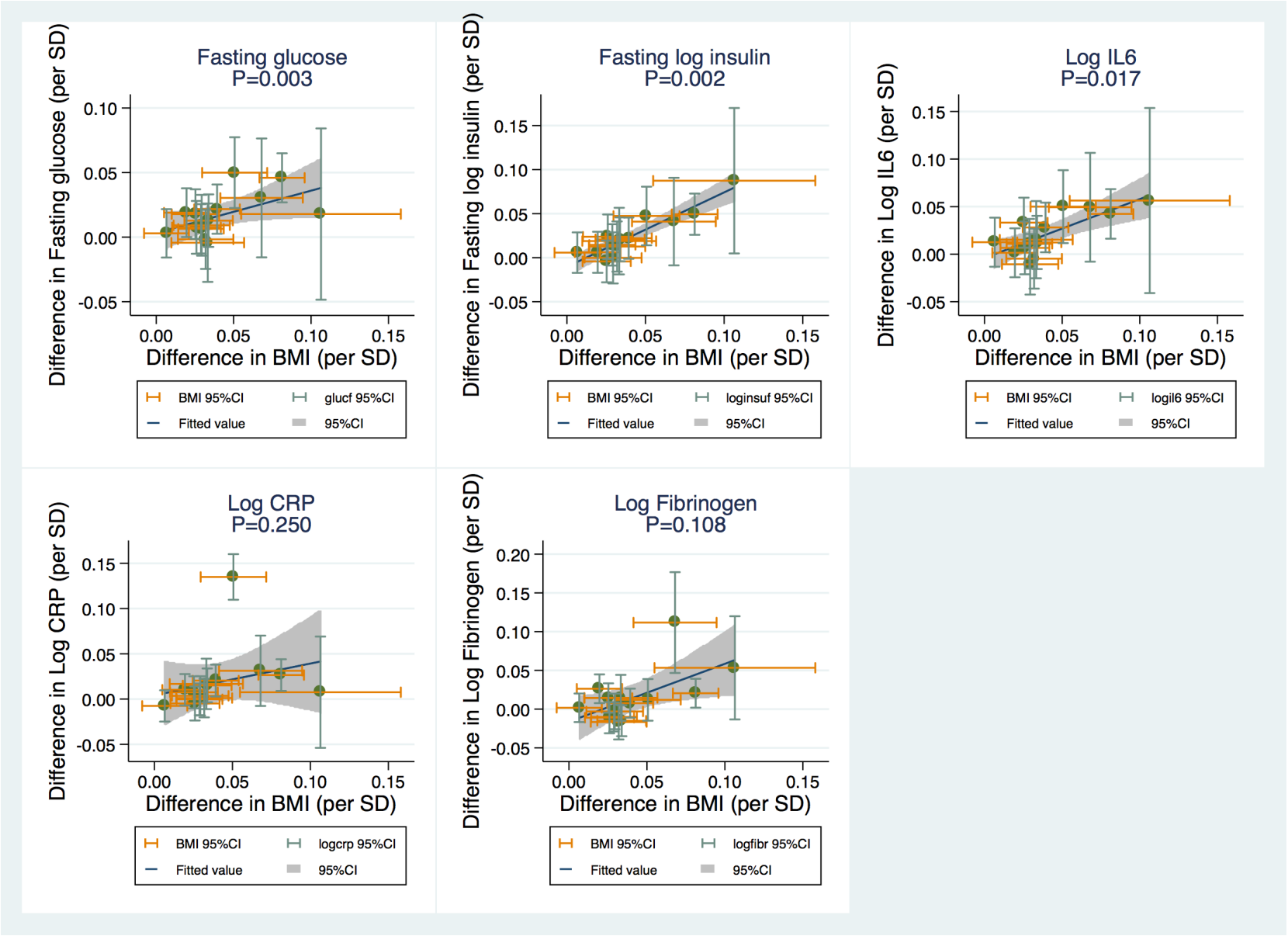


Figure S4. Meta-analysis pooled estimates of the association between each SNP in the genetic score and BMI.



Legend: regression coefficients represent the per-allele difference in BMI (kg/m^2) for each SNP.

Figure S5. Pair-wise association plots of the 14 SNPs in the BMI genetic score with each cardiometabolic trait.
P-values derived from meta-regression.



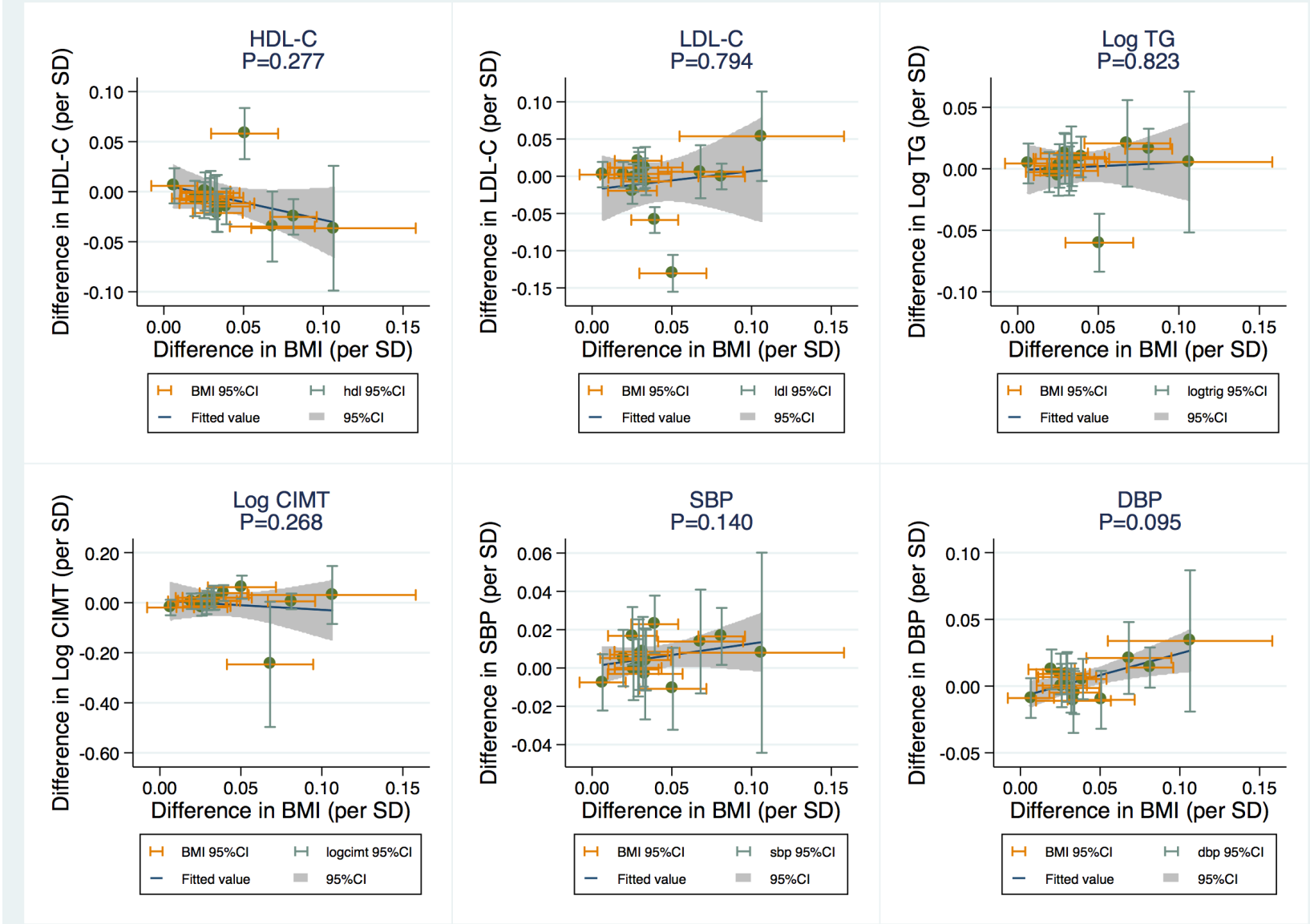
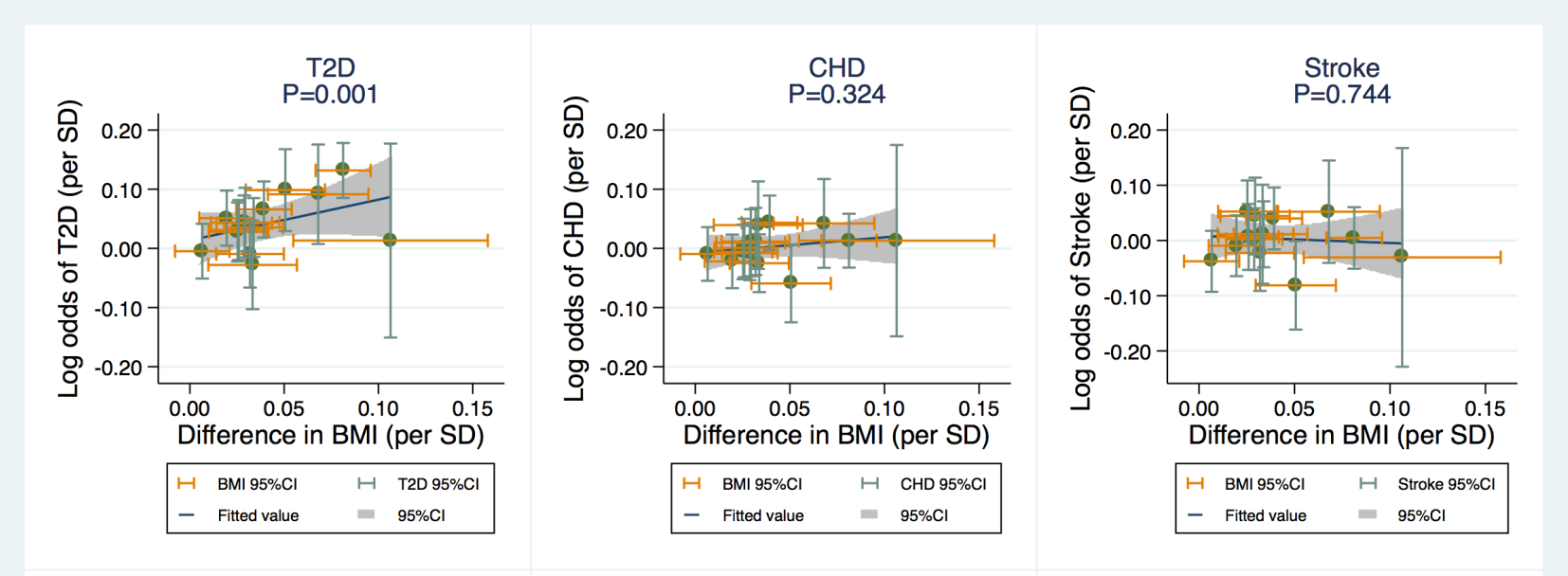
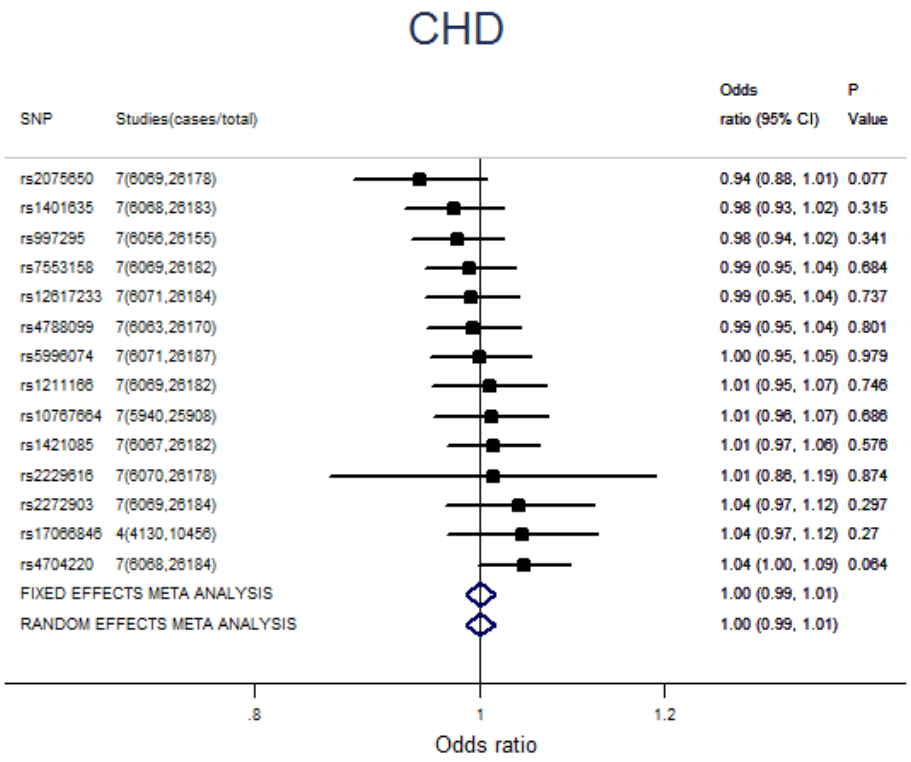

HDL-C
P=0.277
Difference in HDL-C (per SD)
0.10
0.05
0.00
-0.05
-0.10
0.00
0.05
0.10
0.15
Difference in BMI (per SD)
BMI 95%CI
hdl 95%CI
Fitted value
95%CI
LDL-C
P=0.794
Difference in LDL-C (per SD)
0.10
0.05
0.00
-0.05
-0.10
-0.15
0.00
0.05
0.10
0.15
Difference in BMI (per SD)
BMI 95%CI
ldl 95%CI
Fitted value
95%CI
Log TG
P=0.823
Difference in Log TG (per SD)
0.05
0.00
-0.05
-0.10
0.00
0.05
0.10
0.15
Difference in BMI (per SD)
BMI 95%CI
logtrig 95%CI
Fitted value
95%CI
Log CIMT
P=0.268
Difference in Log CIMT (per SD)
0.20
0.00
-0.20
-0.40
-0.60
0.00
0.05
0.10
0.15
Difference in BMI (per SD)
BMI 95%CI
logcimt 95%CI
Fitted value
95%CI
SBP
P=0.140
Difference in SBP (per SD)
0.06
0.04
0.02
0.00
-0.02
-0.04
0.00
0.05
0.10
0.15
Difference in BMI (per SD)
BMI 95%CI
sbp 95%CI
Fitted value
95%CI
DBP
P=0.095
Difference in DBP (per SD)
0.10
0.05
0.00
-0.05
0.00
0.05
0.10
0.15
Difference in BMI (per SD)
BMI 95%CI
dbp 95%CI
Fitted value
95%CI

Figure S6. Pair-wise association plots of the 14 SNPs in the BMI genetic score with cardiometabolic events.



Legend: P-values derived from meta-regression.

Figure S7. Association of the individual SNPs comprising the BMI genetic score with coronary heart disease (CHD) and type 2 diabetes (T2D).



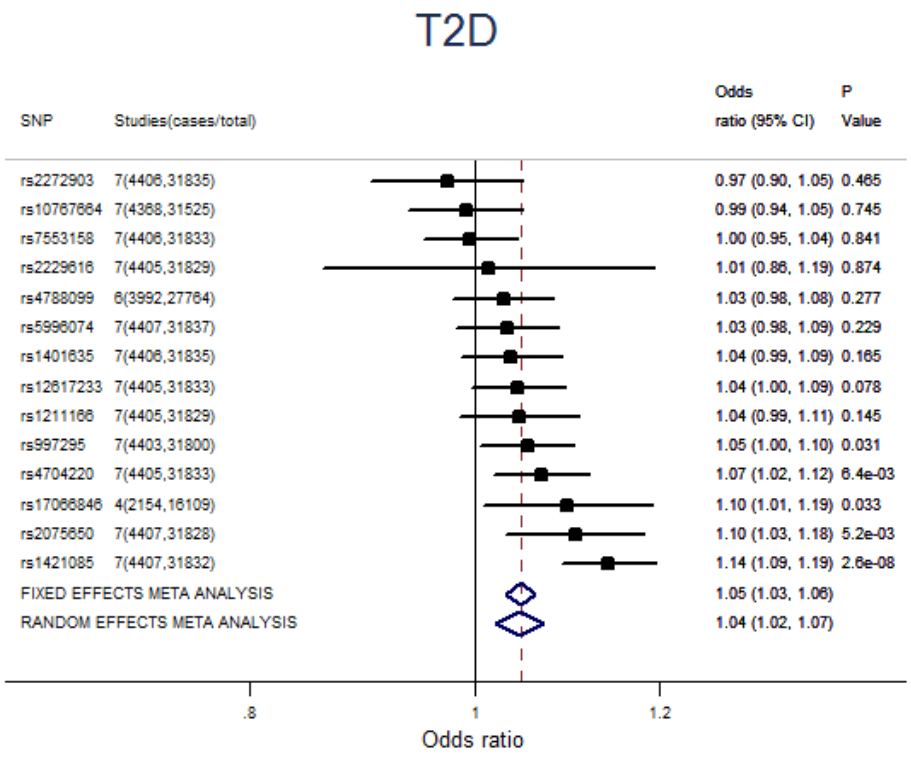


Table S1. Studies contributing towards the Mendelian randomization analysis

Study	Design	Geographical region	Recruitment design	Year of blood sampling for DNA measurement	Participants included	Sex, % female	Age, mean (SD)	Family structure
ARIC	Cohort	USA	Community-based	1987-1989	9549	53.5	54.28(5.69)	No
CARDIA	Cohort	USA	Community-based	1995-1996	1427	53.6	25.58(3.37)	No
CHS	Cohort	USA	Community-based	1992–1993	3924	56.2	72.78(5.60)	No
EPIC-NL	Nested-case control	Netherlands	Population-based	1993 and 1997	5192	78.1	54.05(10.11)	No
FHS	Cohort	USA	Community-based	1948-present	312	56.4	44.43 (10.52)	Yes [a]
MEDAL	RCT	Australia, European, South Africa, USA	Patients with osteoarthritis or rheumatoid arthritis	2002-2006	4054	29.2	63.21 (8.23)	No
MESA	Cohort	USA	Population	2000-2002	2293	52.3	62.70 (10.24)	No
WHI	RCT and cohort	USA	Community	1993-1998	7787	100	67.97(6.59)	No

Footnotes: [a] only the oldest person in each unit was used for analyses

Table S2. SNPs contributing to the BMI genetic score

SNP	Gene	Chromosome	Weights used for gene score [a]
rs2229616	*MC4R*	18q22	0.526
rs1421085	*FTO*	16q12.2	0.418
rs10767664	*BDNF*	11p13	0.202
rs2272903	*TFAP2B*	6p12	0.202
rs2075650	*TOMM40*	19q13	0.197
rs17066846	*MC4R*	18q22	0.179
rs4788099	*SH2B1*	16p11.2	0.146
rs1211166	*NTRK2*	9q22.1	0.136
rs1401635	*BDNF*	11p13	0.136
rs12617233	*FANCL/ FLJ30838*	2p16.1	0.132
rs997295	*MAP2K5*	15q23	0.122
rs5996074	*SREBF2*	22q13	0.122
rs4704220	*COL4A3BP/ HMGCR*	5q13.3	0.118
rs7553158	*TNN13K*	1p31.1	0.071

Footnote: [a] beta coefficients obtained from a large-scale gene-centric analysis[1] (originally reported on the per SD scale) were multiplied by 4.7 (obtained from a pooled dataset of 1,462,958 Caucasian individuals[2]) to yield a regression coefficient on the native units (kg/m^2).

Table S3. Outcome definitions in the contributing studies

Study	Prevalent/incident coronary heart disease	Prevalent/ incident stroke	Prevalent/incident type 2 diabetes
CARe studies (ARIC, CHS, CARDIA, FHS, MESA)	Prevalent MI was defined as history of MI at first study visit; prevalent MI was adjudicated for ARIC and CHS and self-reported for the FHS and MESA. Adjudicated incident MI status was available for all CARe studies.	Prevalent stroke was defined as self-reported history of stroke at first study visit for all CARe studies. Adjudicated incident stroke status was available for all CARe studies.	Prevalent diabetes was based on diabetes status at first visit for all CARe studies. For ARIC, prevalent diabetes was based on diabetes at first visit defined by a fasting glucose of less than 126 mg/dL or self-report of physician-diagnosed type 2 diabetes. Incident diabetes status was based on self-report of physician-diagnosed diabetes at visits 2-4. For CHS, prevalent diabetes was based on American Diabetes Association (ADA) criteria at visit 1. Incident diabetes was based on diabetes status at visits 3-5, 9 and 10, where diabetes status was determined by either self-report or ADA criteria. Diabetes status was only available for the Framingham Heart Study offspring sub-cohort. Prevalent diabetes was based on self-report physician-diagnosed diabetes at first visit and incident diabetes status was determined using self-report of physician-diagnosed diabetes for visit 2-7. For MESA, prevalent diabetes was determined using ADA criteria at first visit and incident diabetes was determined using ADA criteria at visits 2-4.
EPIC-NL	Incident coronary heart disease during follow-up was obtained through linkage with the database of hospital discharge diagnoses from the Dutch National Medical Registry. Mortality information was obtained through linkage with the Cause of Death Registry at Statistics Netherlands. CHD cases were classified as persons with a first event (either fatal or non-fatal of ICD-9:410-414, incl. sub codes 427.5, 798.1, 798.2, 798.9 and ICD-10:I20, I23 t/m I25, incl. all sub codes).	N/A	N/A
MEDAL	CHD was classified as clinical history of myocardial infarction, angina pectoris, angioplasty, carotid artery disease, or coronary artery bypass surgery.	N/A	Type 2 Diabetes was defined from self-report of physician-diagnosed status (we note that MEDAL has been used in a previous T2D study[3] and the positive control *TCF7L2* T2D signal was evident.
WHI	CHD events were defined as nonfatal MI and CHD death initially identified by semi-annual questionnaires followed by central adjudication from medical record review. Definite and probable nonfatal MI required overnight hospitalization and was defined according to an algorithm based on standardized criteria using cardiac pain, cardiac enzymes and troponin levels, and ECG findings. CHD death was defined as death consistent with underlying cause of CHD plus one or more of the following: hospitalization for myocardial infarction within 28 days prior to death, previous angina or myocardial infarction, death due to a procedure related to CHD, or a death certificate consistent with underlying cause of atherosclerotic CHD.[4]	N/A	Type 2 Diabetes was defined from self-report of physician-diagnosed status (we note that WHI was also represented in a previous T2D study[3] and the positive control *TCF7L2* T2D signal was evident.

Table S4. Selection of SNPs for construction of stricter genetic score.

SNP	Gene	Associations of gene in GWAS catalog	Included in strict GS? [a]
rs1421085	*FTO*	T2D, BMI, OA, sex hormone binding globulins, Metabolic syndrome, Weight	No
rs10767664	*BDNF*	BMI, smoking, weight	No
rs2229616	*MC4R*	urate, BMI, BP, height, waist circumference	No
rs2075650	*TOMM40*	macular degeneration, cognition, CRP, Metabolic syndrome , CVD risk factors, Longevity, Alzheimer's, HDL, LDL, TG	No
rs4788099	*SH2B1*	Crohn's disease, BMI, weight	No
rs12617233	*FANCL/ FLJ30838*	Obesity, BMI	**Yes**
rs2272903	*TFAP2B*	renal function, obesity, Metabolic syndrome, BMI	No
rs997295	*MAP2K5*	BMI, restless leg syndrome,	No
rs4704220	*COL4A3BP/ HMGCR*	BMI, LDL-C,	No
rs5996074	*SREBF2*	N/A	**Yes**
rs1211166	*NTRK2*	N/A	**Yes**
rs17066846	*MC4R*	urate, BMI, BP, height, waist circumference	No
rs1401635	*BDNF*	BMI, smoking, weight	No
rs7553158	*TNN13K*	N/A	**Yes**

Footnote: [a] SNPs that associated with additional non-adiposity traits were excluded from the stricter GS.

Table S5. Instrumental variable (causal) estimates for the relationship of BMI with incident CHD and stroke.

Outcome	Studies	Cases	Total	Odds ratio[a]	Lower 95%CI limit	Upper 95%CI limit
Coronary heart disease (incident-only)	6	5422	24079	0.99	0.93	1.07
Stroke (incident-only)	5	3534	18680	1.04	0.96	1.13

Footnotes: [a] estimates represent the causal odds ratio per 1 kg/m^2 increase in BMI

Table S6. Instrumental variable (causal) estimates for the relationship of BMI with cardiometabolic traits and outcomes adjusted for age and sex.

Trait (units)	**Studies**	**Cases**	**Total**	**Regression coefficient[a]**	**Lower 95%CI limit**	**Upper 95%CI limit**
Glucose (mmol/l)	6	N/A	20677	0.18	0.12	0.23
log Insulin (pmol) [b]	3	N/A	12758	8.59	6.04	11.20
log CRP (mg/l) [b]	7	N/A	24319	12.3	8.24	16.51
log IL6 (pg/ml) [b]	5	N/A	9885	7.47	4.43	10.6
log Fibrinogen (g/l) [b]	6	N/A	19041	1.05	0.38	1.73
SBP (mmHg)	6	N/A	30136	0.87	0.42	1.31
HDL-C (mmol/l)	6	N/A	24943	-0.012	-0.023	-0.002
LDL-C (mmol/l)	6	N/A	23364	-0.04	-0.06	-0.01
log TG (mmol/l) [b]	6	N/A	24761	0.80	-0.62	2.24
log cIMT (mm) [b]	3	N/A	6260	1.38	-0.08	2.85
Outcome	**Studies**	**Cases**	**Total**	**Odds ratio[a]**	**Lower 95%CI limit**	**Upper 95%CI limit**
Type 2 diabetes	7	4407	31844	1.28	1.19	1.38
Coronary heart disease	7	6073	26193	1.02	0.95	1.09
Coronary heart disease [c]	6	4161	21610	1.04	0.96	1.14
Stroke	6	3813	23782	1.05	0.96	1.14
Lipid lowering therapy	5	2999	24980	0.94	0.85	1.05

Footnotes: [a] Values represent instrumental variable (causal) estimates for the effect of a 1 kg/m^2 increase in BMI. [b] for log-transformed variables, the regression coefficients are presented as a percentage difference in the geometric mean. [c] additionally adjusted for LDL-C.

Table S7. Instrumental variable (causal) estimates for the relationship of BMI with incident CHD and stroke using a stricter GS.

Outcome	Studies	Cases	Total	Odds ratio[a]	Lower 95%CI limit	Upper 95%CI limit
T2D	7	4407	31844	1.24	1.01	1.52
Coronary heart disease	7	6073	26193	1.00	0.82	1.20
Stroke	6	3813	23782	1.05	0.83	1.32

Footnotes: [a] estimates represent the causal odds ratio per 1 kg/m^2 increase in BMI

Table S8. Consistency of estimates derived from instrumental variable analysis in Mendelian randomization studies.

	Evidence for causality (↑= positive; ↓= negative; X = none; n/a not analysed)								**Consistent evidence across studies? (Y=yes, N=no)**
Study, first name (reference)	Holmes et al (present study)	Fall et al [5]	Nordestgaard et al [6]	Welsh et al [7]	Timpson et al [8]	Kivimaki et al [9]	Timpson et al [10]	Freathy et al [11]	
Sample size	34,538	198,502	75,627	5804	36,867	2230	21,836	16,639	
# of SNPs for BMI genetic instrument (gene)	14 (see **Table S2**)	1 (*FTO*)	3 (*FTO, MC4R, TMEM18*)	2 (*FTO, MC4R*)	2 (*FTO, MC4R*)	1 (*FTO*)	1 (*FTO*)	1 (FTO)	
R^2/F-statistic	0.8%/237	Not reported	Not reported	Not reported	F>60	R^2=0.4%	F=31.1	Not reported	
Ethnicity	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	
Cardio-metabolic traits									
Fasting glucose	↑	X	n/a	n/a	n/a	X /↑ [a]	n/a	↑	N
Insulin	↑	↑	n/a	n/a	n/a	n/a	n/a	↑	Y
CRP	↑	↑	n/a	↑	n/a	n/a	↑	n/a	Y
IL6	↑	X	n/a	n/a	n/a	n/a	n/a	n/a	N
Fibrinogen	↑	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
HDL-C	↓	↓	n/a	n/a	n/a	n/a	n/a	↓	Y
LDL-C	↓	X	n/a	n/a	n/a	n/a	n/a	n/a	N
Triglycerides	X	n/a	n/a	n/a	n/a	n/a	n/a	↑	N
SBP	↑	↑	n/a	n/a	↑	↑/↑ [a]	n/a	n/a	Y
DBP	↑	↑	n/a	n/a	↑	n/a	n/a	n/a	Y
cIMT	X	n/a	n/a	n/a	n/a	X /↑ [a]	n/a	n/a	N
Events									
T2D	↑	↑	n/a	n/a	n/a	n/a	n/a	n/a	Y
CHD	X	X	↑	n/a	n/a	n/a	n/a	n/a	N
Stroke	X	X	n/a	n/a	n/a	n/a	Y	n/a	Y

Footnotes: [a] First estimate refers to patient data, second refers to simulated data (e.g. X/↑ means that in the participant data, no evidence was found, whereas in simulated data, a positive association between BMI and the trait emerged). N/a: not applicable (not investigated).

Supplemental References

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