Borges, C., Oliveira, I. O., Freitas, D. F., Horta, B. L., Ong, K. K., Gigante, D. P., & Barros, A. J. D. (2017). Obesity-induced hypoadiponectinaemia: the opposite influences of central and peripheral fat compartments. International Journal of Epidemiology, [dyx022]. DOI: 10.1093/ije/dyx022

SUPPLEMENTARY MATERIAL

SUPPLEMENTARY METHODS

1982 Pelotas Birth Cohort – Conventional association analysis

Body composition and anthropometric measures

Abdominal fat depots were measured using the ultrasound machine Toshiba Xario (Toshiba Medical Systems Corp., Tokyo, Japan)[1-3]. Briefly, visceral fat thickness was estimated by the distance between the peritoneum and the lumbar spine at the intersection between the xyphoid line and the waist circumference. Subcutaneous abdominal fat thickness was estimated at the same probe site by the distance between the posterior line of dermis and the outer bowel wall. Intra-observer error was 4.1% for visceral and 3.4% for subcutaneous abdominal fat. Inter-observer technical error of measurement was 3.1% for both visceral fat and subcutaneous abdominal fat. Women that were pregnant or three months postpartum were excluded.

Gluteofemoral fat was assessed by Dual-energy X-ray Absorptiometry (DXA) (Lunar Prodigy Advance—GE, Germany). Participants with osteoarticular disabilities, confirmed or suspected pregnancy, non-removable metallic objects, wheelchair users, extremely obese (weight > 120 kg) or extremely tall (height > 192 cm) individuals were excluded. Weight was measured to the nearest 0.1 kg on a calibrated electronic scale (TANITA model BC-418 MA; Tanita, Tokyo, Japan).

Standing height was assessed to the nearest 0.1 cm using a full-length wall-mounted stadiometer (SECA 240; Seca, Birmingham, United Kingdom).

Genomic ancestry

Genomic ancestry was estimated using 370,539 ancestry informative markers shared by samples from the HapMap Project [4], the Human Genome Diversity Project (HGDP) [5] and the Epigen-Brazil study population [6]. The following HapMap samples were used: 266 Africans (176 Yoruba in Ibadan, Nigeria [YRI] and 90 Luhya in Webuye, Kenya [LWK]), 262 Europeans (174 Utah residents with Northern and Western European ancestry [CEU] and 88 from Toscans from Italy [TSI]), 170 admixed individuals (77 Mexicans from Los Angeles, California [MEX] and 83 Afro-African from Southwest USA [ASW]), and 93 Native Americans from the HGDP (25 Pima, 22 Karitiana, 25 Maya and 21 Surui). The software ADMIXTURE [7] was used to estimate the contribution from European, African and Native American ancestry for each cohort participant. SNPs were genotyped using Illumina Omni 2.5M-8v1 array (San Diego, California). Further details can be found in Lima-Costa et al. [6].

GIANT and ADIPOGen consortia – Mendelian randomization analysis

Proportion of phenotypic variance explained by genetic instruments

In order to estimate the strength of our genetic instruments, we estimated the phenotypic variance explained by a given SNP (R^2) for each exposure of interest (waist circumference, hip circumference, and adiponectin concentration). We used ADIPOGen and GIANT summary data to approximate R^2 for a given SNP based on the effect estimate for its association with the trait of interest (beta or $\hat{\beta}$), respective standard error ($se(\hat{\beta})$), minor allele frequency (MAF), and sample size (N). The following formula was used as previously described by Shim et al., 2015[8]:

$$R^2 \cong \frac{2\hat{\beta}^2 MAF(1-MAF)}{2\hat{\beta}^2 MAF(1-MAF) + (se(\hat{\beta}))^2 2NMAF(1-MAF)}$$

The phenotypic variance explained by the composite genetic instrument (combining all SNPs) was estimated by the sum of SNP-specific R^2.

Power calculations

We have estimated power for our Mendelian randomization analyses using the online calculator tool (http://cnsgenomics.com/shiny/mRnd/) and assuming a range of effect sizes for the potential underlying causal association between exposure and outcome. Details on the parameters used and the resulting estimated power are provided below.

Exposure	Outcome	Sample size[1]	Type-I error rate	Effect estimate[2]	Instrument strength (R^2)[3]	Power
WC	Adiponectin	29,347	0.05	0.05	0.012	16%
WC	Adiponectin	29,347	0.05	0.10	0.012	47%
WC	Adiponectin	29,347	0.05	0.20	0.012	97%
HipC	Adiponectin	29,347	0.05	0.05	0.02	23%
HipC	Adiponectin	29,347	0.05	0.10	0.02	68%
HipC	Adiponectin	29,347	0.05	0.20	0.02	100%
Adiponectin	WC or HipC	210,088	0.05	0.05	0.04	100%
Adiponectin	WC or HipC	210,088	0.05	0.10	0.04	100%
Adiponectin	WC or HipC	210,088	0.05	0.20	0.04	100%

[1] Approximate sample size used for estimating SNP-outcome association

[2] Considering the true underlying causal association is unknown, a range of values was used.

[3] Instrument strength relates to the proportion of variance in the exposure explained by the instrument (R^2). This was calculated by the sum of R^2 from each SNP in the instrument (56 SNPs for waist circumference, 75 SNPs for hip circumference and 4 SNPs for adiponectin concentration). SNPs were in linkage equilibrium. The formula used to estimate R^2 for each SNP is detailed in "*Proportion of phenotypic variance explained by genetic instruments"* section.

Inverse-variance weighted (IVW) method

For the unadjusted Mendelian randomization model, the inverse-variance weighted (IVW) method was used to derive the beta coefficient (mean difference in standardized log adiponectin per standard unit increase in waist or hip circumference) and its standard error by using the following formulas:

$$\hat{\beta}\text{IVW} = \frac{\sum_{k=1}^{K} X_k Y_k \sigma_{yk}^{-2}}{\sum_{k=1}^{K} X_k^2 \sigma_{yk}^{-2}} \qquad SE_{\hat{\beta}\text{IVW}} = \sqrt{\frac{1}{\sum_{k=1}^{K} X_k^2 \sigma_{yk}^{-2}}}$$

Where X_k is the mean difference in standardized waist or hip circumference per additional effect allele of SNP k and Y_k is the mean difference in standardized log adiponectin per additional effect allele of SNP k with standard error σ_{Yk}.

For the adjusted Mendelian randomization model, we fitted a model having betas for SNP-adiponectin levels association as the dependent variable, betas for SNP-waist circumference and SNP-hip circumference as independent variables and inverse variance weights (with no intercept) to estimate the

independent association of genetically increased waist or hip circumference with blood adiponectin levels. This method is equivalent to the unadjusted IVW method when there is only one independent variable [9].

In the original ADIPOGen summary dataset, betas for the association of SNPs with adiponectin concentration are provided as changes in log units of adiponectin per SNP allele. In order to have the same scale between Mendelian randomization and conventional association analysis, betas (and standard errors) from ADIPOGen dataset had to be harmonised prior to analysis. As only summary data was available, conversion of log adiponectin to equivalent standardized log adiponectin was made using individual level data from 1982 Pelotas Birth Cohort with similar adiponectin distribution (adiponectin levels in ADIPOGen consortium: mean = 9.8 μg/ml (SD = 5.6); adiponectin levels in 1982 Pelotas Birth Cohort: mean = 9.3 μg/ml (SD = 5.7)).

MR-Egger regression method

The Egger regression has been used for almost two decades to detect small study bias in meta-analyses of randomized clinical trials [10]. In this method, the ratio of the effect estimate by its standard error is regressed against the estimate's precision (the inverse of the standard error). Bowden et al. [11] recently proposed an adaptation of the Egger regression to test for bias from pleiotropy in Mendelian randomisation studies.

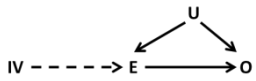
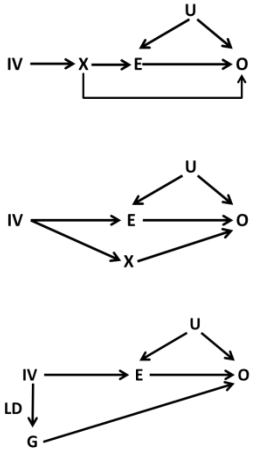
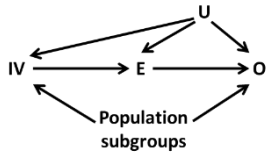
While the IVW estimate is equivalent to the slope of the best fitting line through the observations that pass through the origin, the MR-Egger estimate would be the of the best fitting line through the observations in a model that allows the intercept to vary. In this method, the intercept will reflect the average pleiotropic effect across genetic variants (e.g. mean difference in log adiponectin levels when difference in waist or hip circumference per allele is zero) and the slope coefficient will provide an estimate of the causal effect provided that the InSIDE (Instrument Strength Independent of Direct Effect) assumption holds, which requires that there is no correlation between SNP-exposure association and direct effects of SNP on outcome. The MR-Egger estimate may be underpowered, as it relies on variants having different strengths of association with the risk factor. Bootstrapping (10,000 iterations) was used to derive corrected 95% confidence intervals for MR-Egger intercept and slope using the percentile method [11].

Penalized weighted median estimator

Median-based methods give consistent estimates even when up to half the genetic variants are invalid instrumental variables. The weighted median estimate is defined as the median of an empirical distribution in which each instrumental variable estimate appears with probability proportional to the inverse of its variance[12]. The weighted median estimate is consistent under the assumption that genetic variants representing over 50% of the weight in the analysis are valid instruments. The contribution of heterogeneous variants to the weighted median estimate was downweighted (penalized) by multiplying the inverse-variance weight by the p-value of a chi-squared distribution (1 degree of freedom) corresponding to the *Q* statistics of each SNP when p-value < 0.05 [13]. Bootstrapping (1,000 iterations) was carried out and the bootstrap standard error (the standard deviation of the bootstrap estimates) and a normal approximation (estimate ± 1.96*standard error) were used to derive 95% confidence interval [13].

SUPPLEMENTARY TABLES AND FIGURES

Supplementary table 1. Core instrumental variable assumptions and strategies used to address them

Assumption	*Graphical examples of assumption violation*[*]	*Consequences of potential violation*	*Validation of assumption in the current analysis*
1. IV should be (strongly) associated with the exposure		A weak association between the IV and E can reduce precision and introduce weak instrument bias, which tends to bias the causal estimate towards the OLS estimate in one-sample MR	- Only genetic variants strongly associated with the exposure were selected - In two-sample MR studies with non-overlapping datasets, any bias from weak instruments would be in the direction of the null and, thus, should not result in false positive findings
2. IV should only affect the outcome through the exposure		Bias in MR estimate can result from horizontal pleiotropy (e.g. genetic variant itself or a correlated variant is associated with multiple pathways independent of the exposure) the direction and magnitude of this bias will depend up the direction and magnitude of the association path from IV to O that is not via E	- We extensively investigated heterogeneity and asymmetry in IVW estimates - We compared results from the conventional Mendelian randomization analysis to other Mendelian randomization estimators (Penalized weighted median estimator MR-Egger method) based on a less stringent set of assumptions to assess the validity of our findings
3. IV should be independent of exposure-outcome confounders and IV-outcome exposures		In cases of population stratification, there could be an spurious association between IV and phenotypes	- To reduce the possibility of bias due to population stratification, the analyses were restricted to European-ancestry individuals - All consortia accounted for population structure by adjusting for genomic control inflation factor

IV: instrumental variable; E: exposure; O: outcome; U: unknown confounder; X: other phenotype: G: other genetic variant in LD; LD: linkage disequilibrium. A dashed arrow was used to indicate weak association between IV and E. Adapted from Vanderweele.[14]

Supplementary Table 2. Characteristics of data sources used in the Mendelian randomization analyses

Consortium	ADIPOGen	GIANT
Use	SNP-log adiponectin*	SNP-BMI-adjusted WC and SNP-BMI-adjusted HipC
Studies	16 cohort studies with GWAS data	101 studies of multiple designs with GWAS or Metabochip data
Study population	29,347 individuals of European ancestry	≈ 210,088 individuals of European ancestry
Study-specific mean age (in years) - median [range]	52 [10, 75]	58 [19, 76]
Study-specific mean adiponectin levels (µg/mL) - median [range]	9.8 [4.9, 15.8]	N/A
Study-specific median WC (in cm) - median [range]	N/A	101 [75, 116]
Study-specific median HipC (in cm) - median [range]	N/A	96 [63, 119]
Imputation	IMPUTE, MACH, BIMBAM or Beagle (reference: Phase II CEU HapMap population)	IMPUTE, MACH or Beagle (reference: Phase II CEU HapMap population)
Quality control criteria†	Call rate > 0·95; MAF > 0·01; $p_{HWE} > 10^{-6}$; and quality measures for imputed SNPs ($r^2 \geq 0{\cdot}3$, or proper info ≥ 0·4)	Sample cal rate > 0·85-0·98; SNP call rate > 0·90-0·99; MAF > 0·00-0·01; $p_{HWE} > 10^{-3}$-10^{-7}; and quality measures for imputed SNPs ($r^2 \geq 0{\cdot}3$, proper info ≥ 0·4, or no filtering)
Model	additive	additive
Adjustments	Age, sex, BMI, principal components of genomic ancestry, study site (where appropriate), family structure (one family-based study) and genomic control inflation factor (λ)	Age, age2, BMI and study specific variables (e.g. principal components of genomic ancestry), and genomic control inflation factor (λ)
Data download	https://www.mcgill.ca/genepi/adipogen-consortium	http://www.broadinstitute.org/collaboration/giant/index.php/GIANT_consortium_data_files

* Blood adiponectin concentration was assessed using ELISA or RIA methods. † Quality control criteria may have varied across studies within each consortium. BMI: body mass index; CEU: Centre d'Etude du Polymorphisme Humain collected in Utah; GIANT: Genetic Investigation of ANthropometric Traits; GWAS: genome-wide association study; HipC: hip circumference; HWE: Hardy-Weinberg equilibrium; MAF: minor allele frequency; SNP: single nucleotide polymorphism; WC: waist circumference; N/A: not available. Information on study-specific age, adiponectin levels, WC, and HipC distribution were extracted from the supplementary material of the original publications of GIANT[15] and ADIPOGen[16]. Medians were calculated based only on studies for which information was available in the original publications (all the 16 cohorts from ADIPOGen and 91 out of 101 studies from GIANT)

Supplementary Table 3. SNPs used as instrumental variables for waist circumference in Mendelian randomization analysis

rs ID	Chr	EA	NEA	EAF	R^2	Beta	SE	P-value	N
rs9435732	1	C	T	0.825	0.0003	0.031	0.004	4E-16	228579
rs7536458	1	T	G	0.65	0.0003	0.030	0.004	1E-15	228790
rs12064744	1	T	C	0.3417	0.0002	0.026	0.004	2E-14	231298
rs984222	1	G	C	0.575	0.0005	0.036	0.004	2E-25	231215
rs11205277	1	G	A	0.3898	0.0003	0.027	0.004	1E-13	215898
rs2274432	1	A	G	0.3729	0.0002	0.025	0.004	2E-12	227843
rs12991495	2	T	C	0.675	0.0002	0.028	0.004	6E-14	229964
rs6715793	2	T	C	0.45	0.0001	0.019	0.003	1E-08	231071
rs2052670	2	G	A	0.4083	0.0001	0.020	0.004	2E-08	231210
rs2124969	2	C	T	0.4083	0.0001	0.020	0.003	7E-09	231284
rs13083798	3	A	G	0.5417	0.0002	0.020	0.003	3E-09	230391
rs9864077	3	T	C	0.7583	0.0002	0.022	0.004	1E-09	219478
rs6772896	3	T	C	0.6417	0.0002	0.024	0.004	2E-11	231246
rs7621331	3	A	G	0.6917	0.0001	0.021	0.004	9E-09	231264
rs1344674	3	G	A	0.4833	0.0002	0.024	0.003	4E-13	231241
rs17451107	3	T	C	0.625	0.0002	0.026	0.004	1E-13	227636
rs12493901	3	G	A	0.5417	0.0002	0.021	0.003	8E-10	230668
rs710841	4	T	C	0.2417	0.0003	0.029	0.004	9E-14	230174
rs17541471	5	C	T	0.2333	0.0001	0.023	0.004	4E-08	230478
rs12656497	5	T	C	0.4833	0.0002	0.022	0.003	2E-10	231223
rs459193	5	A	G	0.2167	0.0002	0.025	0.004	8E-11	231220
rs10041657	5	A	G	0.2167	0.0002	0.025	0.004	3E-10	230824
rs272869	5	G	A	0.6583	0.0002	0.021	0.003	7E-10	229935
rs4868125	5	G	C	0.6417	0.0002	0.021	0.004	3E-09	225860
rs10516107	5	A	G	0.2917	0.0002	0.023	0.004	8E-11	231310
rs6556301	5	T	G	0.375	0.0003	0.028	0.004	2E-12	191245
rs1776897	6	G	T	0.075	0.0004	0.061	0.007	6E-20	197374
rs998584	6	A	C	0.475	0.0003	0.029	0.004	6E-15	210814
rs395962	6	T	G	0.3667	0.0003	0.029	0.004	1E-15	231306
rs2745359	6	C	T	0.069	0.0002	0.052	0.009	2E-09	178085
rs2745353	6	T	C	0.55	0.0003	0.029	0.003	8E-19	231143
rs6570507	6	G	A	0.75	0.0002	0.024	0.004	6E-11	228993
rs798489	7	C	T	0.725	0.0002	0.025	0.004	1E-11	230932
rs2214442	7	G	A	0.4417	0.0002	0.026	0.005	4E-09	152053
rs4141278	7	C	T	0.1833	0.0003	0.034	0.004	3E-15	231233
rs7801581	7	T	C	0.2583	0.0002	0.027	0.004	8E-11	216463
rs849140	7	T	C	0.4	0.0003	0.029	0.003	5E-17	228910
rs12679556	8	G	T	0.2083	0.0002	0.026	0.004	1E-11	225056
rs7854560	9	T	C	0.2667	0.0002	0.026	0.004	5E-12	229674
rs10748826	10	T	C	0.5776	0.0002	0.023	0.004	3E-10	195019
rs2071449	12	A	C	0.325	0.0003	0.032	0.004	3E-18	226567
rs7970350	12	C	T	0.5083	0.0001	0.019	0.003	4E-08	229815
rs12317176	12	T	C	0.6167	0.0001	0.020	0.004	6E-09	230924
rs12372180	12	A	G	0.0667	0.0001	0.041	0.007	3E-08	219175
rs7166081	15	A	G	0.8083	0.0002	0.024	0.004	2E-09	230255
rs4886782	15	G	A	0.7333	0.0002	0.024	0.004	6E-12	228446
rs4246302	15	G	A	0.3333	0.0002	0.022	0.004	6E-09	227205
rs4567683	15	A	G	0.2833	0.0001	0.022	0.004	8E-09	228589
rs16957304	16	A	G	0.95	0.0002	0.059	0.011	3E-08	151917
rs3760318	17	G	A	0.6417	0.0002	0.021	0.004	9E-10	228998
rs757608	17	A	G	0.3	0.0002	0.027	0.004	1E-13	229039
rs4239436	18	G	A	0.7417	0.0004	0.040	0.004	1E-22	229607
rs12608504	19	A	G	0.3417	0.0001	0.020	0.004	2E-08	228998
rs3786897	19	G	A	0.4083	0.0001	0.020	0.004	9E-09	228567
rs979012	20	T	C	0.3583	0.0004	0.033	0.004	5E-20	229815
rs2179129	22	A	G	0.55	0.0001	0.019	0.003	3E-08	228844

Chr: chromosome; EA: effect allele (trait-increasing allele); NEA: non-effect allele; R^2: proportion of phenotypic variance explained by SNP; Beta: increase in standardized waist circumference per EA; SE: standard error; N: sample size.

Supplementary Table 4. SNPs used as instrumental variables for hip circumference in Mendelian randomization analysis

rs ID	Chr	EA	NEA	EAF	R^2	Beta	SE	P-value	N
rs6657613	1	T	A	0.53	0.0004	0.031	0.004	4E-18	210917
rs12086130	1	T	C	0.10	0.0002	0.037	0.006	3E-09	206610
rs3748656	1	C	T	0.80	0.0002	0.024	0.004	6E-09	210890
rs11205303	1	C	T	0.36	0.0005	0.041	0.004	6E-25	196314
rs17346473	1	G	A	0.22	0.0003	0.030	0.004	3E-14	210431
rs12075079	1	G	A	0.16	0.0002	0.031	0.004	5E-13	211016
rs2301453	1	A	G	0.54	0.0002	0.022	0.004	6E-10	210882
rs1046934	1	C	A	0.38	0.0002	0.023	0.004	6E-10	210450
rs2820443	1	C	T	0.30	0.0007	0.048	0.004	2E-35	211030
rs6672530	1	A	C	0.77	0.0002	0.028	0.005	8E-10	208172
rs1545552	2	G	A	0.71	0.0003	0.029	0.004	6E-13	208132
rs10195252	2	C	T	0.44	0.0002	0.023	0.004	1E-10	210403
rs4973517	2	T	C	0.75	0.0002	0.029	0.005	2E-10	175930
rs11242	3	T	C	0.43	0.0003	0.027	0.004	6E-14	204637
rs1388251	3	A	G	0.74	0.0002	0.023	0.004	2E-08	211029
rs10804591	3	C	A	0.15	0.0004	0.038	0.004	7E-18	210953
rs724016	3	G	A	0.48	0.0009	0.048	0.004	8E-43	211032
rs4243400	3	G	A	0.50	0.0002	0.025	0.004	3E-12	210478
rs2098771	3	G	A	0.33	0.0001	0.022	0.004	4E-08	196732
rs6845078	4	C	T	0.84	0.0002	0.035	0.005	9E-12	207534
rs9993613	4	T	G	0.51	0.0003	0.027	0.005	7E-10	143494
rs1662837	4	C	T	0.28	0.0003	0.028	0.004	1E-13	210825
rs12648786	4	A	G	0.41	0.0003	0.032	0.004	2E-16	199289
rs11736535	4	G	A	0.30	0.0003	0.029	0.005	1E-10	143695
rs11730399	4	A	C	0.95	0.0003	0.060	0.008	5E-13	173372
rs1173771	5	A	G	0.47	0.0002	0.026	0.004	6E-13	210986
rs7703857	5	T	C	0.41	0.0003	0.028	0.005	5E-10	143721
rs1294410	6	T	C	0.38	0.0003	0.029	0.004	2E-15	210861
rs13216391	6	G	A	0.15	0.0002	0.028	0.005	3E-09	199240
rs11754288	6	A	G	0.47	0.0002	0.021	0.004	3E-09	210954
rs12210905	6	A	G	0.88	0.0002	0.033	0.006	1E-08	210929
rs1759645	6	C	T	0.13	0.0002	0.029	0.005	9E-09	209671
rs16894959	6	C	T	0.10	0.0002	0.036	0.005	3E-13	210242
rs975496	6	G	A	0.84	0.0002	0.031	0.005	1E-09	199331
rs6903448	6	C	T	0.84	0.0002	0.034	0.005	5E-12	211031
rs12207675	6	C	T	0.13	0.0003	0.041	0.006	1E-13	211077
rs7759938	6	C	T	0.36	0.0003	0.028	0.004	2E-13	211029
rs1538170	6	T	C	0.38	0.0002	0.026	0.004	3E-12	201926
rs9491696	6	C	G	0.47	0.0002	0.023	0.004	1E-10	210813
rs9388766	6	T	C	0.33	0.0002	0.028	0.004	8E-13	211072
rs6570509	6	G	T	0.74	0.0006	0.045	0.004	1E-29	197803
rs798497	7	A	G	0.72	0.0004	0.035	0.004	4E-20	210942
rs849141	7	A	G	0.29	0.0003	0.032	0.004	2E-16	211081
rs42235	7	T	C	0.34	0.0004	0.036	0.004	8E-20	208455
rs3731321	7	T	C	0.87	0.0002	0.029	0.005	5E-08	182525
rs7008867	8	A	G	0.21	0.0002	0.024	0.004	6E-09	211066
rs10958476	8	C	T	0.14	0.0002	0.028	0.005	9E-10	199716
rs6984782	8	T	C	0.88	0.0002	0.033	0.005	2E-09	210592
rs6470764	8	C	T	0.81	0.0004	0.039	0.005	8E-18	210864
rs7007820	8	A	G	0.63	0.0001	0.020	0.004	1E-08	211016
rs4448343	9	G	A	0.32	0.0002	0.024	0.004	3E-11	210984
rs10123368	9	C	T	0.20	0.0002	0.026	0.004	5E-09	210933
rs686320	11	G	C	0.91	0.0002	0.038	0.006	7E-12	199308
rs1351394	12	T	C	0.48	0.0002	0.025	0.004	5E-13	210068
rs10748128	12	T	G	0.36	0.0002	0.023	0.004	4E-09	197305
rs7953508	12	T	C	0.25	0.0002	0.024	0.004	4E-09	210624
rs12817549	12	T	C	0.57	0.0003	0.029	0.004	2E-16	210856
rs1727294	12	A	G	0.20	0.0003	0.032	0.004	8E-14	208707
rs3118906	13	G	A	0.76	0.0003	0.030	0.004	8E-15	211005
rs558003	13	A	G	0.04	0.0003	0.049	0.006	4E-15	199267

Supplementary Table 4 (continued)

rs10140922	14	G	T	0.63	0.0003	0.030	0.005	5E-11	143568
rs1254263	14	C	T	0.28	0.0003	0.029	0.005	9E-10	143808
rs17193922	16	G	C	0.38	0.0002	0.024	0.004	1E-09	198589
rs9890032	17	C	G	0.63	0.0002	0.026	0.004	2E-12	207385
rs561341	17	G	T	0.17	0.0002	0.031	0.005	3E-10	211106
rs7223966	17	A	G	0.32	0.0003	0.029	0.004	2E-13	211080
rs1120297	17	T	C	0.48	0.0002	0.021	0.004	2E-09	210991
rs4369779	18	C	T	0.74	0.0003	0.035	0.004	3E-15	210787
rs181553	18	A	G	0.68	0.0003	0.029	0.004	9E-15	210832
rs12980348	19	G	T	0.38	0.0003	0.029	0.004	9E-16	210456
rs169797	20	A	G	0.75	0.0002	0.024	0.004	1E-09	204594
rs6088619	20	G	A	0.13	0.0003	0.039	0.005	9E-13	199166
rs143384	20	G	A	0.40	0.0006	0.044	0.004	1E-31	209682
rs6060717	20	C	T	0.16	0.0002	0.032	0.005	6E-12	211073
rs6141600	20	C	T	0.28	0.0003	0.035	0.005	3E-11	142740

Chr: chromosome; EA: effect allele (trait-increasing allele); NEA: non-effect allele; R^2: proportion of phenotypic variance explained by SNP; Beta: increase in standardized hip circumference per EA; SE: standard error; N: sample size.

Supplementary Table 5. SNPs used as instrumental variables for adiponectin concentration in Mendelian randomisation analysis and association with adiponectin concentration

rs ID	Chr	EA	NEA	EAF	R^2	Beta	SE	P-value	N
rs6810075	3	T	C	0.63	0.0066	0.108	0.0078	4.E-41	29140
rs16861209	3	A	C	0.08	0.0125	0.313	0.0163	3.E-77	29199
rs17366568	3	G	A	0.91	0.0125	0.252	0.0142	3.E-66	24865
rs3774261	3	A	G	0.40	0.0080	0.114	0.0075	1.E-49	29081

Chr: chromosome; EA: effect allele (trait-increasing allele); NEA: non-effect allele; R^2: proportion of phenotypic variance explained by SNP; Beta: increase in standardized log adiponectin concentration per EA; SE: standard error; N: sample size.

Supplementary Table 6. Association of fat depots and adiponectin concentration with covariates according to sex

	Visceral fat			Deep subcutaneous abdominal fat			Superficial subcutaneous abdominal fat			Gluteofemoral fat			Adiponectin concentration		
	β	95% CI		β	95% CI		β	95% CI		β	95% CI		β	95% CI	
							Males								
African ancestry (%)															
0.00-4.59	Ref			Ref			Ref			Ref			Ref		
4.60-10.99	0.01	-0.12	0.13	-0.02	-0.14	0.10	-0.03	-0.16	0.10	-0.09	-0.22	0.04	0.00	-0.13	0.14
11.00-87.91	-0.11	-0.24	0.02	-0.19	-0.31	-0.07	-0.06	-0.18	0.07	-0.27	-0.40	-0.14	-0.12	-0.25	0.01
Leisure-time physical activity															
Inactive	Ref			Ref			Ref			Ref			Ref		
Insufficiently active	-0.02	-0.15	0.11	-0.02	-0.15	0.11	-0.08	-0.22	0.05	-0.08	-0.22	0.06	0.04	-0.10	0.18
Active	-0.18	-0.30	-0.06	-0.10	-0.22	0.02	-0.09	-0.21	0.04	-0.13	-0.26	0.00	-0.07	-0.20	0.06
Smoking															
Never smoker	Ref			Ref			Ref			Ref			Ref		
Ex-smoker	0.13	-0.01	0.27	-0.10	-0.24	0.03	-0.17	-0.31	-0.03	-0.05	-0.20	0.10	-0.04	-0.19	0.11
1-9 cigarettes/day	-0.04	-0.23	0.16	-0.33	-0.51	-0.14	-0.37	-0.57	-0.18	-0.39	-0.60	-0.19	0.00	-0.21	0.20
≥ 10 cigarettes/day	-0.03	-0.17	0.11	-0.39	-0.52	-0.26	-0.46	-0.59	-0.32	-0.40	-0.55	-0.26	0.04	-0.11	0.19
Alcohol drinking															
< 1 dose/day	Ref			Ref			Ref			Ref			Ref		
≥ 1 dose/day	0.18	0.08	0.29	-0.03	-0.14	0.07	-0.09	-0.20	0.02	0.01	-0.11	0.12	0.00	-0.11	0.12
							Females								
African ancestry (%)															
0.00-4.59	Ref			Ref			Ref			Ref			Ref		
4.60-10.99	0.06	-0.07	0.18	-0.06	-0.18	0.07	-0.02	-0.15	0.10	-0.13	-0.26	0.00	-0.13	-0.26	-0.01
11.00-87.91	0.21	0.09	0.33	0.02	-0.10	0.15	0.10	-0.02	0.23	-0.03	-0.16	0.10	-0.27	-0.39	-0.14
Leisure-time physical activity															
Inactive	Ref			Ref			Ref			Ref			Ref		
Insufficiently active	-0.21	-0.35	-0.07	0.02	-0.12	0.16	0.03	-0.11	0.17	-0.05	-0.19	0.10	0.02	-0.13	0.16
Active	-0.30	-0.42	-0.17	-0.08	-0.21	0.05	-0.10	-0.23	0.02	-0.07	-0.20	0.06	0.09	-0.04	0.22
Smoking															
Never smoker	Ref			Ref			Ref			Ref			Ref		
Ex-smoker	0.07	-0.07	0.20	-0.02	-0.16	0.12	-0.03	-0.16	0.11	-0.08	-0.22	0.06	-0.11	-0.25	0.02
1-9 cigarettes/day	-0.04	-0.22	0.14	0.00	-0.18	0.19	-0.16	-0.34	0.02	-0.28	-0.47	-0.09	-0.14	-0.33	0.04
≥ 10 cigarettes/day	0.15	0.00	0.31	-0.13	-0.29	0.03	-0.25	-0.40	-0.09	-0.31	-0.47	-0.15	-0.28	-0.43	-0.12
Alcohol drinking															
< 1 dose/day	Ref			Ref			Ref			Ref			Ref		
≥ 1 dose/day	-0.01	-0.12	0.09	0.04	-0.07	0.15	-0.04	-0.14	0.07	0.05	-0.06	0.16	0.06	-0.05	0.16

Fat depots and adiponectin concentration are expressed as standard deviation units.

Supplementary Table 7. Pearson's correlation coefficients between different measures of adiposity

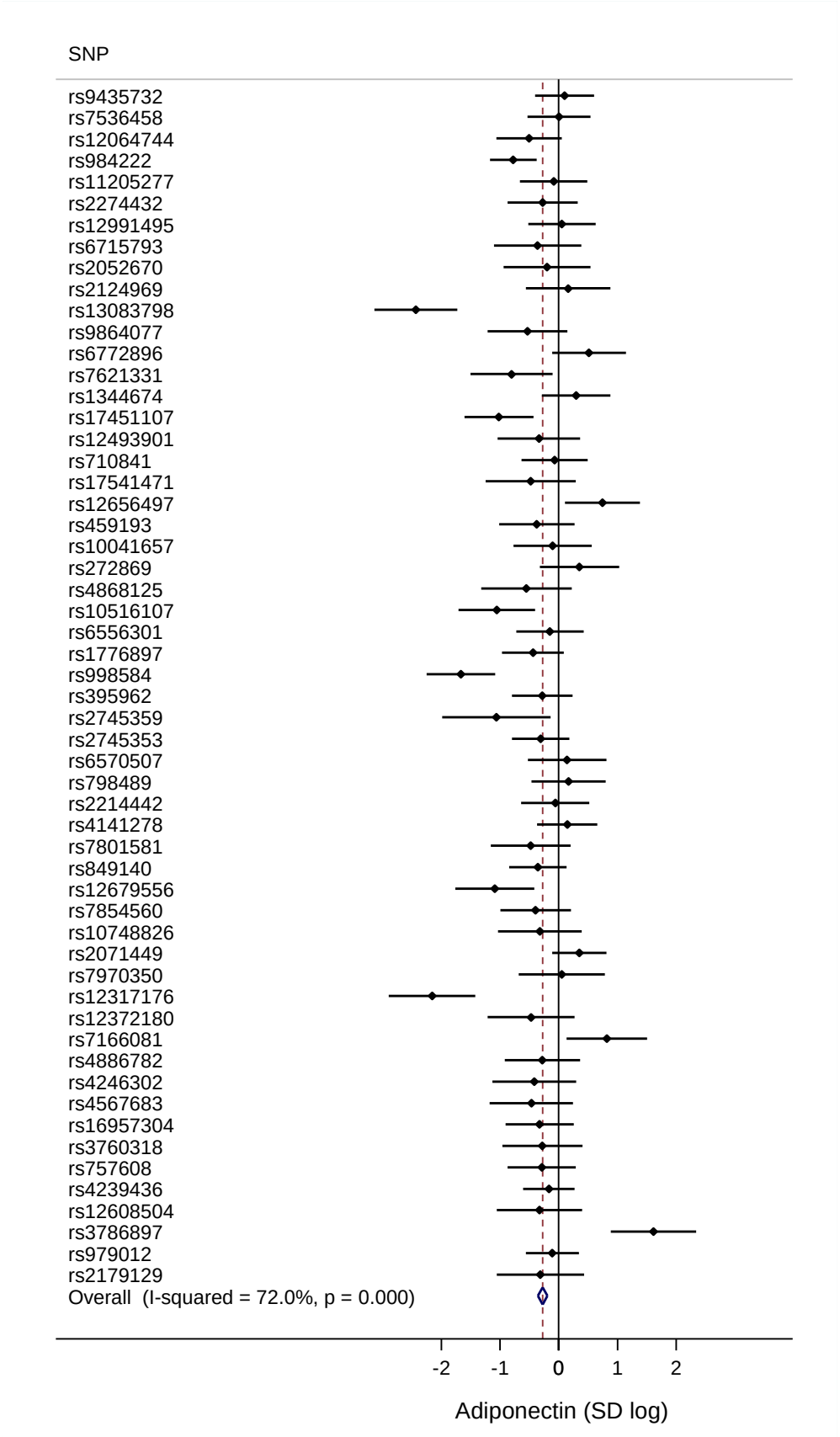
		BMI	Total fat	VAT	dSCAAT	sSCAAT	GFAT
MALE	BMI	1.00	0.89	0.67	0.66	0.60	0.84
	Total fat	0.89	1.00	0.62	0.75	0.71	0.97
	VAT	0.67	0.62	1.00	0.35	0.30	0.53
	dSCAAT	0.66	0.75	0.35	1.00	0.59	0.71
	sSCAAT	0.60	0.71	0.30	0.59	1.00	0.69
	GFAT	0.84	0.97	0.53	0.71	0.69	1.00
FEMALE	BMI	1.00	0.94	0.63	0.71	0.66	0.89
	Total fat	0.94	1.00	0.55	0.73	0.68	0.97
	VAT	0.63	0.55	1.00	0.34	0.31	0.48
	dSCAAT	0.71	0.73	0.34	1.00	0.46	0.65
	sSCAAT	0.66	0.68	0.31	0.46	1.00	0.61
	GFAT	0.89	0.97	0.48	0.65	0.61	1.00

BMI: body mass index; GFAT: gluteofemoral adipose tissue; dSCAAT: deep subcutaneous adipose tissue; sSCAAT: superficial subcutaneous adipose tissue; VAT: visceral adipose tissue. Data from the 2012 follow-up of the 1982 Pelotas Birth Cohort.

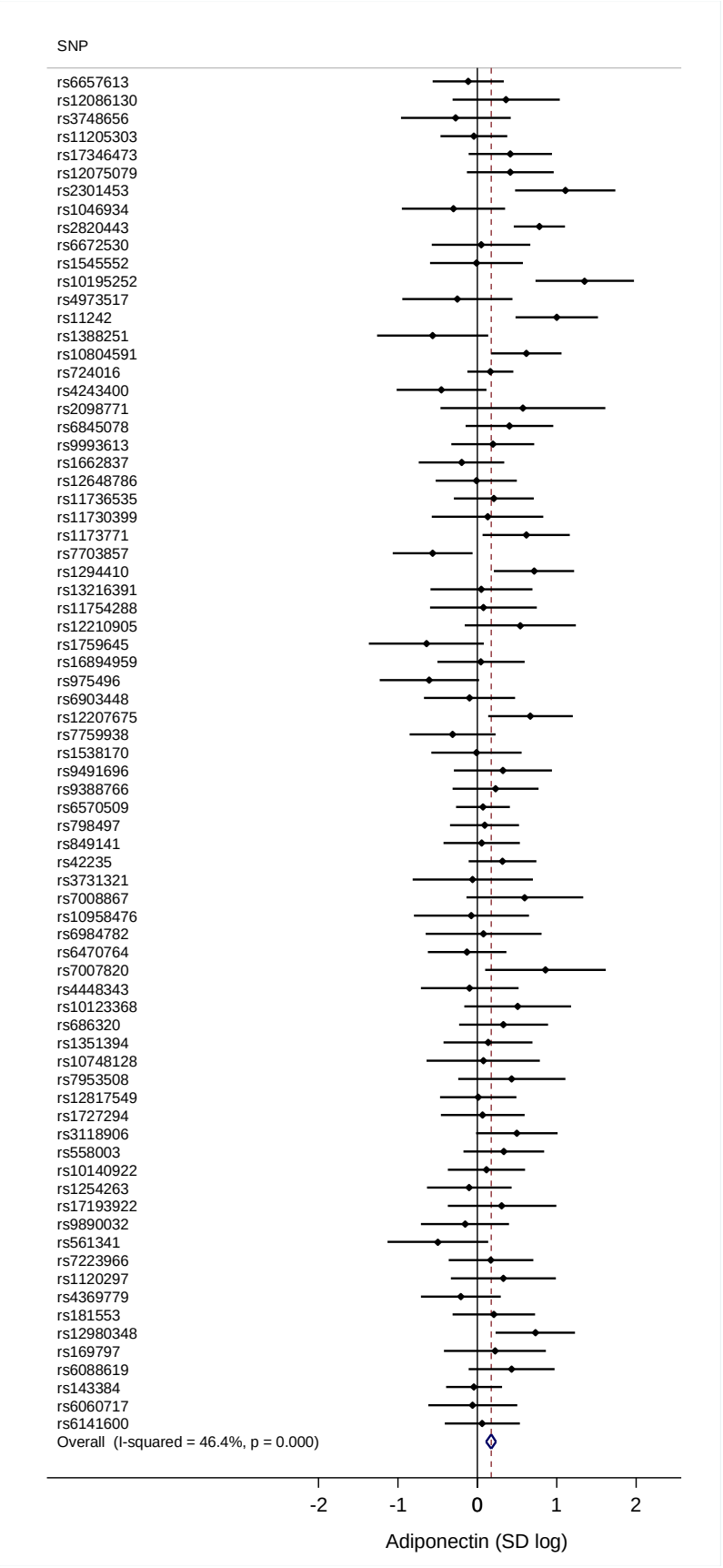
Supplementary Table 8. P values for the association of study variables with missingness indicator

	P values	
Variable	Males	Females
African ancestry	0.13	0.64
Leisure-time physical activity	0.10	0.02
Smoking	0.47	0.48
Alcohol drinking	0.03	0.70
Body mass index	$\mathbf{1*10^{-10}}$	0.74
Visceral fat	$\mathbf{2*10^{-4}}$	0.74
Deep subcutaneous abdominal fat	$\mathbf{3*10^{-5}}$	0.72
Superficial subcutaneous abdominal fat	**0.01**	0.41
Gluteofemoral fat	0.16	0.34
Adiponectin	0.80	0.67
Glucose	0.12	0.83
C reactive protein	0.16	0.19

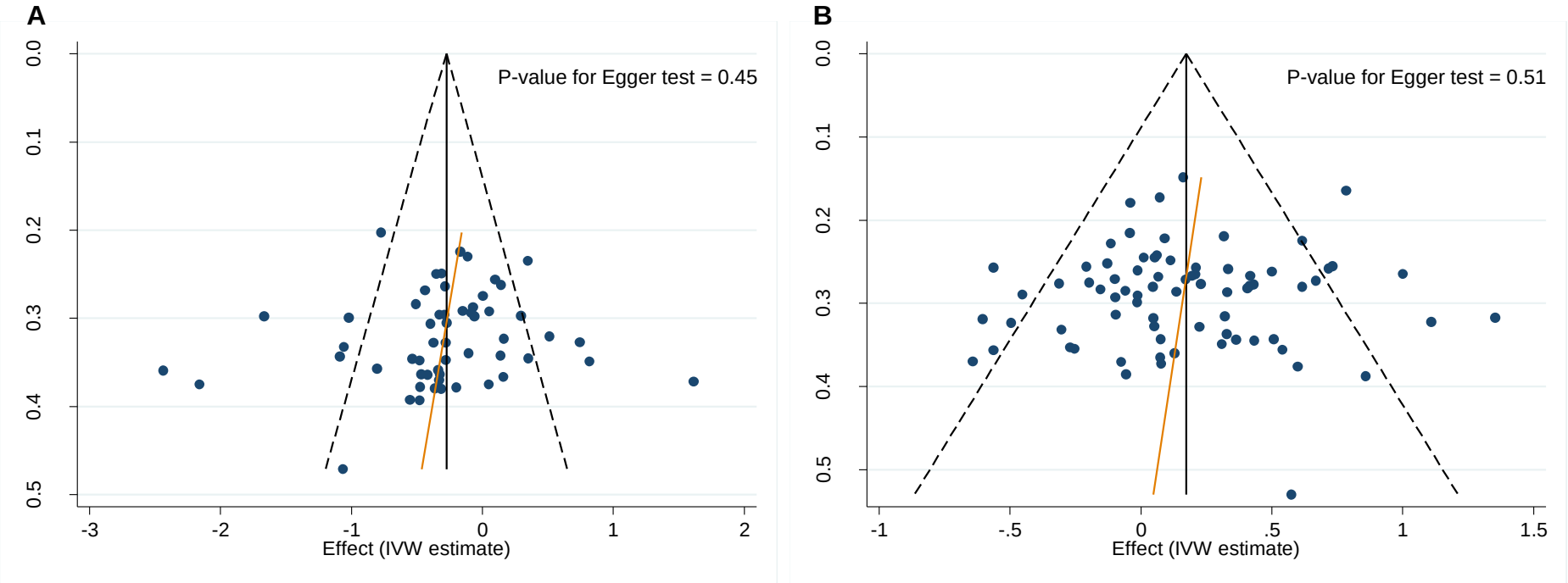
Data from the 2012 follow-up of the 1982 Pelotas Birth Cohort



Supplementary Figure 1. Metanalysis and heterogeneity analysis of Mendelian randomization estimates of each SNP for the association of waist circumference with blood adiponectin levels. Data from GIANT (n = up to 210,088 individuals) and ADIPOGen (n = 29,347 individuals) consortia.



Supplementary Figure 2. Metanalysis and heterogeneity analysis of Mendelian randomization estimates of each SNP for the association of hip circumference with blood adiponectin levels. Data from GIANT (n = up to 210,088 individuals) and ADIPOGen (n = 29,347 individuals) consortia.



Supplementary Figure 3. Funnel plot of instrument precision (standard error of IVW estimate) against IVW estimates for each genetic variant for Mendelian randomization analysis of the influence of waist (A) or hip (B) circumference on adiponectin levels. Each blue dot corresponds to estimates of one genetic variant. Full vertical line represents the overall IVW estimate and dashed lines represent pseudo 95% confidence limits. Red line indicates the presence of asymmetry. IVW: inverse-variance weighted method. Data from GIANT (n = up to 210,088 individuals) and ADIPOGen (n = 29,347 individuals) consortia.

References

1. Rolfe Ede L, Loos RJ, Druet C, et al. Association between birth weight and visceral fat in adults. *Am J Clin Nutr* 2010; **92**: 347-52.
2. Stolk RP, Wink O, Zelissen PM, Meijer R, van Gils AP, Grobbee DE. Validity and reproducibility of ultrasonography for the measurement of intra-abdominal adipose tissue. *Int J Obes Relat Metab Disord* 2001; **25**: 1346-51.
3. Araujo de Franca GV, Lucia Rolfe E, Horta BL, et al. Associations of birth weight, linear growth and relative weight gain throughout life with abdominal fat depots in adulthood: the 1982 Pelotas (Brazil) birth cohort study. *Int J Obes (Lond)* 2015.
4. International HapMap C, Altshuler DM, Gibbs RA, et al. Integrating common and rare genetic variation in diverse human populations. *Nature* 2010; **467**: 52-8.
5. Li JZ, Absher DM, Tang H, et al. Worldwide human relationships inferred from genome-wide patterns of variation. *Science* 2008; **319**: 1100-4.
6. Lima-Costa MF, Rodrigues LC, Barreto ML, et al. Genomic ancestry and ethnoracial self-classification based on 5,871 community-dwelling Brazilians (The Epigen Initiative). *Sci Rep* 2015; **5**: 9812.
7. Alexander DH, Novembre J, Lange K. Fast model-based estimation of ancestry in unrelated individuals. *Genome Res* 2009; **19**: 1655-64.
8. Shim H, Chasman DI, Smith JD, et al. A multivariate genome-wide association analysis of 10 LDL subfractions, and their response to statin treatment, in 1868 Caucasians. *PLoS One* 2015; **10**: e0120758.
9. Burgess S, Dudbridge F, Thompson SG. Re: "Multivariable Mendelian randomization: the use of pleiotropic genetic variants to estimate causal effects". *Am J Epidemiol* 2015; **181**: 290-1.
10. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ* 1997; **315**: 629-34.
11. Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. *Int J Epidemiol* 2015; **44**: 512-25.
12. Bowden J, Davey Smith G, Haycock PC, Burgess S. Consistent Estimation in Mendelian Randomization with Some Invalid Instruments Using a Weighted Median Estimator. *Genet Epidemiol* 2016; **40**: 304-14.
13. Bowden J, Smith GD, Haycock PC, Burgess S. Consistent estimation in Mendelian randomization with some invalid instruments using a weighted median estimator. *Genetic Epidemiology (in press)* 2016.
14. VanderWeele TJ, Tchetgen Tchetgen EJ, Cornelis M, Kraft P. Methodological challenges in mendelian randomization. *Epidemiology* 2014; **25**: 427-35.
15. Shungin D, Winkler TW, Croteau-Chonka DC, et al. New genetic loci link adipose and insulin biology to body fat distribution. *Nature* 2015; **518**: 187-96.
16. Dastani Z, Hivert MF, Timpson N, et al. Novel loci for adiponectin levels and their influence on type 2 diabetes and metabolic traits: a multi-ethnic meta-analysis of 45,891 individuals. *PLoS Genet* 2012; **8**: e1002607.