Assessing the causal role of body mass index on cardiovascular health in young adults: Mendelian randomization and recall-by-genotype analyses

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Short title: Causal role of body mass index and cardiovascular health

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ABSTRACT

Background: Mendelian randomization (MR) studies of body mass index (BMI) and cardiovascular health in mid-to-late life suggest causal relationships, but the nature of these has not been explored systematically at younger ages. Using complementary MR and recall-by-genotype (RbG) methodologies, our objective was to estimate the causal effect of BMI on detailed measures of cardiovascular health in a population of young healthy adults.

Methods and Findings: Data from the Avon Longitudinal Study of Parents and Children were used. For MR analyses, a genetic risk score (GRS) comprising 97 independent single nucleotide polymorphisms (SNPs) and constructed using external weighting was used as an instrument to test the causal effect of each unit increase in BMI (kg/m^2) on selected cardiovascular phenotypes measured at age 17 (N=7909). An independent enriched sample from the same cohort participated in a RbG study at age 21, which enabled more detailed cardiovascular phenotyping (N=418; 191/227 from the lower/upper ~30% of a genome-wide GRS distribution predicting variation in BMI). The causal effect of BMI on the additional cardiovascular phenotypes was assessed by comparing the two recalled groups. Difference in mean BMI between RbG groups was $3.85 kg/m^2$ (95% CI: 2.53, 4.63; $P=6.09 \times 10^{-11}$). In both MR and RbG analyses, results indicated that higher BMI causes higher blood pressure (BP) and left ventricular mass (indexed to $height^{2.7}$, LVMI) in young adults (e.g. difference in LVMI per kg/m^2 using MR: $1.07 g/m^{2.7}$; 95% CI: 0.62, 1.52; $P=3.87 \times 10^{-06}$ and per $3.58 kg/m^2$ using RbG: $1.65 g/m^{2.7}$ 95% CI: 0.83, 2.47; $P=0.0001$). Additionally, RbG results indicated a causal role of higher BMI on higher stroke volume (SV; difference per $3.58 kg/m^2$: $1.49 ml/m^{2.04}$; 95% CI: 0.62, 2.35; $P=0.001$) and cardiac output (CO; difference per

3.58kg/m^2: 0.11l/min/m$^{1.83}$; 95% CI: 0.03, 0.19; *P*=0.01). Neither analysis supported a causal role of higher BMI on heart rate.

Conclusions: Complementary MR and RbG causal methodologies, together with a range of appropriate sensitivity analyses, showed that higher BMI is likely to cause worse cardiovascular health, specifically higher BP and LVMI, even in youth. These consistent results support efforts to prevent or reverse obesity in the young.

Key words: Body mass index, cardiovascular traits, ALSPAC, Mendelian randomization, recall-by-genotype, causality

Introduction

Higher body mass index (BMI) in adulthood is likely to be causally associated with numerous cardiovascular risk factors and disease outcomes[1-6]. Whilst chronic conditions may have origins early on, these relationships have been assessed predominantly from the 5th decade of life[7]. It is assumed that these relationships reflect long-term exposure to adiposity and other co-morbidities, which result in adverse structural and functional cardiovascular changes that are different from adaptations encountered earlier in the disease's evolution.

Numerous observational studies have reported associations between higher BMI and the presence of various subclinical markers of cardiovascular disease (CVD) even during young adulthood[8-11]. However, these observational study designs preclude a distinction between correlation and causation due to issues such as confounding or possible reverse causation (particularly in studies of older adults, whereby the 'outcome' is responsible for the variation in the 'exposure'). Whilst recent results from patients who have had bariatric surgery provide supportive evidence for a causal role of greater adiposity on risk of major cardiovascular events[4, 5], no large studies have

assessed the causal impact between BMI and cardiovascular phenotypes in early life where risk emerges.

One method for establishing evidence for causal relationships between an exposure and outcome of interest is Mendelian Randomization (MR), a technique which uses genetic variations as instrumental variables (IVs) in observational epidemiological studies[12, 13]. Due to the technique's requirement for relatively large sample sizes to provide adequate statistical power, however, MR studies traditionally use either routinely collected clinical measures or data generated from high-throughput technologies. Detailed and precise subclinical measures of early structural and functional vascular adaptations are not commonly carried out in large population studies, as these are expensive, time-consuming and require highly skilled operators. This has limited their suitability for analyses using MR methodology.

Recall-by-genotype (RbG) studies are an innovative extension of MR designed to improve study efficiency through the creation of subgroups selected based on genotypes possessing known correlations with exposures of interest (e.g. BMI), rather than random sampling based on extremes of BMI itself. The added statistical power of this technique enables the efficient collection of extremely precise phenotypic data that may be otherwise impractical at the scale necessary for MR analyses[14, 15].

Using data from the Avon Longitudinal Study of Parents and Children (ALSPAC), we aimed to employ these two complementary analytical approaches (MR and RbG), alongside conventional multivariable regression analyses, to support the hypothesis that BMI causally influences variations in multiple clinically relevant measures of cardiovascular structure and function in adolescence and early adulthood, when risk emerges.

METHODS

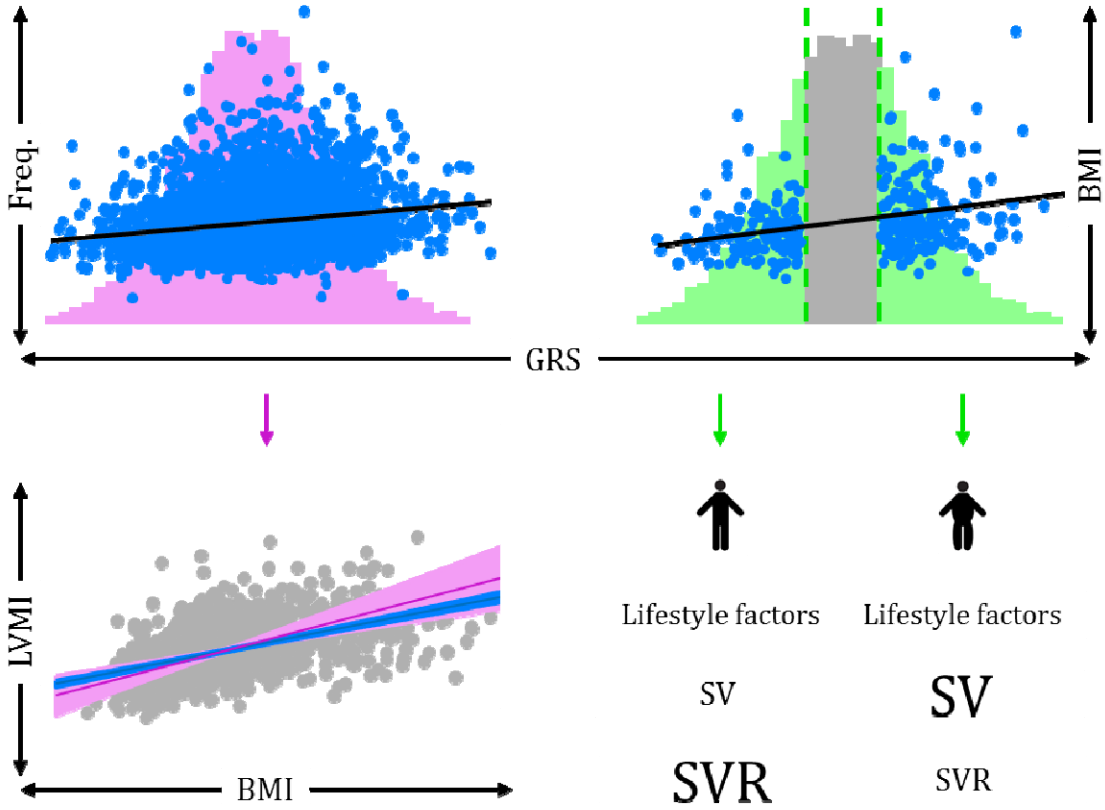
Cohort description

The Avon Longitudinal Study of Parents and Children (ALSPAC) is a prospective birth cohort study investigating factors that influence normal childhood development and growth. The cohort and study design have been described in detail previously[16, 17] and are available at the ALSPAC website (http://www.alspac.bris.ac.uk). The study website contains details of all data that is available through a fully searchable data dictionary (http://www.bris.ac.uk/alspac/researchers/data-access/data-dictionary). Briefly, 14541 pregnant women resident in a defined area of the South West of England, with an expected delivery date of 1st of April 1991 - 31st of December 1992 were enrolled to the cohort. Of these, 13988 live-born children who were still alive 1 year later have been followed-up to date with regular questionnaires and clinical measures, providing behavioural, lifestyle and biological data. Ethical approval for the study was obtained from the ALSPAC Ethics and Law Committee and the Local Research Ethics Committee and written informed consent was obtained from both the parent/guardian and, after the age of 16, children provided written assent.

Study design

The two complementary analytical approaches (MR and RbG) were used to support the hypothesis that BMI causally influences variations in multiple clinically relevant measures of cardiovascular structure and function in adolescence and early adulthood (Figure 1). Firstly, we used a genetic risk score (GRS) comprising 97 BMI-associated single nucleotide polymorphisms (SNPs) constructed using external weighting as an IV within an MR framework to investigate the causal effect of BMI on a range of vascular measures collected from 17-year-old participants[18].

Figure 1. Mendelian randomization (left) and recall-by-genotype (right) methodologies



Top left: *The entire distribution of a genetic risk score (GRS, in pink) is used as an instrumental variable (IV) for body mass index (BMI) in a Mendelian randomization (MR) analysis to assess the causal nature of association between BMI and cardiovascular phenotypes (e.g. left ventricular mass index, LVMI).*
Bottom left: *Comparison of observational multivariable regression and MR-derived estimates using conventional MR methodology.*
Top right: *Instead of using the entire distribution of a GRS, the recall-by-genotype (RbG) method creates genetically recalled samples from the tails of a GRS distribution (green).*
Bottom right: *The RbG groups show a difference in mean BMI, however, there are no differences in confounding factors ("lifestyle factors"). The RbG method allows us to assess the change in detailed cardiovascular measures obtained through precise techniques, which would not otherwise be feasible in large enough studies needed for MR methodology (here, stroke volume [SV] and systemic vascular resistance [SVR]), between the recalled groups.*

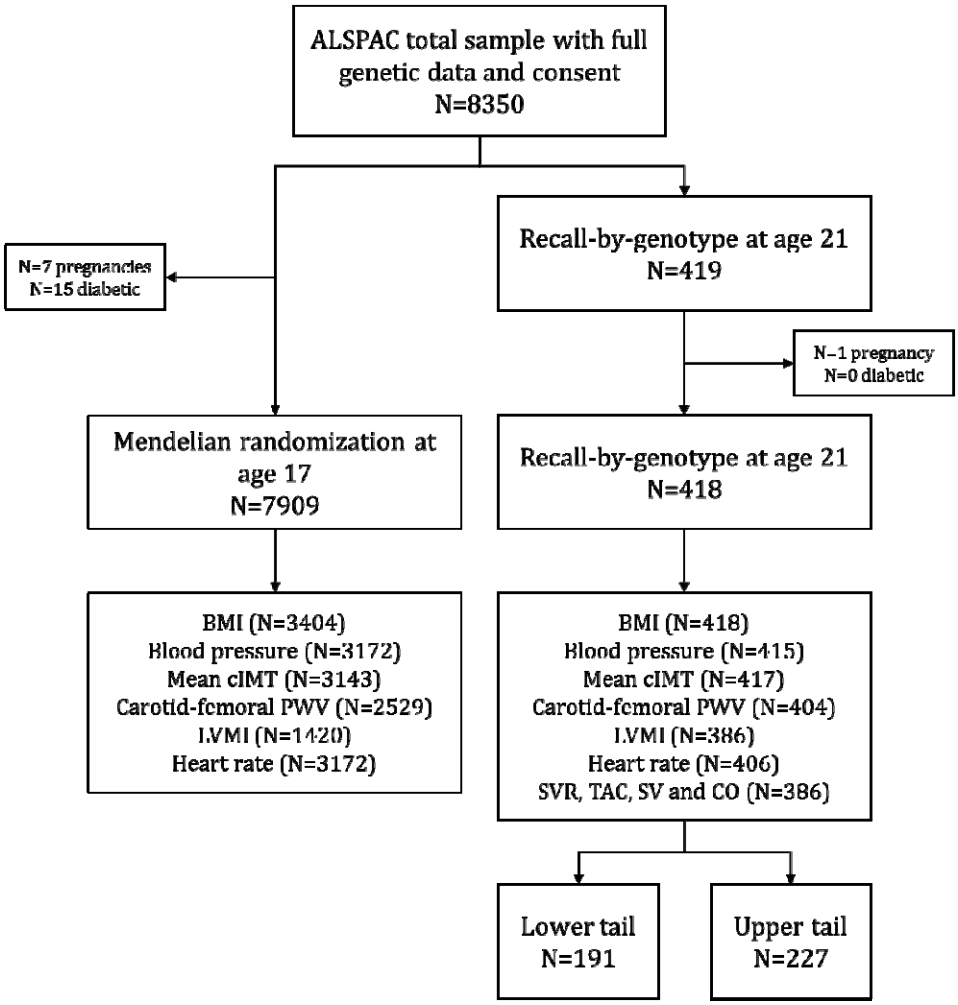
In parallel with this approach, we examined the ability of a highly-powered RbG design to reproduce these findings and explore their underlying mechanisms further through the extensive phenotyping of a smaller group of independent individuals, recalled specifically on a genome-wide GRS distribution[19].

Of those with full genetic data and consent (N=8,350), individuals were invited to the RbG study based on the lower and upper ~30% of a genome-wide GRS distribution, constructed from results from a genome-wide association study (GWAS) of BMI conducted by Speliotes *et al.*[19]. Individuals were recalled based on their appearance in the sampling groups, from the most extreme to the least to maximise power and difference in BMI. Of those invited (N=4602), 419 individuals were successfully recalled across the distribution of invited sampling groups (Supplementary Methods).

We excluded data of all females who were pregnant or individuals who had diabetes at both the 17-year clinic (N=7 pregnancies and 15 diabetics) and the 21-year recall (N=1 pregnancy and 0 diabetics). After exclusions, 7,909 individuals at age 17 were included in MR-analyses, which used a GRS comprising 97 SNPs (constructed using external weighting) shown to be associated with BMI from a large-scale GWAS[18]. The independent sample of 418 individuals were used in the 21-year RbG analyses (Figure 2).

153 Figure 2. Flow of samples used for Mendelian randomization and recall-by-genotype

154 studies



155

156 *The total number of individuals in ALSPAC with full genetic data and consent was 8,350. Of*

157 *these, 7,909 individuals were used in MR analyses at age 17, which used a GRS comprising*

158 *97 SNPs (and constructed using external weighting) shown to be associated with BMI from*

159 *a large-scale GWAS[18]. The independent sample of 418 individuals was used in the RbG*

160 *study, based on the lower and upper ~30% of a continuous genome-wide GRS distribution*

161 *for BMI, constructed on the basis of results from a GWAS of BMI[19]. A total of 191 were*

162 *within the lower tail and 227 were in the upper tail. The number of individuals with*

163 *available data on the exposure (BMI) and cardiovascular outcomes are also presented.*

Genotyping

Participants were genotyped using the Illumina HumanHap550 quad genome-wide SNP genotyping platform[20]. Participants were excluded due to having at least one of: incorrectly recorded sex, minimal or excessive heterozygosity, disproportionate levels of individual missingness, evidence of cryptic relatedness or non-European ancestry[20, 21]. SNPs with a minor allele frequency (MAF) of <1% and call rate of <95% were removed and only SNPs that passed an exact test of Hardy-Weinberg equilibrium ($P<5x10^{-7}$) were included. Imputation of genotypes was conducted with MACH 1.0.16 Markov Chain Haplotyping software, using CEPH individuals from phase 2 of the HapMap project as a reference (release #22).

Measures of adiposity at age 17 and 21

At both ages, height was measured to the nearest centimetre using a stadiometer (SECA 213, Birmingham, UK) and weight to the nearest 0.1 kilogram, unshod and in light clothing, using electronic weighing scales (Marsden M-110, Rotherham, UK). BMI was calculated as weight (kg) divided by height-squared (m^2).

Cardiovascular phenotypes at age 17

The following cardiovascular phenotypes were used in MR analyses to assess the causal role of BMI on cardiovascular health at age 17.

Blood pressure and heart rate

Sitting BP and heart rate were measured in both arms with an Omron 705 IT oscillometric BP monitor in accordance with European Society of Hypertension guidelines[22]. The average of the final two of three readings was used and the arm

189 with the greatest number of valid observations was used for analyses. From the systolic
190 and diastolic BP readings (SBP and DBP, respectively), mean arterial pressure (MAP)
191 was calculated (DBP + (SBP-DBP)/3). Pulse pressure (PP) was calculated as the
192 difference between SBP and DBP.
193
194 *Pulse-wave velocity (PWV)*
195 Aortic stiffness (carotid-femoral PWV) was assessed using a Vicorder device
196 (Skidmore Medical, UK). Participants rested supine on a couch with their head raised to
197 30°. Real-time pulse-wave measures were recorded between proximal (right carotid)
198 and distal (the upper right thigh) sensor cuffs and with the time delay between the two
199 simultaneously measured cardiac cycles measured. Transit distance was measured from
200 suprasternal notch directly to the top of the thigh cuff. Measurements were taken until
201 pressure waveforms over the carotid and thigh area were of high quality and
202 reproducible. Three carotid-femoral PWV measurements, within ≤0.5 m/s of each other,
203 were averaged.
204
205 *Carotid intima-media thickness (cIMT)*
206 Common carotid artery B-mode ultrasound images were acquired in the ear–to-
207 ear plain with the head rotated to 45° from the midpoint using a Zonare Z.OneUltra
208 system equipped with a L10-5 linear transducer (Zonare Medical Systems, CA, US).
209 Images were recorded in Digital Imaging and Communications in Medicine (DICOM)
210 format as 10 second cine-loop files for offline analysis using the Carotid Analyzer
211 (Medical Imaging Applications, Coralville, IA). Left and right cIMT were taken to be the
212 average of three end-diastolic measurements located on the far-wall of a single segment

of arterial wall 5–10 mm in length and 10 mm proximal to the bifurcation. The mean of left and right cIMT was calculated and used in analyses.

Left ventricular mass (LVM)

A sub-sample of study participants from the 17-year clinic underwent echocardiography using a HDI 5000 ultrasound machine (Phillips) and P4-2 Phased Array ultrasound transducer using a standard examination protocol. Left ventricular mass (LVM) was estimated according to American Society of Echocardiography (ASE) guidelines[23].

Cardiovascular phenotypes at age 21

The following detailed cardiovascular phenotypes were also measured in the two RbG groups at age 21.

Blood pressure and heart rate

BP and heart rate were measured in the right arm in the supine position using a digital automated sphygmomanometer (Omron M6, Omron Healthcare, Netherlands) and the mean of two values used for analyses. MAP and PP were calculated in the same way as at 17 years.

Pulse-wave velocity

Arterial stiffness was assessed using applanation tonometry (SphygmoCor Vx, AtCor Medical, NSW, Australia), with PWV estimated using ECG-gated pulse waves travelling between carotid-femoral sites. The patient was rested in a supine position and a handheld tonometer was placed over the left carotid artery in order to allow the

238 recording of 10-12 clear and reproducible pressure waveforms. The same tonometer
239 was then used to measure a similar number of femoral arterial pulse waveforms in the
240 inguinal crease at the top of the right leg. Transit distance was measured between the
241 upper edge of the suprasternal notch and the femoral pulse measurement site via the
242 umbilicus using a tape measure. The device software calculated the mean transit time
243 (in milliseconds) from the recorded pulse waveforms and PWV was calculated as the
244 transit distance/transit time.
245
246 *Carotid intima-media thickness*
247 Ultrasound assessment of the thickness of intima media interface was measured
248 using an identical protocol to that described for age 17.
249
250 *Cardiovascular Magnetic Resonance Imaging (MRI)*
251 All measures were made using a 1.5T MR scanner (Avanto, Siemens Medical
252 Solutions, Erlangen, Germany). Endocardial borders of the left ventricle (LV) were
253 traced manually on short axis stacks at end-diastole and end-systole to evaluate end-
254 diastolic volume (EDV) and end-systolic volume (ESV). Stroke volume (SV) was
255 obtained by subtracting ESV from EDV. Epicardial borders were traced in end-diastole
256 to calculate an epicardial volume. The EDV was subtracted from this volume, multiplied
257 by assumed myocardial density to obtain LVM.
258 Flow quantification was performed through-plane in a cross-section of the
259 ascending aorta as it passes the bifurcation of the pulmonary arteries using an ECG-
260 gated spiral phase-contrast MR sequence, as described previously[24]. This technique
261 allows images to be acquired within a short breath-hold (0.5 seconds) with a spatial
262 resolution of 1.6 x 1.6 mm and a temporal resolution of 30 milliseconds. All images were

263 processed using in-house plug-ins for the Open source software OsiriX (OsiriX
264 Foundation, Geneva, Switzerland). Flow images were manually segmented (using the
265 modulus images) and SV (ml) was measured and cardiac output (CO, L/min) was
266 calculated as SV x heart rate. At the time of flow imaging, BP was simultaneously
267 measured using MRI-compatible oscillometric sphygmomanometer (Datex Ohmeda).
268 Systemic vascular resistance (SVR; measured in $mmHg.L^{-1}.min^{-1}$) was calculated by
269 dividing the measured mean BP by CO. Total arterial compliance (TAC) was calculated
270 by optimisation of the two-element Windkessel model, as previously described[25].
271 Briefly, the flow curves and SVR were used as inputs to the model. PP was calculated for
272 a series of modelled pressure curves generated using a range of TAC values from 0.1 to
273 5.0 $mL.mmHg^{-1}$ in increments of 0.01. The compliance value that gave the smallest error
274 between the modelled PP and the true PP was taken to be the true compliance.
275
276 ***Confounders***
277 Due to potentially confounding effects, the following variables were added as
278 covariates in the multivariable regression analyses: maternal education and household
279 occupation, plus current smoking status and the most recent records of physical activity
280 and, where available, dietary intake.
281 At enrolment, mothers reported their educational attainment and both her and
282 her partner's occupation. Highest household occupation was used to assign participants
283 a household social class, using the 1991 British Office of Population Census Statistics
284 classification[26]. Both maternal education and household social class were used as a
285 general indication of socioeconomic position.
286 The most recent measurement of dietary intake of the participant was total
287 energy intake at age 13, previously estimated from linear spline multi-level models of

the combination of food frequency questionnaires and diet diaries[27]. Additionally, the most recent record of physical activity was defined as the counts per minute (CPM) and minutes spent in moderate-to-vigorous activity (MVPA) obtained from a subsample of participants at the 15-year clinic by an MTI Actigraph AM7164 2.2 accelerometer worn for 7 days[28]. Smoking status was obtained by questionnaire and participants were coded as "ever" or "never" smokers.

For analyses using data from the 21-year RbG group, physical activity and smoking status were obtained via questionnaire at the time of cardiovascular phenotyping; however, information on recent dietary intake was not available. Participants at this age were similarly classed as "ever" or "never" smokers and weekly exercise was categorised as either "never/rarely/<2 times a week" or "≥2 times a week".

STATISTICAL ANALYSES

Pre-analysis transformations and adjustments

As the distribution of residuals from the linear regression of BMI on carotid-femoral PWV was positively skewed, values of these variables were log transformed. LVM measured at each age was indexed to height to the power of 2.7 (LVMI)[23]. To assess the impact of adiposity on central vascular measures over and above that caused by stature[29], SVR and TAC were adjusted for height using SVR x height$^{1.83}$, TAC/height$^{1.83}$[30], and both CO and SV measures were indexed to height by dividing CO by height$^{1.83}$ and SV by height$^{2.04}$. The use of "positive" and "inverse" throughout the text refer to directional association rather than clinical implication. Stata 14 (Stata Corp, Texas) and R (https://cran.r-project.org/) were used for all analyses.

313

314 ***Multivariable regression***

315 Observational associations between BMI and each cardiovascular phenotype at
316 age 17 were assessed using multivariable linear regression in three models: (i)
317 unadjusted, (ii) adjusted for age, sex, smoking status and dietary intake of the
318 participant, household social class and maternal education and (iii) additionally
319 adjusted for physical activity (added as a separate model due to sample size).
320 Associations of the confounders with BMI, cardiovascular measures and weighted GRSs
321 at each age were tested using linear regression.

322

323 ***Mendelian randomization***

324 The externally weighted GRS used as an instrument for BMI in MR analyses was
325 generated from 97 independent SNPs shown to be reliably associated with BMI in the
326 GWAS conducted by the Genetic Investigation of ANthropometric Traits (GIANT)
327 consortium[18]. To generate the GRS, the dosage of each BMI-increasing allele at each
328 locus in ALSPAC was weighted by the external effect size of the variant in the GWAS
329 results[31, 32]. The doses were then added together and multiplied by the average
330 external effect size of all the SNPs on BMI to reflect the number of average BMI-
331 increasing alleles carried by each individual.

332 Two-stage least squares (2SLS) analysis was performed using the GRS as an
333 instrument for BMI at age 17 (*ivreg2* command in Stata). *F*-statistics for the first-stage
334 regression between the GRS and BMI were examined to check that the instruments
335 were valid, satisfying the assumption that the instrument was sufficiently associated
336 with the exposure[33]. The Durbin-Wu-Hausman (DWH) test for endogeneity was used

337 to compare multivariable regression and IV effect estimates (*ivendog* command in
338 Stata)[34].

339 ***Recall-by-genotype***

340 Linear regression was used to assess the association of the genome-wide GRS
341 group allocation (upper vs. lower ~30% of the genome-wide GRS distribution, as
342 described above) with BMI and each of the cardiovascular phenotypes measured at age
343 21. Each estimate therefore represents the mean difference in each variable with the
344 corresponding mean difference in BMI between RbG groups.

345

346 ***Sensitivity analyses***

347 Both BP and heart rate are correlated with other cardiovascular measures,
348 namely PWV, cIMT and LVMI[35-37]. To assess the causal association between BMI and
349 these cardiovascular measures, independent of BP and heart rate, we took the residuals
350 of the regression between each of these variables and both BP and heart rate and
351 repeated MR and RbG main analyses using these residuals.

352 Additionally, evidence suggests that some of the cardiovascular phenotypes used
353 in these analyses are not independent of height[23, 38, 39]. To account for this and the
354 inconsistent residual correlation between BMI and height throughout the
355 lifecourse[40], we assessed the association between the weighted GRS on height at age
356 17 and explored the impact of adjustment for height and height-squared on the
357 association between the weighted GRS and BMI. We also adjusted both multivariable
358 regression and MR analyses for height and height-squared measured at the same time of
359 the BMI exposure and cardiovascular phenotype and compared these to the main
360 analyses.

The use of multiple alleles in MR analyses increases the potential for unbalanced pleiotropic effects due to aggregation of invalid genetic instruments having an effect in one particular direction[13, 32]. To investigate the validity of the weighted GRS as an IV, the MR-Egger[41] approach was used to detect and accommodate violations of the MR assumptions, where the intercept of the MR-Egger test can be interpreted as an estimate of the average pleiotropic effect across the genetic variants, with a non-zero intercept term indicating overall directional pleiotropy. MR-Egger estimates were compared to those obtained from the inverse-variance weighted (IVW)[41, 42] and weighted median methods[43], which provide estimates of the causal effect of BMI on cardiovascular phenotypes under varying assumptions of instrument validity. As in the main analyses, the estimates of the association between each SNP and BMI were obtained from an independent external source, as to not induce weak instrument bias in a two-sample MR setting[44, 45].

Consistent with previous studies[46], we performed a sensitivity analysis using a weighted GRS that was limited to the genetic variants that were associated with BMI in the analysis of all people of European descent and excluded those that only reached genome-wide significance in only one sex or stratum (n=77) in the GIANT consortium[18]. Additionally, a previous study in a large sample based in the UK[46] suggested exclusion of three variants owing to pleiotropy (rs11030104, rs13107325 and rs3888190) and three SNPs that are not in Hardy-Weinberg equilibrium ($P<1x10^{-6}$; rs17001654, rs2075650 and rs9925964). Therefore, as a sensitivity analysis, we excluded these additional SNPs, resulting in an instrument consisting of 71 independent SNPs.

Additionally, to assess the validity of the genome-wide GRS (derived from the results of the BMI GWAS conducted by Speliotes *et al.*[19] and used in main RbG

386 analyses), MR analyses were conducted using the same genome-wide GRS as an
387 instrument for BMI, scaled to represent the same difference in mean BMI per unit
388 increase in the genome-wide GRS compared to the Locke *et al.* score, comprising 97
389 SNPs, used in main MR analyses.
390
391 ***In silico recall-by-genotype analyses***
392 To support the main RbG analyses at age 21 and the use of a genome-wide GRS,
393 we conducted two *in silico* RbG analyses with available cardiovascular phenotypes at
394 age 17. From the larger sample of individuals originally used in the MR analyses who
395 had no missing data on BMI or cardiovascular outcomes at age 17 (N=1190), we
396 randomly sampled 200 individuals from the lower and upper ~30% of the distribution
397 of i) the Locke *et al.* GRS (N=97 SNPs, constructed using external weighting)[18] and ii)
398 the genome-wide GRS from the Speliotes *et al.* GWAS used in main RbG analyses[19]. To
399 do this, each of the scores was sorted by value and 400 individuals were kept from both
400 the lower and upper ~30% of the distribution. Of those, 50% were randomly selected
401 for analyses, leaving 200 at each of the lower and upper tails (comparable to the
402 selection criteria for the main RbG study). This was also tested with an iterative random
403 sampling approach (results not shown).
404
405 ***RESULTS***
406 The MR cohort were 17.8 years old (SD = 0.4), consisted of 47.8% females and
407 had an average BMI of 22.7kg/m^2 (SD = 4) (Table 1). In the RbG study, individuals were
408 21.5 years old (SD = 0.9), had an average BMI of 24.5kg/m^2 (SD = 5.7) and 65.8% were
409 females (Table 2).
410

411 Table 1. Descriptive statistics for ALSPAC 17-year clinic

Variable	**N**	**Mean (SD) or percentage**
Participant's phenotypes		
Age (years)	3493	17.79 (0.42)
Sex (% female)	7909	47.81
BMI (kg/m^2)	3404	22.73 (3.99)
SBP (mmHg)	3172	118.61 (10.95)
DBP (mmHg)	3172	63.65 (6.56)
PP (mmHg)	3172	54.96 (10.08)
MAP (mmHg)	3172	81.97 (6.78)
Mean cIMT (mm)	3143	0.48 (0.04)
Carotid-femoral PWV (m/s)	2529	5.75 (0.69)
LVMI (g/m$^{2.7}$)	1420	28.93 (6.11)
Heart rate (bpm)	3172	64.45 (9.80)
Smoking status (% ever smoked)	2844	51.55
Physical activity		
CPM (counts)	1687	484.09 (180.99)
MVPA (minutes)	1687	23.73 (18.81)
Dietary intake (kcal)	7141	2260.24 (184.61)
Parental phenotypes		
Highest household social class	6598	
I	947	14.35
II	2902	43.98
III (non-manual)	1651	25.02
III (manual)	769	11.66
IV	290	4.40
V	39	0.59
Maternal education	6982	
CSE	1156	16.56
Vocational	635	9.09
O-Level	2453	35.13
A-Level	1700	24.35
Degree	1038	14.87

412 *ALSPAC = Avon Longitudinal Study of Parents and Children; BMI = body mass index; BP =*
413 *blood pressure; cIMT = carotid intima-media thickness; CSE = certificate of secondary*
414 *education; DBP = diastolic blood pressure; LVMI = left ventricular mass indexed to*
415 *height$^{2.7}$; MAP = mean arterial pressure; PWV = pulse wave velocity; SBP = systolic blood*
416 *pressure; SD = standard deviation*
417

418 Table 2. Descriptive statistics for ALSPAC 21-year RbG group

Variable	**N**	**Mean (SD) or percentage**
Participant's phenotype		
Age (years)	418	21.51 (0.94)
Sex (% female)	418	65.79
BMI (kg/m^2)	418	24.52 (5.70)
SBP (mmHg)	415	116.97 (10.28)
DBP (mmHg)	415	67.23 (6.64)
PP (mmHg)	415	49.74 (9.57)
MAP (mmHg)	415	83.81 (6.65)
Mean cIMT (mm)	417	0.46 (0.04)
Carotid-femoral PWV (m/s)	404	5.58 (0.74)
SVR (mmHg/L/min)	386	15.62 (3.02)
TAC (mL/mmHg)	386	1.02 (0.23)
LVMI (g/m$^{2.7}$)	386	21.42 (4.15)
Heart rate (bpm)	406	61.24 (9.43)
SV (ml)	386	89.69 (16.96)
CO (l/min)	386	5.70 (1.16)
Smoking status (% ever smoked)	417	17.27
Weekly exercise (% never/rarely/<2 per week)	299	38.46
Parental phenotypes		
Highest household social class	370	
I	91	24.59
II	169	45.68
III (non-manual)	76	20.54
III (manual)	27	7.30
IV	7	1.89
Maternal education	380	
CSE	31	8.16
Vocational	28	7.37
O-Level	109	28.68
A-Level	120	31.58
Degree	92	24.21

419 *ALSPAC = Avon Longitudinal Study of Parents and Children; BMI = body mass index; BP =*
420 *blood pressure; cIMT = carotid intima-media thickness; CO = cardiac output; CSE =*
421 *certificate of secondary education; DBP = diastolic blood pressure; LVMI = left ventricular*
422 *mass indexed to height$^{2.7}$; MAP = mean arterial pressure; PP = pulse pressure; PWV = pulse*
423 *wave velocity; SBP = systolic blood pressure; SD = standard deviation; SV = stroke volume;*
424 *SVR = systemic vascular resistance; TAC = total arterial compliance*
425

426 ***Confounder analyses***

427 BMI and all of the cardiovascular phenotypes were associated with a majority of
428 the confounding factors (Supplementary Tables S1 and S2, respectively).

429

430 ***Multivariable regression***

431 After adjusting for potential confounders, multivariable regression analyses
432 provided evidence for positive associations of measured BMI with SBP, DBP, PP, MAP,
433 LVMI and heart rate at age 17 (Table 3), as well as an inverse association with carotid-
434 femoral PWV.

435

436 Table 3. Multivariable regression associations between BMI and cardiovascular phenotypes in ALSPAC 17-year clinic

Outcome (units)	**N**	**Difference in mean outcome per 1kg/m^2 higher BMI (95% CI)**	***P*-value**	**N**	**Difference in mean outcome per 1kg/m^2 higher BMI (95% CI)[1]**	***P*-value**	**N**	**Difference in mean outcome per 1kg/m^2 higher BMI (95% CI)[2]**	***P*-value**
SBP (mmHg)	3108	0.86 (0.76, 0.95)	$7.43x10^{-74}$	2389	0.84 (0.74, 0.94)	$6.31x10^{-64}$	1033	0.83 (0.68, 0.98)	$1.00x10^{-25}$
DBP (mmHg)	3108	0.52 (0.47, 0.58)	$4.67x10^{-77}$	2389	0.49 (0.42, 0.56)	$2.73x10^{-44}$	1033	0.47 (0.36, 0.58)	$1.44x10^{-17}$
PP (mmHg)	3108	0.33 (0.25, 0.42)	$1.98x10^{-13}$	2389	0.35 (0.26, 0.44)	$6.08x10^{-15}$	1033	0.36 (0.22, 0.49)	$3.97x10^{-07}$
MAP (mmHg)	3108	0.63 (0.58, 0.69)	$1.13x10^{-109}$	2389	0.61 (0.54, 0.68)	$2.89x10^{-68}$	1033	0.59 (0.48, 0.69)	$5.64x10^{-27}$
Mean cIMT (mm)	3079	0.0001 (0.0003, 0.0005)	0.72	2368	-0.0001 (-0.001, 0.0004)	0.65	1028	0.0003 (-0.0005, 0.001)	0.48
Carotid-femoral PWV (log(m/s))	2495	-0.001 (-0.002, 0.0004)	0.20	1957	-0.001 (-0.002, 0.001)	0.20	867	-0.003 (-0.005, -0.0004)	0.02
LVMI (g/m$^{2.7}$)	1420	0.79 (0.72, 0.86)	$7.66x10^{-108}$	1151	0.83 (0.75, 0.91)	$2.02x10^{-99}$	569	0.95 (0.83, 1.07)	$5.40x10^{-57}$
Heart rate (bpm)	3108	0.21 (0.12, 0.29)	$3.50x10^{-06}$	2389	0.20 (0.10, 0.31)	0.0001	1033	0.24 (0.08, 0.39)	0.003

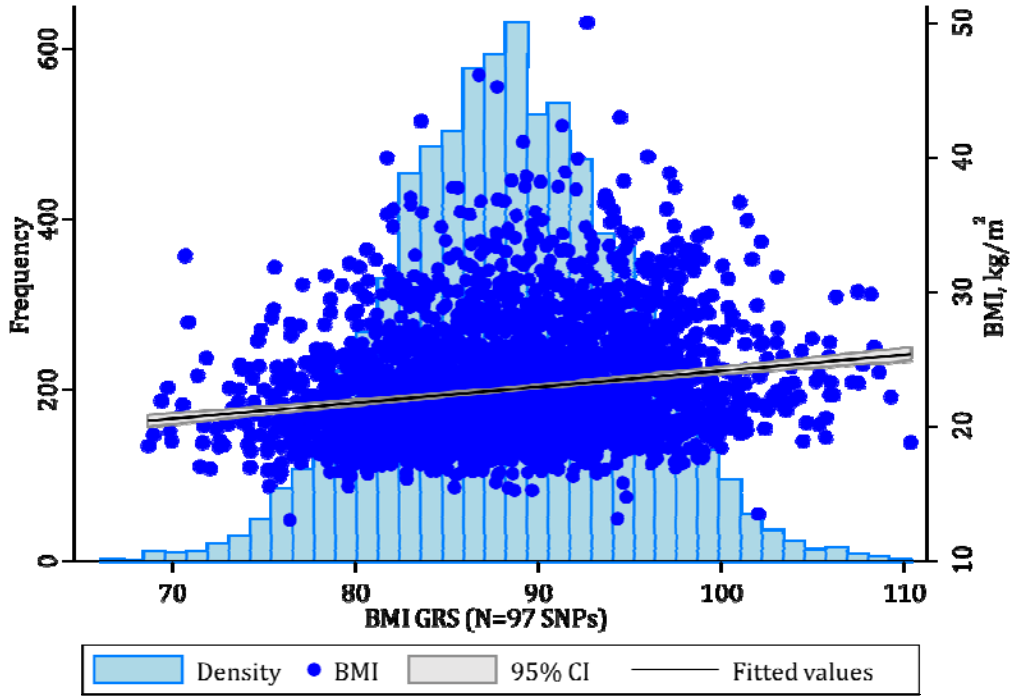
437 *ALSPAC = Avon Longitudinal Study of Parents and Children; BMI = body mass index; BP = blood pressure; CI = confidence interval; cIMT =*
438 *carotid intima-media thickness; DBP = diastolic blood pressure; LVMI = left ventricular mass indexed to height$^{2.7}$; MAP = mean arterial*
439 *pressure; PP = pulse pressure; PWV = pulse wave velocity; SBP = systolic blood pressure*
440 *[1]Adjusted for age, gender, smoking and dietary intake of the participant and maternal education and household social class*
441 *[2]Additionally adjusted for physical activity of the participant*

442 ***Mendelian randomization***

443 Each allele increase in the weighted GRS (comprising 97 SNPs) was associated
444 with a 0.12kg/m^2 (95% CI: 0.10, 0.14; P=9.53x10^{-28}) higher BMI, explaining 3% of the
445 variance (Figure 3). Unlike the direct measure of BMI and the cardiovascular
446 phenotypes, the GRS was not associated with a majority of confounders (Supplementary
447 Table S3).There was evidence for a positive effect of each kg/m^2 higher BMI on SBP
448 (difference: 0.79mmHg; 95% CI: 0.30, 1.28; P=0.002), DBP (difference: 0.29mmHg; 95%
449 CI: 0.0002, 0.59; P=0.05), PP (difference: 0.49mmHg; 95% CI: 0.03, 0.96; P=0.04), MAP
450 (difference: 0.46mmHg; 95% CI: 0.16, 0.75; P=0.002) and LVMI (difference: 1.07g/m$^{2.7}$;
451 95% CI: 0.62, 1.52; P=3.87x10^{-06}) (Table 4). F-statistics for these analyses ranged from
452 36 to 123. There was no strong evidence that the results from MR analyses were
453 different to those from the multivariable regression analyses (all P-values for
454 comparison > 0.12).
455

456 Figure 3. Association between weighted GRS (comprising 97 SNPs) and BMI in ALSPAC

457 17-year clinic



458

459 *The light blue histogram represents the weighted GRS (comprising 97 SNPs) distribution*

460 *(frequency, left-hand axis). The mid-blue scatter plot and linear trend with corresponding*

461 *95% confidence intervals represent the association between the same weighted GRS and*

462 *BMI (kg/m², right-hand axis).*

463

464 Table 4. Mendelian randomization analyses of the association between BMI and cardiovascular phenotypes in ALSPAC 17-year clinic

Outcome (units)	**N**	**Difference in mean outcome per 1kg/m^2 higher BMI (95% CI)**	***P*-value**	***F*-stat**	***P*-value for difference in multivariable regression and MR analyses[1]**
SBP (mmHg)	3108	0.79 (0.30, 1.28)	0.002	115.76	0.78
DBP (mmHg)	3108	0.29 (0.0002, 0.59)	0.05	115.76	0.12
PP (mmHg)	3108	0.49 (0.03, 0.96)	0.04	115.76	0.49
MAP (mmHg)	3108	0.46 (0.16, 0.75)	0.002	115.76	0.24
Mean cIMT (mm)	3079	0.002 (-0.001, 0.004)	0.14	122.97	0.15
Carotid-femoral PWV (log(m/s))	2495	-0.001 (-0.01, 0.01)	0.74	86.30	0.92
LVMI (g/m$^{2.7}$)	1420	1.07 (0.62, 1.52)	3.87x10^{-06}	36.24	0.21
Heart rate (bpm)	3108	-0.05 (-0.51, 0.41)	0.82	115.76	0.26

465 *ALSPAC = Avon Longitudinal Study of Parents and Children; BMI = body mass index; BP = blood pressure; CI = confidence interval; cIMT =*
466 *carotid intima-media thickness; DBP = diastolic blood pressure; LVMI = left ventricular mass indexed to height$^{2.7}$; MR = Mendelian*
467 *randomization; MAP = mean arterial pressure; PP = pulse pressure; PWV = pulse wave velocity; SBP = systolic blood pressure; SNP = single*
468 *nucleotide polymorphism*
469 *[1]P-value obtained from Durbin-Wu-Hausman test of heterogeneity*

Recall-by-genotype

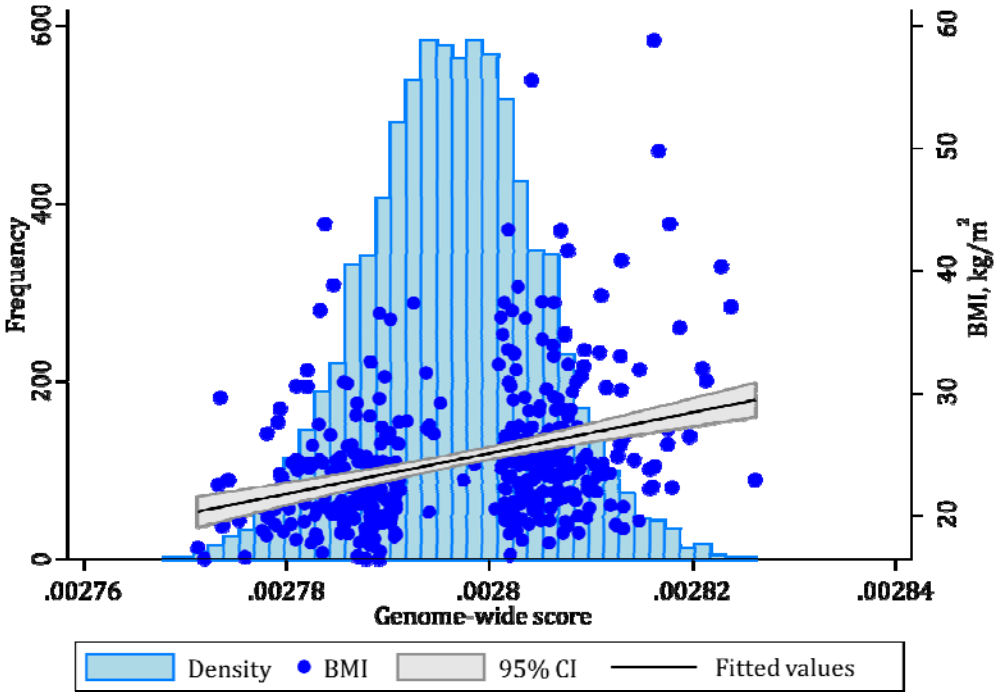
Difference in mean BMI between RbG groups was 3.85kg/m^2 (95% CI: 2.53, 4.63; *P*=6.09x10^{-11}) (Table 5, Figure 4). Measures of both BMI and cardiovascular outcomes were associated with a majority of confounders when assessed as a whole sample (Supplementary Tables S4 and S5, respectively). There was no strong evidence that the RbG group allocation was associated with confounders (Supplementary Table S6).

Table 5. Association between RbG groups and cardiovascular measures in ALSPAC 21-year RbG group

Outcome (units)	**N**	**Difference in mean outcome per 3.58kg/m^2 higher BMI (95% CI)**	***P*-value**
BMI (kg/m^2)	418	3.58 (2.53, 4.63)	6.09x10^{-11}
Cardiovascular outcomes			
SBP (mmHg)	415	3.70 (1.74, 5.66)	0.0002
DBP (mmHg)	415	2.25 (0.98, 3.52)	0.001
PP (mmHg)	415	1.45 (-0.40, 3.30)	0.12
MAP (mmHg)	415	2.73 (1.47, 3.99)	0.00003
Mean cIMT (mm)	417	0.001 (-0.01, 0.01)	0.82
Carotid-femoral PWV (log(m/s))	404	0.03 (0.01, 0.06)	0.01
SVR (mmHg/L/min.m$^{1.83}$)	386	-1.03 (-2.49, 0.43)	0.17
TAC (mL/mmHg/m$^{1.83}$)	386	0.01 (-0.01, 0.02)	0.37
LVMI (g/m$^{2.7}$)	386	1.65 (0.83, 2.47)	0.0001
Heart rate (bpm)	406	-0.08 (-1.93, 1.77)	0.93
SV (ml/m$^{2.04}$)	386	1.49 (0.62, 2.35)	0.001
CO (l/min/m$^{1.83}$)	386	0.11 (0.03, 0.19)	0.01

ALSPAC = Avon Longitudinal Study of Parents and Children; BMI = body mass index; BP = blood pressure; CI = confidence interval; cIMT = carotid intima-media thickness; CO = cardiac output; DBP = diastolic blood pressure; LVMI = left ventricular mass indexed to height$^{2.7}$; MAP = mean arterial pressure; PP = pulse pressure; PWV = pulse wave velocity; SBP = systolic blood pressure; SV = stroke volume; SVR = systemic vascular resistance; TAC = total arterial compliance

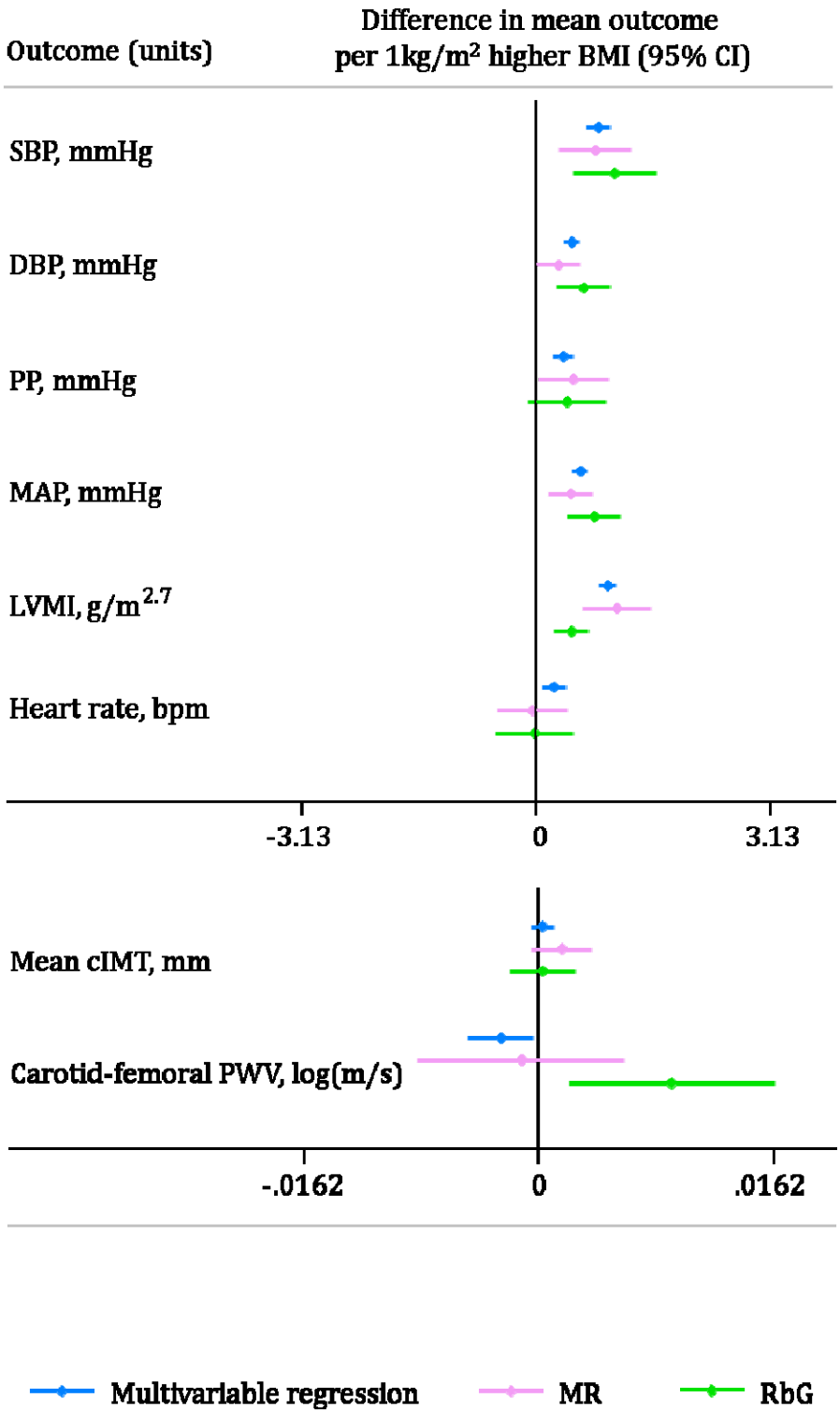
Figure 4. Association between RbG groups and BMI in ALSPAC 21-year RbG group



The light blue histogram represents the genome-wide GRS distribution (frequency, left-hand axis). The mid-blue scatter plot and linear trend with corresponding 95% confidence intervals represent the association between the same genome-wide GRS and BMI (kg/m^2, right-hand axis) included in the RbG groups.

493 Of the cardiovascular measures that overlapped between the two methods (MR
494 and RbG), the RbG groups were associated with higher SBP (difference in mean between
495 higher vs. lower RbG groups: 3.70mmHg; 95% CI: 1.74, 5.66; *P*=0.0002), DBP
496 (difference: 2.25mmHg; 95% CI: 0.98, 3.52; *P*=0.001), MAP (difference: 2.73mmHg; 95%
497 CI: 1.47, 3.99; *P*=0.00003) and carotid-femoral PWV (difference: 0.03log(m/s); 95% CI:
498 0.01, 0.06; *P*=0.01) (Table 5). Scaling the effect estimates to represent a kg/m^2 higher
499 BMI, as in the MR analyses, these results are equivalent to a 1.03mmHg higher SBP, a
500 0.63mmHg higher DBP, a 0.76mmHg higher MAP and a 0.01log(m/s) higher carotid-
501 femoral PWV. There was therefore considerable consistency between effect estimates
502 on the overlapping phenotypes at both ages, i.e. each kg/m^2 higher BMI had a causal
503 effect of similar magnitude on SBP, DBP and MAP, whilst showing no association with
504 heart rate or cIMT (Figure 5). However, there was evidence for a positive causal effect of
505 BMI on carotid-femoral PWV in RbG analyses at age 21 that was not evident in MR
506 analyses at 17 years.
507

508 Figure 5. Comparison of estimates from multivariable regression, MR and RbG
509 methodologies for the difference of all cardiovascular phenotypes available at both ages
510 (graphs are separated by scale similarities) per 1kg/m^2 higher BMI.



511

In addition to these cardiovascular measures, the RbG framework enabled the collection of more precise cardiovascular phenotypes and showed a positive causal role of higher BMI on MRI-derived LVMI (difference in mean between higher vs. lower RbG groups: 1.65g/$m^{2.7}$; 95% CI: 0.83, 2.47; *P*=0.0001), SV (difference: 1.49ml/$m^{2.04}$; 95% CI: 0.62, 2.35; *P*=0.001) and CO (difference: 0.11l/min/$m^{1.83}$; 95% CI: 0.03, 0.20; *P*=0.01), with no evidence of a difference in SVR or TAC.

Sensitivity analyses

After adjusting for SBP and heart rate, both MR and RbG results for the effect of BMI on cIMT, carotid-femoral PWV and LVMI were mostly consistent with main analysis (Supplementary Table S7). The one exception was the positive effect of BMI on carotid-femoral PWV shown in RbG analysis, which attenuated to the null following adjustment for SBP and heart rate (estimate: 0.02(log(m/s)); 95% CI: -0.01, 0.04; *P*=0.18).

The weighted GRS (comprising 97 SNPs), used in MR analyses, was not associated with height or height-squared at age 17. Adjusting for both height and height-squared made no difference to the association between the GRS and BMI at age 17 (Supplementary Table S8a), multivariable regression analyses (Supplementary Table S8b) or MR analyss (Supplementary Table S8c).

Where pleiotropy is perfectly balanced, an informative GRS is sufficient in an MR analysis, but this method is less able to cope with unbalanced pleiotropic effects (where invalid genetic instruments have an aggregate effect in one particular direction)[41]. The MR-Egger can go some way to accommodate violation of MR assumptions through pleiotropy, where the intercept term provides an estimate for unbalanced pleiotropy. This test provided no evidence for unbalanced pleiotropic effects of the genetic variants included within the GRS on any cardiovascular outcome (all *P*-values for the intercept ≥

537 0.24) (Supplementary Table S9). The MR effect estimates from IVW, MR-Egger and
538 weighted median analyses for the causal effect of BMI on the cardiovascular phenotypes
539 were largely consistent with the main analyses, though the effect estimate of BMI on
540 SBP did not agree for the weighted median analyses compared to the main MR, IVW and
541 MR-Egger estimates, albeit with very wide confidence intervals (Supplementary Table
542 S9 and Supplementary Figures S1a-S1e).

543 Both the instrument containing 77 SNPs (found in Europeans and analyses of
544 both sexes only in the GIANT GWAS, Supplementary Figure S2) and 71 SNPs (found in
545 Europeans only and removing potentially pleiotropic SNPs and those that were not in
546 HWE in a large sample based in the UK, Supplementary Figure S3) were associated with
547 BMI to a comparable extent as the GRS comprising the full set of 97 SNPs
548 (Supplementary Table S10) and produced similar results to the main analyses
549 (Supplementary Tables S11b and S11b, respectively). Similarly, when the genome-wide
550 GRS initially used to recall individuals to the RbG study was implemented in MR
551 analyses, the GRS was associated with a comparable change in BMI (Supplementary
552 Table S10, Supplementary Figure S4) and produced similar results to main MR analyses
553 (Supplementary Table S12).

554

555 ***In silico recall-by-genotype analyses***

556 In both *in silico* RbG analyses, the difference in mean BMI between the two RbG
557 groups was similar (1.63kg/m^2; 95% CI: 0.93, 2.32; P=5.89x10^{-06} and 1.65kg/m^2; 95%
558 CI: 0.91, 2.38; P=1.43x10^{-05} between RbG groups sampled from lower and upper ~30%
559 of the tails of the Locke *et al.* GRS and the Speliotes *et al.* genome-wide GRS
560 distributions, respectively), each explaining 5% of the variance in BMI (Supplementary
561 Table S13 and Supplementary Figures S5 and S6, respectively). Most results from both

562 *in silico* RbG analyses were in the same direction of effect and also in the same direction
563 as results from the main RbG analyses (Supplementary Table S14a and S14b,
564 respectively). For example, scaling the effect estimates to represent a kg/m^2 higher BMI,
565 as in the main analyses, the equivalent effect estimates using the weighted GRS
566 (comprising 97 SNPs) and the genome-wide GRS, respectively, were 0.87mmHg (95%
567 CI: -1.23, 2.97; *P*=0.42) and 0.67mmHg (95% CI: -1.46, 2.79; *P*=0.54) for SBP, 1.45g/m$^{2.7}$
568 (95% CI: 0.27, 2.63; *P*=0.02) and 1.83g/m$^{2.7}$ (95% CI: 0.68, 2.98; *P*=0.002) for LVMI and
569 -0.01log(m/s) (95% CI: -0.03, 0.02; *P*=0.59) and -0.01log(m/s) (95% CI: -0.03, 0.01;
570 *P*=0.22) for carotid-femoral PWV. However, the direction of effect for DBP, MAP and
571 heart rate differed between the two *in silico* sensitivity analyses (with one always being
572 consistent with the main analyses), but these *in silico* analyses had wide confidence
573 intervals. The iterative random sampling produced the same results (not shown).
574
575 ***Discussion***
576 *Summary and comparison of findings*
577 In a large cohort of young adults, we employed two complementary analyses
578 (MR and RbG) to investigate the causal effect of higher BMI on measures of
579 cardiovascular structure and function and compared these to adjusted multivariable
580 regression results. Alongside multivariable regression and MR analyses, the RbG
581 method predominantly allowed the collection of extremely precise cardiovascular
582 phenotypes that would otherwise be impractical at scale.
583 Regarding the cardiovascular phenotypes that were used across MR, RbG and
584 multivariable regression analyses in the current study, our results are consistent with
585 previous observational studies in children and adults[47-49] and across all three
586 methodologies used. Results suggest that higher BMI causes higher BP (SBP, DBP, PP

and MAP) and higher LVMI, the latter suggesting adverse cardiac structure, even in young adults. Furthermore, the similarity of findings across these methods, given different sources of bias between the MR and RbG on the one hand[41, 50] and multivariable regression on the other[13], strongly support causality in this instance. If further sustained through adulthood, these cardiovascular effects of higher BMI are likely to increase CVD risk and CVD-specific mortality in later life[51-55].

Previous multivariable regression results from smaller observational studies in children and adolescents have found higher BMI to be associated with faster PWV and thicker cIMT[56-59]. In contrast to this, our three methods gave results that did not support a causal effect for cIMT. This suggests that previous studies may have been influenced by residual confounding or bias, for which we have been better able to control here. This conclusion is also supported to some extent by analysis of the same variables at a lower age in an observational context[47]. With less consistent results across our analysis approaches, higher BMI was associated with slower PWV at age 17 years (i.e. healthier PWV) in multivariable regression analysis and a faster PWV (i.e. worse PWV) in RbG analysis, with MR analysis showing a null association (Figure 5). Indeed, another previous study suggests that the relationship between BMI and PWV may be inverse in youth and becomes positive at older ages[60].

As might be expected, higher BMI resulted in increased CO in our RbG study and, although contrary to other observational studies at this age[61, 62], this appeared to be solely driven by SV, as neither our MR or RbG analyses suggested a causal effect of BMI on heart rate. It is possible that previously reported associations between BMI and heart rate may be a result of unmeasured confounding. The BMI-mediated change in SV (and consequently CO) seen at age 21 is therefore likely to at least partially account for

611 the cardiac hypertrophy and higher BP that we see in multivariable regression, MR and
612 RbG both ages in the data analysed here.
613
614 *Strengths and limitations*
615 A key strength is the comparison of results from confounder-adjusted
616 multivariable regression, MR and RbG, along with a range of appropriate sensitivity
617 analyses, which together provide strong evidence for a causal effect of higher BMI on BP
618 and cardiac structure (LVMI) in young adulthood. This is one of the first uses of RbG for
619 BP and cardiac structure, where we could compare results directly to multivariable
620 regression and MR within the same general population. The consistency between
621 results found from RbG with 418 participants to those from MR with 7,909 participants
622 suggests this approach is valid and statistically efficient. Furthermore, the RbG method
623 allowed the collection of precise cardiovascular phenotypes that would otherwise not
624 have been possible in sample sizes required for MR. For example, we could explore the
625 impact of BMI on SV and CO, measures that are prohibitively expensive to undertake in
626 several thousands of participants.
627 In contrast to these strengths, it is of course the case that both the MR and RbG
628 analyses may be biased if the IV analyses assumptions are violated[13]. These require,
629 firstly, that the genetic instruments need to be robustly related to the exposure (here
630 BMI). We used variants in both MR and RbG that have been shown to be genome-wide
631 significant and replicated; the first-stage F-statistics, a measure of instrument strength,
632 were high for all the MR analyses. Secondly, it is assumed that confounders of the
633 observational BMI-cardiovascular outcome association are not related to the genetic
634 instrument. There is empirical evidence that this is unlikely to be the case[63] and for
635 observed confounders we demonstrate it in our analyses here. Thirdly, it is assumed

636 that there is no path from the genetic instrument to the outcomes other than through
637 BMI, which may result from horizontal pleiotropy. Using an aggregate allelic score
638 increases the possibility of horizontal pleiotropy and we therefore undertook sensitivity
639 analyses to explore this[41]. Across a range of sensitivity analysis (including the MR-
640 Egger and weighted median approaches in the MR analyses, limiting the GRS to different
641 subsets of genetic instruments and performing *in silico* RbG analyses using different
642 genetic proxies), provided broadly similar results to main analyses, lending more
643 confidence to the causal estimates and direction of effect with higher BMI and
644 suggesting that these were not largely driven by horizontal pleiotropy.

645 Although the RbG approach enabled sampling from lower and upper ~30% of a
646 genome-wide GRS, which produced a difference of ~3.5kg/m^2 in BMI, the genome-wide
647 nature of the score could be considered less refined than the GRS used in MR analyses,
648 comprising 97 SNPs shown to be robustly associated with BMI in a large meta-analyses
649 of GWASs[18]. Despite this, sensitivity analyses performed showed that the genome-
650 wide GRS provided comparable results to MR-analyses and *in silico* RbG analyses.

651 One complication in some of the sensitivity analyses performed (specifically,
652 adjusting for variables including BP, heart rate and height in multivariable and MR
653 analyses) is the potential for inducing collider bias[64, 65]. However, due to the overall
654 consistency in effect estimates generated from the various sensitivity analyses, this is
655 unlikely to be the case in this study. More generally, it is possible that some of the
656 differences in effect size of BMI on cardiovascular outcomes (for example, carotid-
657 femoral PWV) between methodologies may relate to the difference in age at which the
658 methods were applied. In addition, given the small range of some of the cardiovascular
659 outcomes (for example, cIMT) in these young individuals and the potentially small effect
660 size of BMI, power to detect such small effect sizes in this context may be limited.

Further, we adjusted for a range of potentially confounding factors in multivariable regression analyses but even in such a comprehensive longitudinal cohort, it can be difficult to accurately measure (and therefore appropriately account for) all confounders. Indeed, this illustrates the need for better methods (such as MR and RbG employed here) to assess the causal nature of the association between BMI and cardiovascular health.

Conclusion

With this innovative study design, using complementary multivariable regression, MR and RbG analyses, together with a range of appropriate sensitivity analyses, we found results that strongly suggest a causal role of higher BMI resulting in adverse levels of BP and LVMI. These findings support efforts to reduce BMI and prevent the obesity burden from a young age, with the aim of attenuating the development of vascular and cardiac changes, known to be the precursors of long-term adverse cardiovascular outcomes, and preventing the development of additional peripheral vascular damage not yet evident at this early stage of life.

Acknowledgements

We are extremely grateful to all the families who took part in this study, the midwives for their help in recruiting them, and the whole ALSPAC team, which includes interviewers, computer and laboratory technicians, clerical workers, research scientists, volunteers, managers, receptionists and nurses. We'd also like to thank Frank Dudbridge for ongoing conversations regarding genetic risk scores used in Mendelian randomization methodologies. The UK Medical Research Council (MRC) and the Wellcome Trust (Grant ref: 102215/2/13/2) and the University of Bristol provide core

686 support for ALSPAC. This publication is the work of the authors and KHW and NJT serve
687 as guarantors for the contents of this paper. This research was specifically funded
688 through grants from the British Heart Foundation (grant numbers: RG/10/004/28240,
689 PG/06/145 and CS/15/6/31468) and the UK MRC, University of Bristol and Wellcome
690 Trust (grant numbers: MC_UU_12013/1-9, 096989/Z11/Z, 086676/7/08/Z, MR-
691 M009351/1, MR/M020894/1 and 202802/Z/16/Z). GWAS data was generated by
692 Sample Logistics and Genotyping facilities at Wellcome Sanger Institute and LabCorp
693 (Laboratory Corporation of America) using support from 23andMe.

694 References

695 1. Calle EE, Thun MJ, Petrelli JM, Rodriguez C, Heath CWJ. Body-Mass Index and
696 Mortality in a Prospective Cohort of U.S. Adults. New England Journal of Medicine.
697 1999;341(15):1097-105.
698 2. Prospective Studies Collaboration. Body-mass index and cause-specific mortality
699 in 900,000 adults: collaborative analyses of 57 prospective studies. The Lancet.
700 373(9669):1083-96.
701 3. Nordestgaard BG, Palmer TM, Benn M, Zacho J, Tybjærg-Hansen A, Davey Smith
702 G, et al. The Effect of Elevated Body Mass Index on Ischemic Heart Disease Risk: Causal
703 Estimates from a Mendelian Randomisation Approach. PLOS Medicine.
704 2012;9(5):e1001212.
705 4. Adams TD, Gress RE, Smith SC, Halverson RC, Simper SC, Rosamond WD, et al.
706 Long-Term Mortality after Gastric Bypass Surgery. New England Journal of Medicine.
707 2007;357(8):753-61.
708 5. Christou NV SJ, Liberman M, Look D, Auger S, McLean APH, MacLean LD. Surgery
709 decreases long-term mortality, morbidity, and health care use in morbidly obese
710 patients. Ann Surg. 2004;240:416-24.
711 6. Hägg S, Fall T, Ploner A, Mägi R, Fischer K, Draisma HH, et al. Adiposity as a cause
712 of cardiovascular disease: a Mendelian randomization study. International Journal of
713 Epidemiology. 2015;44:578-586.
714 7. Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, et al. Heart
715 Disease and Stroke Statistics—2015 Update. Circulation. 2015:131:e29-e322.
716 8. Halcox JPJ DJ. Childhood origins of endothelial dysfunction. Heart. 2005;91:1272-
717 4.
718 9. Oren A, Vos LE, Uiterwaal CM, Grobbee DE, Bots ML. Cardiovascular risk factors
719 and increased carotid intima-media thickness in healthy young adults: The
720 atherosclerosis risk in young adults (arya) study. Archives of Internal Medicine.
721 2003;163(15):1787-92.
722 10. Wildman RP, Mackey RH, Bostom A, Thompson T, Sutton-Tyrrell K. Measures of
723 Obesity Are Associated With Vascular Stiffness in Young and Older Adults.
724 Hypertension. 2003;42(4):468.

11. Lorber R, Gidding SS, Daviglus ML, Colangelo LA, Liu K, Gardin JM. Influence of systolic blood pressure and body mass index on left ventricular structure in healthy African-American and white young adults: the CARDIA study. Journal of the American College of Cardiology. 2003;41(6):955-60.
12. Haycock PC, Burgess S, Wade KH, Bowden J, Relton C, Davey Smith G. Best (but oft-forgotten) practices: the design, analysis, and interpretation of Mendelian randomization studies. The American Journal of Clinical Nutrition. 2016:103:965-978.
13. Davey Smith G HG. Mendelian randomization: genetic anchors for causal inference in epidemiology studies. Hum Mol Genet. 2014:23:R89-R98.
14. Ware JJ, Timpson N, Davey Smith G, Munafò MR. A recall-by-genotype study of CHRNA5-A3-B4 genotype, cotinine and smoking topography: study protocol. BMC Medical Genetics. 2014;15:13
15. Hellmich C, Durant C, Jones MW, Timpson NJ, Bartsch U, Corbin LJ. Genetics, sleep and memory: a recall-by-genotype study of ZNF804A variants and sleep neurophysiology. BMC Medical Genetics. 2015;16:96.
16. Boyd A GJ, Macleod J, Lawlor DA, Fraser A, Henderson J, Molloy L, Ness A, Ring S, Davey Smith G. Cohort Profile: the 'children of the 90s'--the index offspring of the Avon Longitudinal Study of Parents and Children. International Journal of Epidemiology. 2013;42:111-27.
17. Fraser A, Macdonald-Wallis C, Tilling K, Boyd A, Golding J, Davey Smith G, et al. Cohort Profile: The Avon Longitudinal Study of Parents and Children: ALSPAC mothers cohort. International Journal of Epidemiology. 2013;42(1):97-110.
18. Paternoster L HL, Tilling K, Weedon MN, Freathy RM, Frayling TM, Kemp JP, Davey Smith G, Timpson NJ, Ring SM, Evans DM, Lawlor DA. Adult height variants affect birth length and growth rate in children. Hum Mol Genet. 2011;20:4069-75.
19. Sieradzka D PR, Freeman D, Cardno AG, McGuire P, Plomin R, Meaburn EL, Dudbridge F, Ronald A. Are genetic risk factors for psychosis also associated with dimension-specific psychotic experiences in adolescence? PLoS ONE. 2014;9:e94398.
20. Speliotes EK, Willer CJ, Berndt SI, Monda KL, Thorleifsson G, Jackson AU, et al. Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index. Nat Genet. 2010;42(11):937-48.
21. Locke AE, Kahali B, Berndt SI, Justice AE, Pers TH, Day FR, et al. Genetic studies of body mass index yield new insights for obesity biology. Nature. 2015;518(7538):197-206.
22. O'Brien E, Asmar R, Beilin L, Imai Y, Mallion J-M, Mancia G, et al. European Society of Hypertension recommendations for conventional, ambulatory and home blood pressure measurement. Journal of Hypertension. 2003;21(5):821-48. P
23. Lang RM BM, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, Picard MH, Roman MJ, Seward J, Shanewise JS, Solomon SD, Spencer KT, Sutton MS, Stewart WJ. Recommendations for chamber quantification: A report from the american society of echocardiography's guidelines and standards committee and the chamber quantification writing group, developed in conjunction with the european association of echocardiography, a branch of the european society of cardiology. J Am Soc Echocardiogr. 2005;18:1440-63.
24. Steeden JA, Atkinson D, Hansen MS, Taylor AM, Muthurangu V. Rapid Flow Assessment of Congenital Heart Disease with High-Spatiotemporal-Resolution Gated Spiral Phase-Contrast MR Imaging. Radiology. 2011;260(1):79-87.

772 25. Stergiopulos N MJ, Westerhof N. Simple and accurate way for estimating total
773 and segmental arterial compliance: the pulse pressure method. Ann Biomed Eng.
774 1994;22:392-7.
775 26. OPCS. Standard Occupational Classification Volume 3. London: HMSO; 1991.
776 27. Anderson EL TK, Fraser A, Macdonald-Wallis C, Emmett P, Cribb V, Northstone K,
777 Lawlor DA, Howe LD. Estimating trajectories of energy intake through childhood and
778 adolescence using linear-spline multilevel models. Epidemiology. 2013;24:507-15.
779 28. Mattocks C NA, Leary S, Tilling K, Blair SN, Shield J, Deere K, Saunders J, Kirkby J,
780 Smith GD, Wells J, Wareham N, Reilly J, Riddoch C. Use of accelerometers in a large field-
781 based study of children: protocols, design issues, and effects on precision. J Phys Act
782 Health. 2008;5:S98-S111.
783 29. Palmieri V, de Simone G, Arnett DK, Bella JN, Kitzman DW, Oberman A, et al.
784 Relation of various degrees of body mass index in patients with systemic hypertension
785 to left ventricular mass, cardiac output, and peripheral resistance (The Hypertension
786 Genetic Epidemiology Network Study). The American Journal of Cardiology.
787 2001;88(10):1163-8.
788 30. Chinali M, Devereux RB, Howard BV, Roman MJ, Bella JN, Liu JE, et al. Comparison
789 of cardiac structure and function in American Indians with and without the metabolic
790 syndrome (the Strong Heart Study). The American Journal of Cardiology.
791 2004;93(1):40-4.
792 31. Palmer TM LD, Harbord RM, Sheehan NA, Tobias JH, Timpson NJ, Davey Smith G,
793 Sterne JAC. Using multiple genetic variants as instrumental variables for modifiable risk
794 factors. Stat Methods Med Res. 2012;21:223-42.
795 32. Richmond RC DSG, Ness AR, den Hoed M, McMahon G, Timpson NJ. Assessing
796 causality in the association between child adiposity and physical activity levels: a
797 Mendelian randomization analysis. PLOS Med. 2014;11:e1001618.
798 33. JH SDaS. Instrumental variables regression with weak instruments.
799 Econometrica. 1997;65:557-86.
800 34. Baum C SM, Stillman S. IVENDOG: Stata module to calculate Durbin-Wu-Hausman
801 endogeneity test after ivreg. In: S494401 SSC, editor. Boston: Boston Collge Department
802 of Economics 2007.
803 35. Ferreira JP, Girerd N, Bozec E, Machu JL, Boivin JM, London GM, et al. Intima–
804 Media Thickness Is Linearly and Continuously Associated With Systolic Blood Pressure
805 in a Population-Based Cohort (STANISLAS Cohort Study). Journal of the American Heart
806 Association. 2016;5(6).
807 36. Kim EJ, Park CG, Park JS, Suh SY, Choi CU, Kim JW, et al. Relationship between
808 blood pressure parameters and pulse wave velocity in normotensive and hypertensive
809 subjects: invasive study. J Hum Hypertens. 2006;21(2):141-8.
810 37. Malcolm DD, Burns TL, Mahoney LT, Lauer RM. Factors Affecting Left Ventricular
811 Mass in Childhood: The Muscatine Study. Pediatrics. 1993;92(5):703-9.
812 38. Johnson W, Kuh D, Tikhonoff V, Charakida M, Woodside J, Whincup P, et al. Body
813 Mass Index and Height From Infancy to Adulthood and Carotid Intima-Media Thickness
814 at 60 to 64 Years in the 1946 British Birth Cohort Study. Arteriosclerosis, Thrombosis,
815 and Vascular Biology. 2014;34(3):654-60.
816 39. de Simone G, Devereux RB, Daniels SR, Koren MJ, Meyer RA, Laragh JH. Effect of
817 growth on variability of left ventricular mass: Assessment of allometric signals in adults
818 and children and their capacity to predict cardiovascular risk. Journal of the American
819 College of Cardiology. 1995;25(5):1056-62.

820 40. Stergiakouli E, Gaillard R, Tavaré JM, Balthasar N, Loos RJ, Taal HR, et al.
821 Genome-wide association study of height-adjusted BMI in childhood identifies
822 functional variant in ADCY3. Obesity (Silver Spring, Md). 2014;22(10):2252-9.
823 41. Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid
824 instruments: effect estimation and bias detection through Egger regression.
825 International Journal of Epidemiology. 2015. 512-525.
826 42. Corbin LJ, Richmond RC, Wade KH, Burgess S, Bowden J, Smith GD, et al. Body
827 mass index as a modifiable risk factor for type 2 diabetes: Refining and understanding
828 causal estimates using Mendelian randomisation. Diabetes. 2016:65:3002-7.
829 43. Bowden J, Davey Smith G, Haycock PC, Burgess S. Consistent Estimation in
830 Mendelian Randomization with Some Invalid Instruments Using a Weighted Median
831 Estimator. Genetic Epidemiology. 2016;40(4):304-14.
832 44. Hartwig FP, Davies NM. Why internal weights should be avoided (not only) in
833 MR-Egger regression. International Journal of Epidemiology. 2016:45:1676-1678.
834 45. Bowden FJ, Burgess S, Davey Smith G. Response to Hartwig and Davies.
835 International Journal of Epidemiology. 2016:45:1679-1680.
836 46. Yaghootkar H, Lotta LA, Tyrrell J, Smit RAJ, Jones SE, Donnelly L, et al. Genetic
837 Evidence for a Link Between Favorable Adiposity and Lower Risk of Type 2 Diabetes,
838 Hypertension, and Heart Disease. Diabetes. 2016;65(8):2448-60.
839 47. Charakida M JA, Falaschetti E, Khan T, Finer N, Sattar N, Hingorani A, Lawlor DA,
840 Davey Smith G, Deanfield JE. Childhood obesity and vascular phenotypes: a population
841 study. Journal of the American College of Cardiology. 2012;60:2643-50.
842 48. Lavie CJ AC, Ventura HO, Messerli FH. Left atrial abnormalities indicating
843 diastolie ventricular dysfunction in cardiopathy of obesity. Chest. 1967;92:1042-6.
844 49. Vaziri SM LM, Lauer MS, Benjamin EJ, Levy D. Influence of blood pressure on left
845 atrial size. The Framingham Heart Study. Hypertension. 1995;25:1155-60.
846 50. Burgess S, Timpson NJ, Ebrahim S, Davey Smith G. Mendelian randomization:
847 where are we now and where are we going? International Journal of Epidemiology.
848 2015;44(2):379-88.
849 51. Gray L, Lee IM, Sesso HD, Batty GD. Blood pressure in early adulthood,
850 hypertension in middle-age, and future cardiovascular disease mortality: the Harvard
851 Alumni Health Study. Journal of the American College of Cardiology. 2011;58(23):2396-
852 403.
853 52. Levy D, Garrison RJ, Savage DD, Kannel WB, Castelli WP. Prognostic Implications
854 of Echocardiographically Determined Left Ventricular Mass in the Framingham Heart
855 Study. New England Journal of Medicine. 1990;322(22):1561-6.
856 53. Dhuper S AR, Weichbrod L, Mahdi E, Cohen HW. Association of obesity and
857 hypertension with left ventricular geometry and function in children and adolescents.
858 Obesity. 2011;19:128-33.
859 54. Friedemann C, Heneghan C, Mahtani K, Thompson M, Perera R, Ward AM.
860 Cardiovascular disease risk in healthy children and its association with body mass
861 index: systematic review and meta-analysis. BMJ : British Medical Journal.
862 2012;345:e4759.
863 55. Bogers RP, Bemelmans WE, Hoogenveen RT, et al. Association of overweight with
864 increased risk of coronary heart disease partly independent of blood pressure and
865 cholesterol levels: A meta-analysis of 21 cohort studies including more than 300 000
866 persons. Archives of Internal Medicine. 2007;167(16):1720-8.
867 56. Meyer AA, Kundt G, Steiner M, Schuff-Werner P, Kienast W. Impaired Flow-
868 Mediated Vasodilation, Carotid Artery Intima-Media Thickening, and Elevated

869 Endothelial Plasma Markers in Obese Children: The Impact of Cardiovascular Risk
870 Factors. Pediatrics. 2006;117(5):1560-7.
871 57. Woo KS, Chook P, Yu CW, Sung RYT, Qiao M, Leung SSF, et al. Overweight in
872 children is associated with arterial endothelial dysfunction and intima-media
873 thickening. Int J Obes Relat Metab Disord. 2004;28(7):852-7.
874 58. Iannuzzi A, Licenziati MR, Acampora C, Salvatore V, Auriemma L, Romano ML, et
875 al. Increased Carotid Intima-Media Thickness and Stiffness in Obese Children. Diabetes
876 Care. 2004;27(10):2506.
877 59. Urbina EM, Kimball TR, Khoury PR, Daniels SR, Dolan LM. Increased Arterial
878 Stiffness is Found in Adolescents with Obesity or Obesity-Related Type 2 Diabetes
879 Mellitus. Journal of hypertension. 2010;28(8):1692-8.
880 60. Corden B, Keenan NG, de Marvao ASM, Dawes TJW, DeCesare A, Diamond T, et al.
881 Body Fat Is Associated With Reduced Aortic Stiffness Until Middle Age. Hypertension.
882 2013;61(6):1322-7.
883 61. Sorof JM, Poffenbarger T, Franco K, Bernard L, Portman RJ. Isolated systolic
884 hypertension, obesity, and hyperkinetic hemodynamic states in children. The Journal of
885 Pediatrics. 2002;140(6):660-6.
886 62. Jiang X, Srinivasan SR, Urbina E, Berenson GS. Hyperdynamic Circulation and
887 Cardiovascular Risk in Children and Adolescents. Circulation. 1995;91(4):1101-6.
888 63. Smith GD, Lawlor DA, Harbord R, Timpson N, Day I, Ebrahim S. Clustered
889 Environments and Randomized Genes: A Fundamental Distinction between
890 Conventional and Genetic Epidemiology. PLOS Medicine. 2007;4(12):e352.
891 64. Cole SR, Platt RW, Schisterman EF, Chu H, Westreich D, Richardson D, et al.
892 Illustrating bias due to conditioning on a collider. International Journal of Epidemiology.
893 2010;39(2):417-20.
894 65. Aschard H, Vilhjálmsson Bjarni J, Joshi Amit D, Price Alkes L, Kraft P. Adjusting
895 for Heritable Covariates Can Bias Effect Estimates in Genome-Wide Association Studies.
896 American Journal of Human Genetics. 2015;96(2):329-39.