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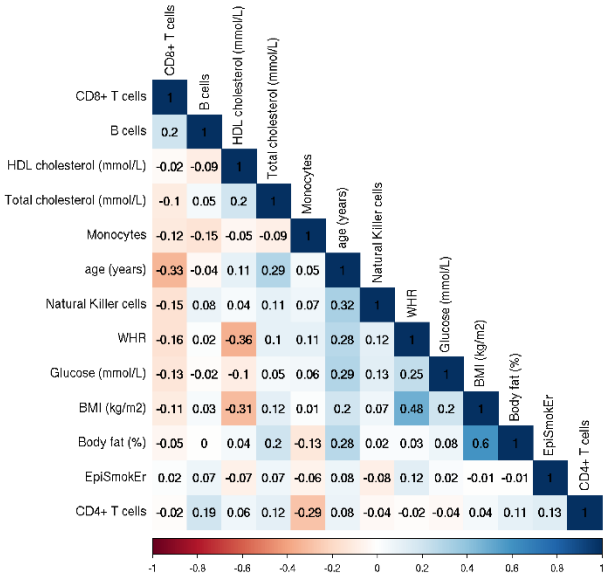
Supplemental information

DNA methylation-based predictors of metabolic traits in Scottish and Singaporean cohorts

Hannah M. Smith, Hong Kiat Ng, Joanna E. Moodie, Danni A. Gadd, Daniel L. McCartney, Elena Bernabeu, Archie Campbell, Paul Redmond, Adele Taylor, Danielle Page, Janie Corley, Sarah E. Harris, Darwin Tay, Ian J. Deary, Kathryn L. Evans, Matthew R. Robinson, John C. Chambers, Marie Loh, Simon R. Cox, Riccardo E. Marioni, and Robert F. Hillary

A)



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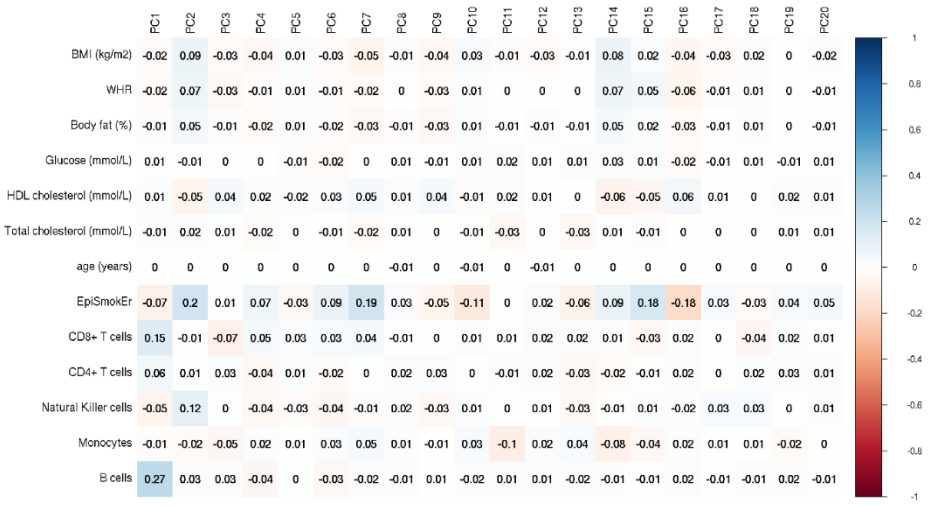


Figure S1: Metabolic trait, covariate and 20 DNAm PCs correlations in Generation Scotland. Figure S1A shows a heatmap of Pearson correlations between metabolic traits (BMI in kg/m^2; HDL cholesterol, total cholesterol and glucose in mmol/L; body fat in percentage; WHR) and covariates in Generation Scotland. Figure S1B shows a heatmap of Pearson correlations between metabolic traits/covariates and the first 20 DNAm PCs. BMI = body mass index; WHR = waist-hip ratio; HDL cholesterol = high-density lipoprotein cholesterol; EpiSmokEr = epigenetic smoking score; DNAm = DNA methylation; PCs = principal components.

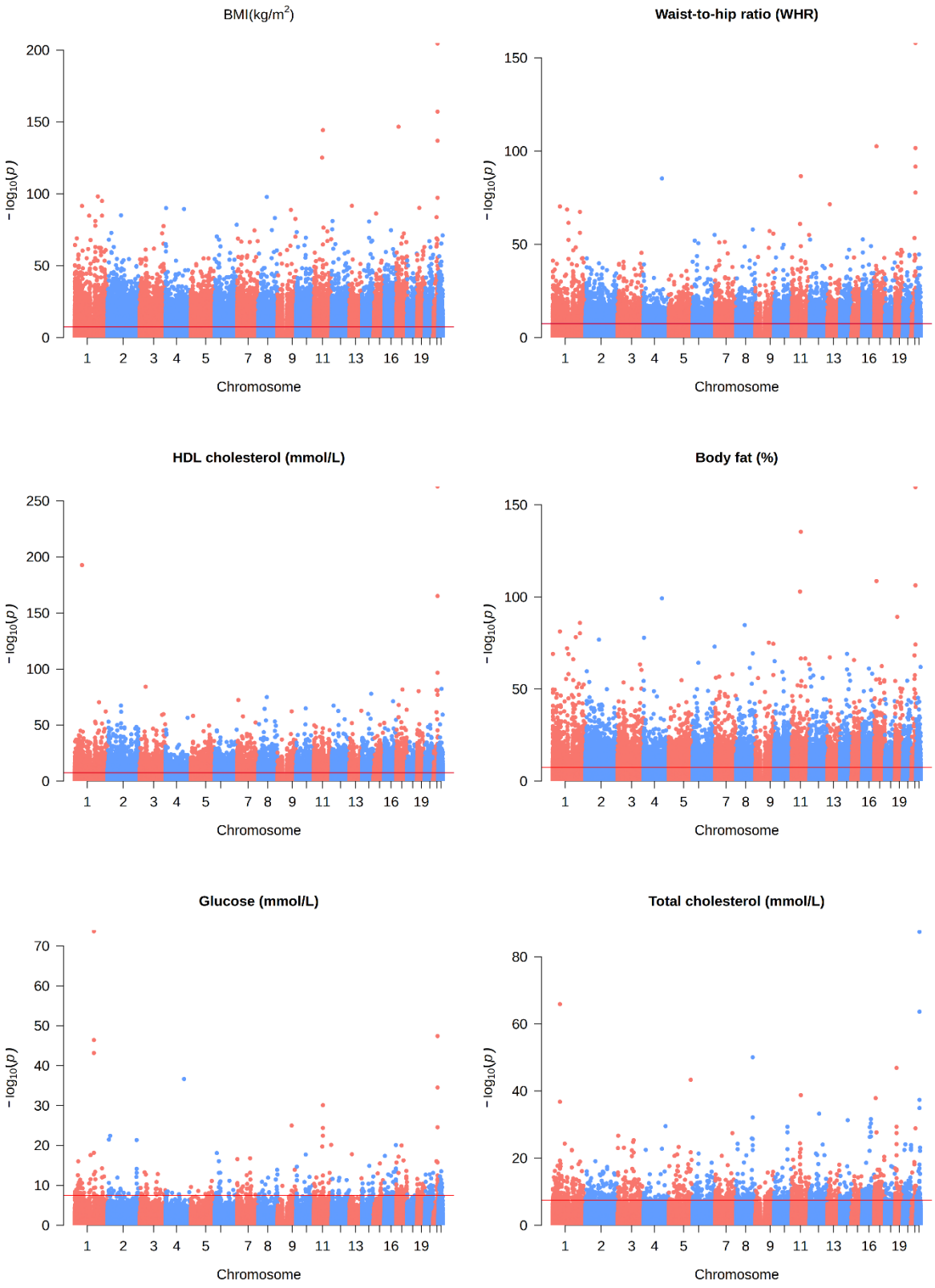
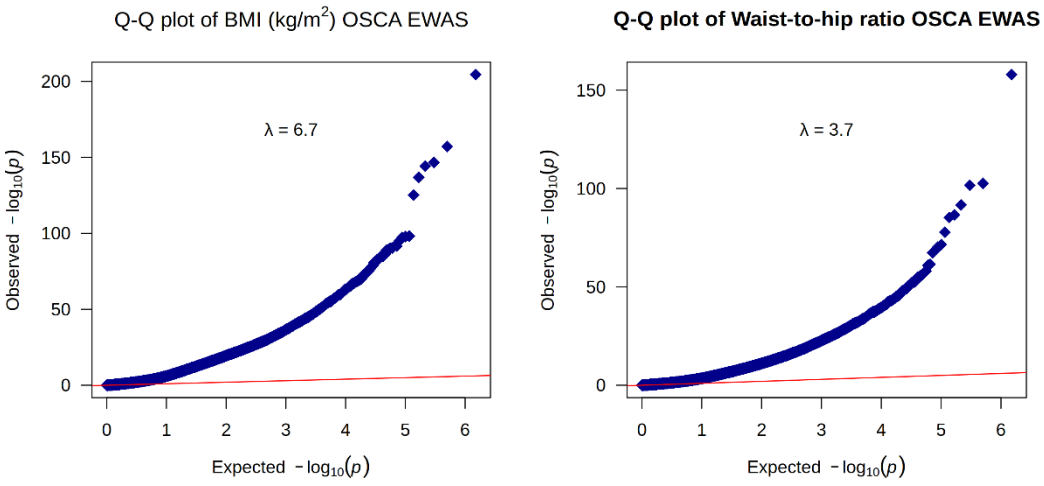
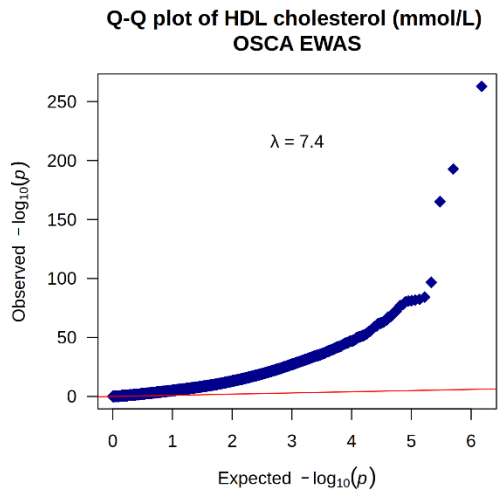
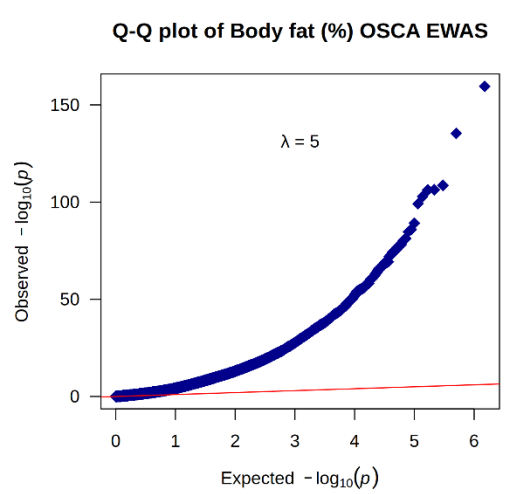


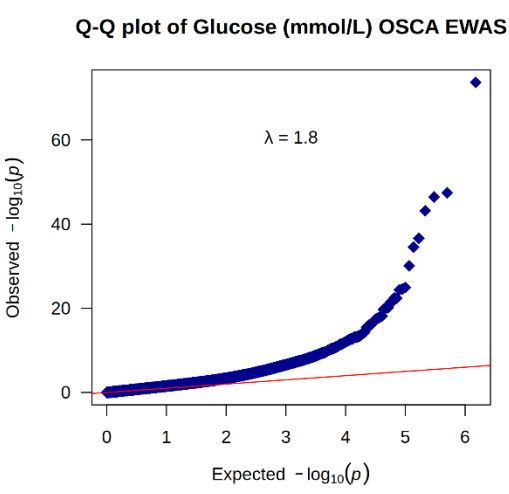
Figure S2: Manhattan plots of the results from the non-PC-adjusted marginal linear regression epigenome-wide association studies of six metabolic traits in Generation Scotland. The Manhattan plots for each of the six metabolic traits show each CpG as a data point. Outcomes in each EWAS are the residuals from metabolic traits regressed on age, age^2, sex and family structure. Original outcome units are indicated in the plot titles. The x-axis shows the chromosome position, and the y-axis shows the association significance (-$\log_{10}$(P)) for each CpG site. The horizontal red line indicates the significance threshold ($P < 3.6 \times 10^{-8}$). BMI =

body mass index; WHR = waist-hip ratio; HDL cholesterol = high-density lipoprotein cholesterol; PC = principal component.









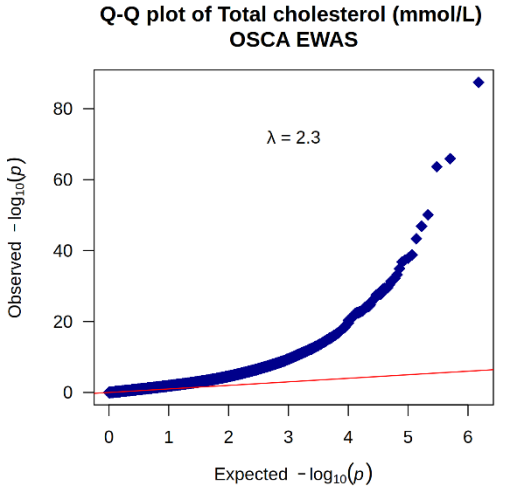


Figure S3: Quantile-Quantile plots of the results from the non-PC-adjusted marginal linear regression epigenome-wide association studies of six metabolic traits in Generation Scotland. The plots show expected $-\log_{10}(P)$ by the observed $-\log_{10}(P)$ for each metabolic trait. Outcomes in each EWAS are the residuals from metabolic traits regressed on age, age^2, sex and family structure. Original outcome units are indicated in the plot titles. The red line shows a trend line of where the observed and expected values are the same. The inflation factor, lambda (λ), is indicated on each plot. BMI = body mass index; WHR = waist-hip ratio; HDL cholesterol = high-density lipoprotein cholesterol; PC = principal component.

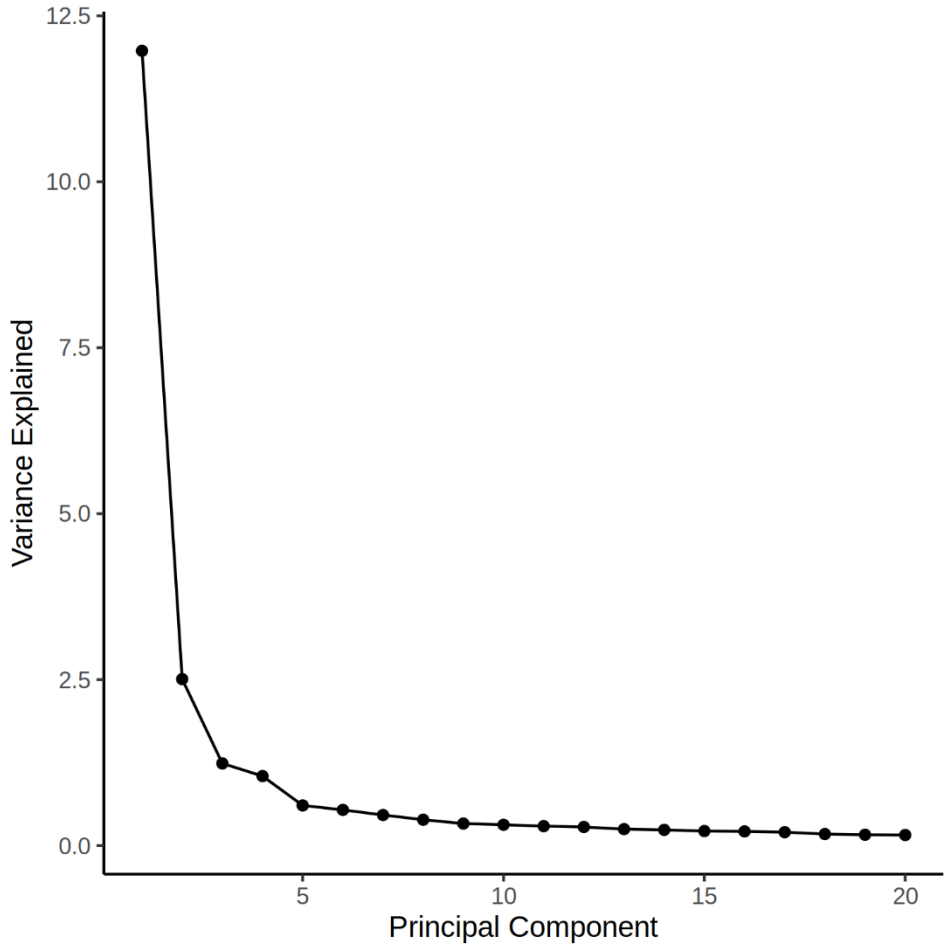


Figure S4: Variance explained in DNA methylation by the first 20 principal components. The plot shows the variance explained in the DNA methylation data by each of the first 20 principal components in Generation Scotland.

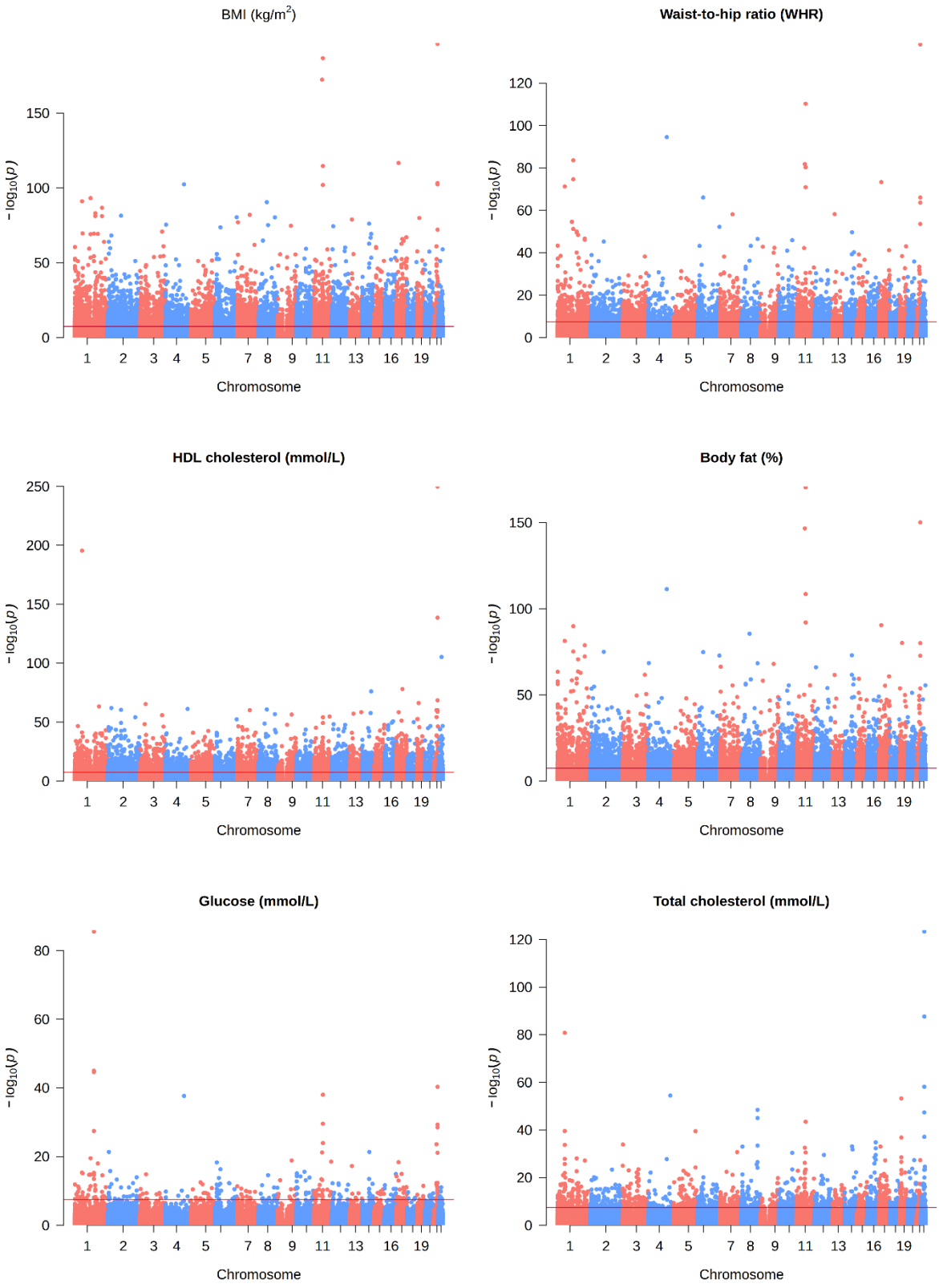


Figure S5: Manhattan plots of the results from the DNAm-PC-adjusted marginal linear regression epigenome-wide association studies of six metabolic traits in Generation Scotland. The Manhattan plots for each of the six metabolic traits show each CpG as a data point. Outcomes in each EWAS are the residuals from metabolic traits regressed on age, age^2, sex and family structure. Original outcome units are indicated in the plot titles. The x-axis shows the chromosome position, and the y-axis shows the association significance (-$\log_{10}$(P)) for each CpG site. The horizontal red line indicates the significance threshold ($P < 3.6 \times 10^{-8}$). BMI =

body mass index; WHR = waist-hip ratio; HDL cholesterol = high-density lipoprotein cholesterol; DNAm = DNA methylation; PC = principal component.

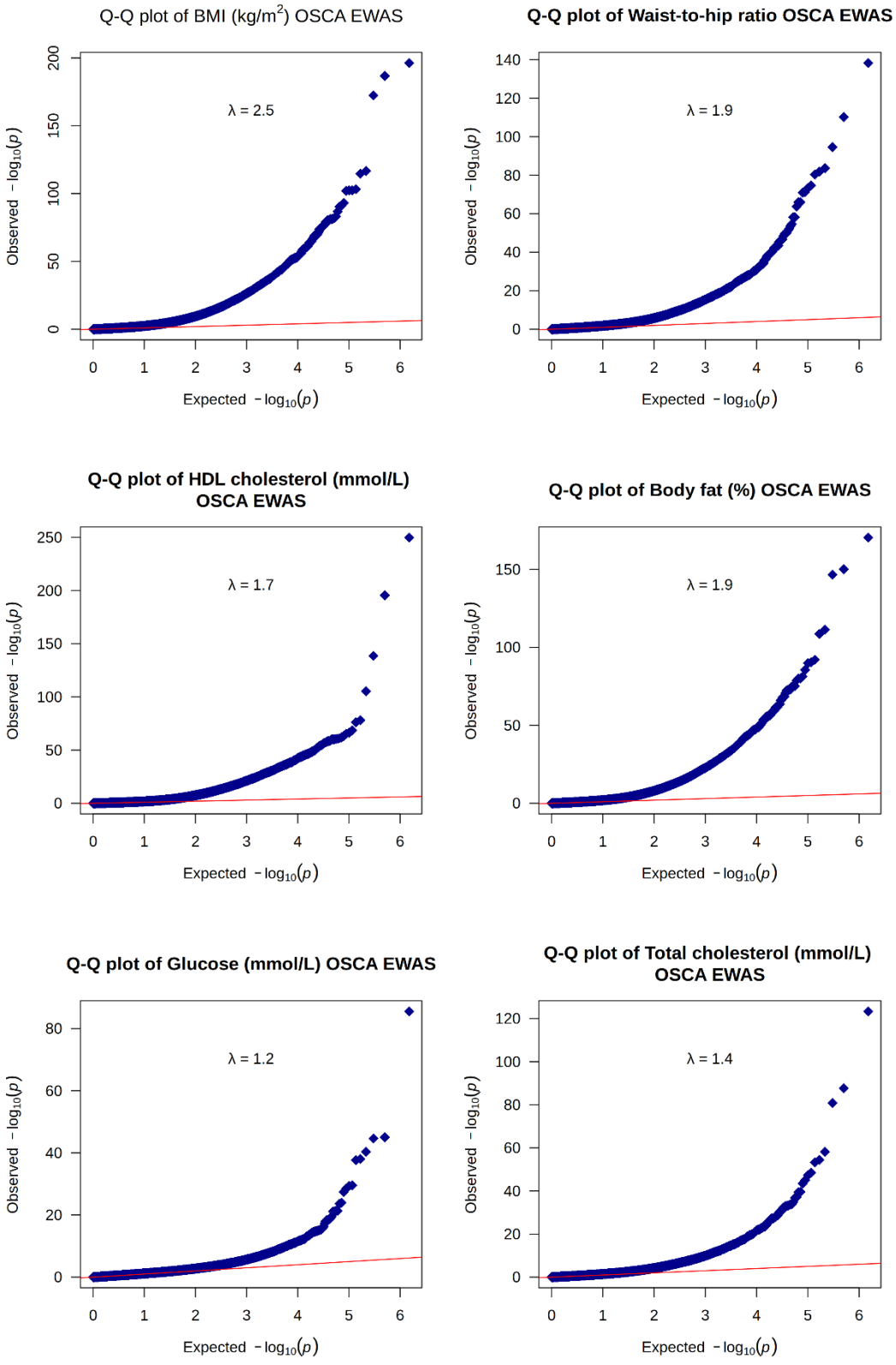


Figure S6: Quantile-Quantile plots of the results from the DNAm-PC-adjusted marginal linear regression epigenome-wide association studies of six metabolic traits in Generation Scotland. The plots show

expected $-\log_{10}(P)$ by the observed $-\log_{10}(P)$ for each metabolic trait. Outcomes in each EWAS are the residuals from metabolic traits regressed on age, age^2, sex and family structure. Original outcome units are indicated in the plot titles The red line shows a trend line of where the observed and expected values are the same. The inflation factor, lambda (λ), is indicated on each plot. BMI = body mass index; WHR = waist-hip ratio; HDL cholesterol = high-density lipoprotein cholesterol; DNAm = DNA methylation; PC = principal component.

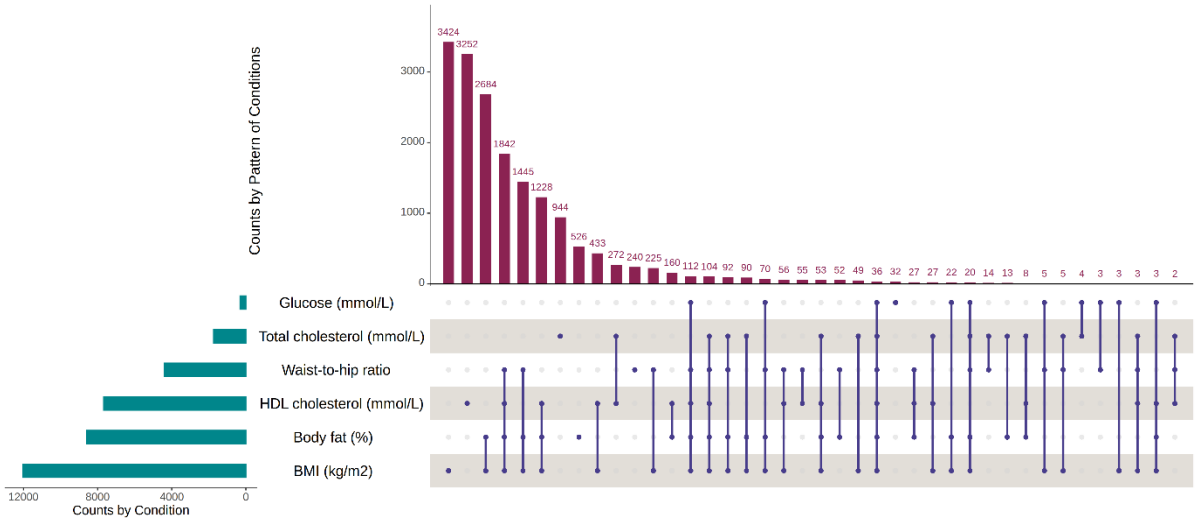


Figure S7: An upset plot of significant CpGs for the six metabolic traits in Generation Scotland. The figure shows the number of unique and overlapping CpGs for the six metabolic traits from the DNAm-PC-adjusted marginal linear regression models. Outcomes in each EWAS are the residuals from metabolic traits regressed on age, age^2, sex and family structure. Original outcome units are indicated in the plot. BMI = body mass index; WHR = waist-hip ratio; HDL cholesterol = high-density lipoprotein cholesterol; DNAm = DNA methylation; PC = principal component.

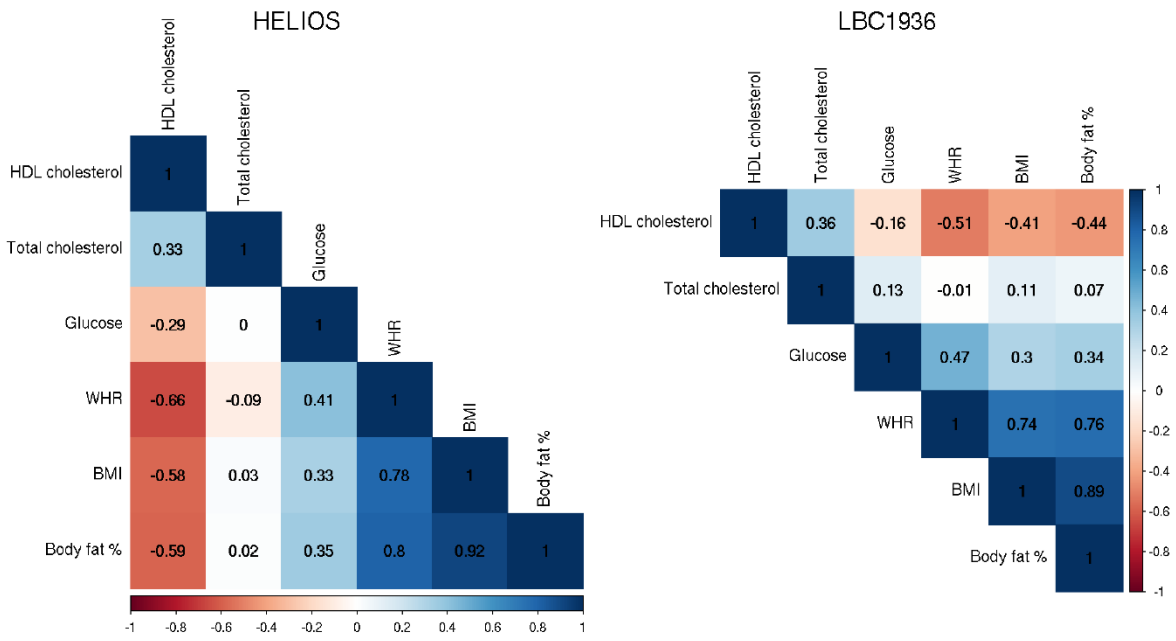


Figure S8: Metabolic EpiScore correlations for the Health for Life in Singapore (HELIOS) study and the Lothian Birth Cohort 1936 (LBC1936). The heatmaps show the Pearson correlation between each metabolic EpiScore in HELIOS and LBC1936. BMI = body mass index; WHR = waist-hip ratio; HDL cholesterol = high-density lipoprotein cholesterol.

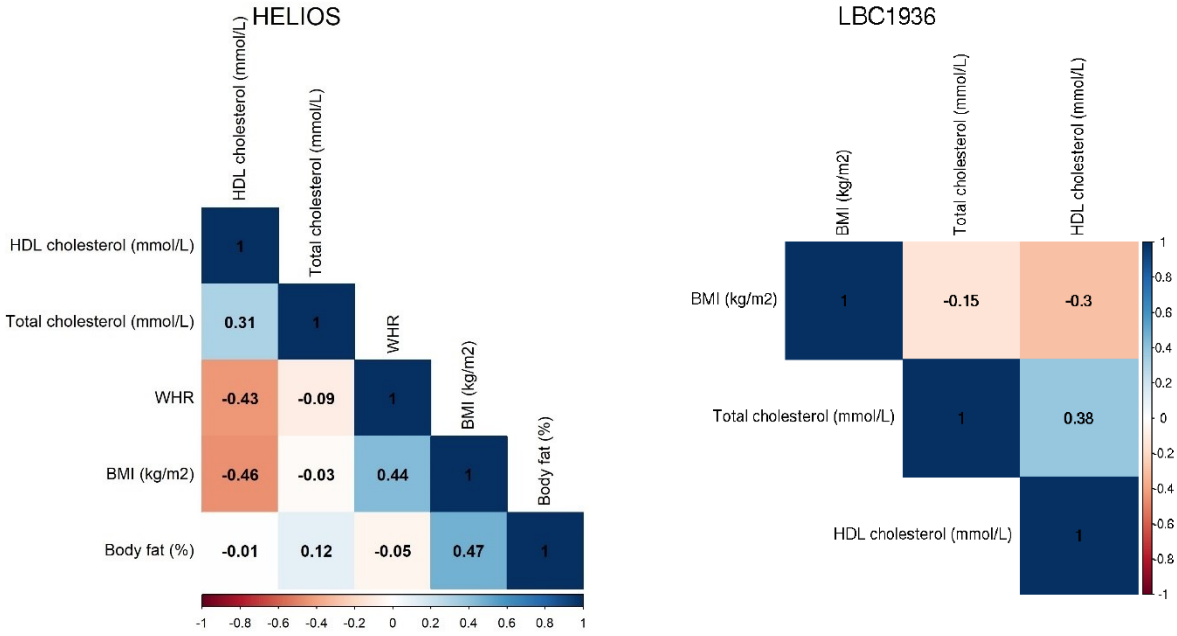


Figure S9: Metabolic trait correlations in the Health for Life in Singapore (HELIOS) study and the Lothian Birth Cohort 1936 (LBC1936). The heatmaps show the Pearson correlation between measured metabolic traits in HELIOS and LBC1936. BMI = body mass index; WHR = waist-hip ratio; HDL cholesterol = high-density lipoprotein cholesterol.

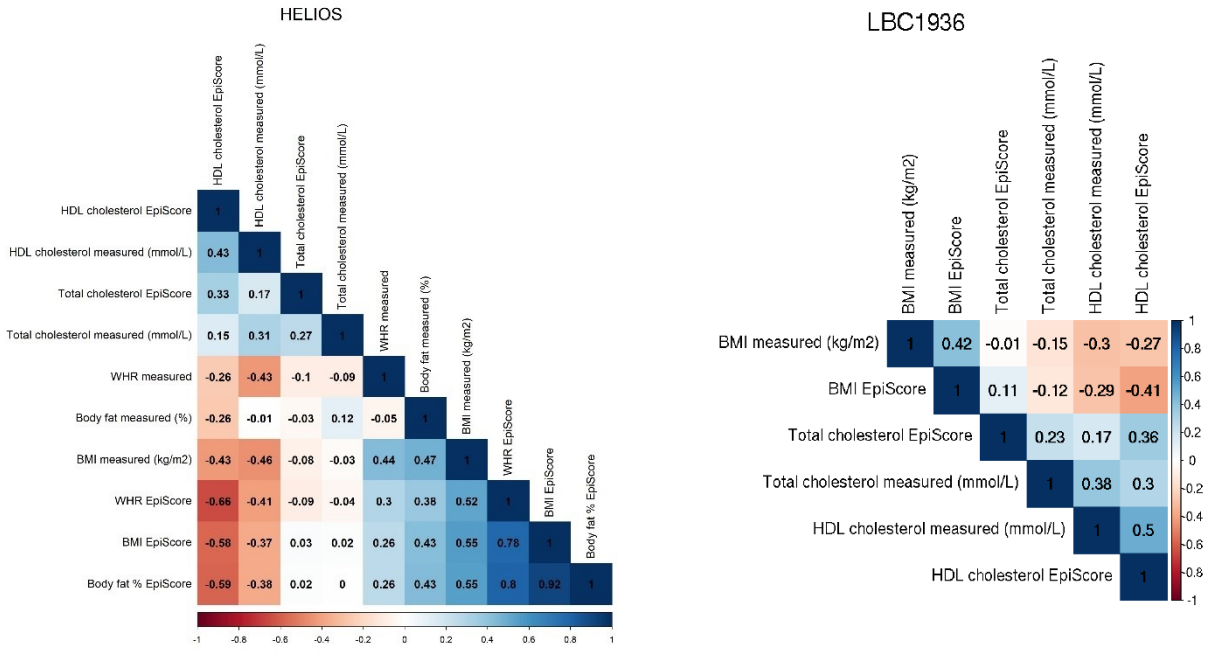


Figure S10: EpiScore-metabolic trait correlations in the Health for Life in Singapore (HELIOS) and the Lothian Birth Cohort (LBC1936). The heatmaps show the Pearson correlations between metabolic EpiScores and measured metabolic traits in HELIOS and LBC1936. BMI = body mass index; WHR = waist-hip ratio; HDL cholesterol = high-density lipoprotein cholesterol.

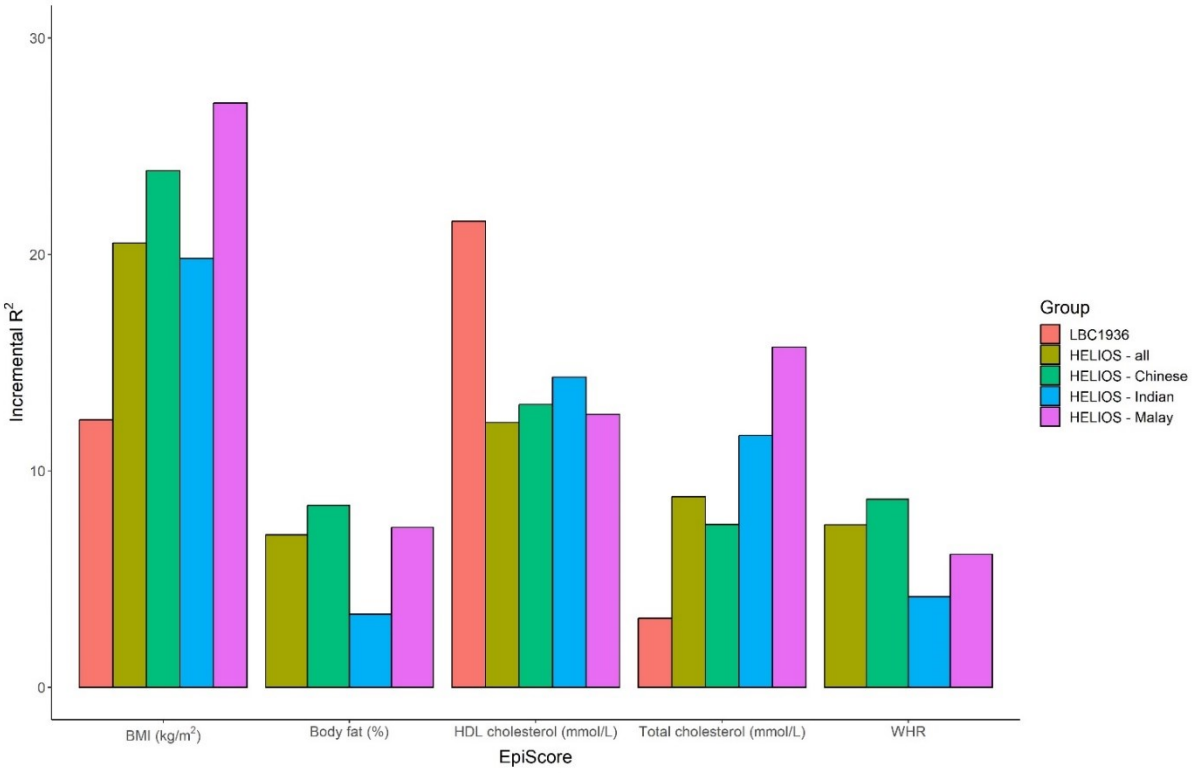


Figure S11: The variance explained in measured metabolic traits by Bayesian EpiScores in the Health for Life in Singapore (HELIOS) study and the Lothian Birth Cohort 1936 (LBC1936). The figure shows the incremental R^2 for each metabolic trait (BMI in kg/m^2; HDL cholesterol and total cholesterol in mmol/L; body fat in percentage; WHR) accounted for by their corresponding Bayesian metabolic EpiScores over and above age and sex-adjusted linear regression models in LBC1936 and HELIOS. The incremental R^2 was calculated for each subset and in the whole cohort for the HELIOS study. Full cohort models in HELIOS were additionally adjusted for subgroup (Chinese, Malay and Indian). BMI = body mass index; WHR = waist-hip ratio; HDL cholesterol = high-density lipoprotein cholesterol.

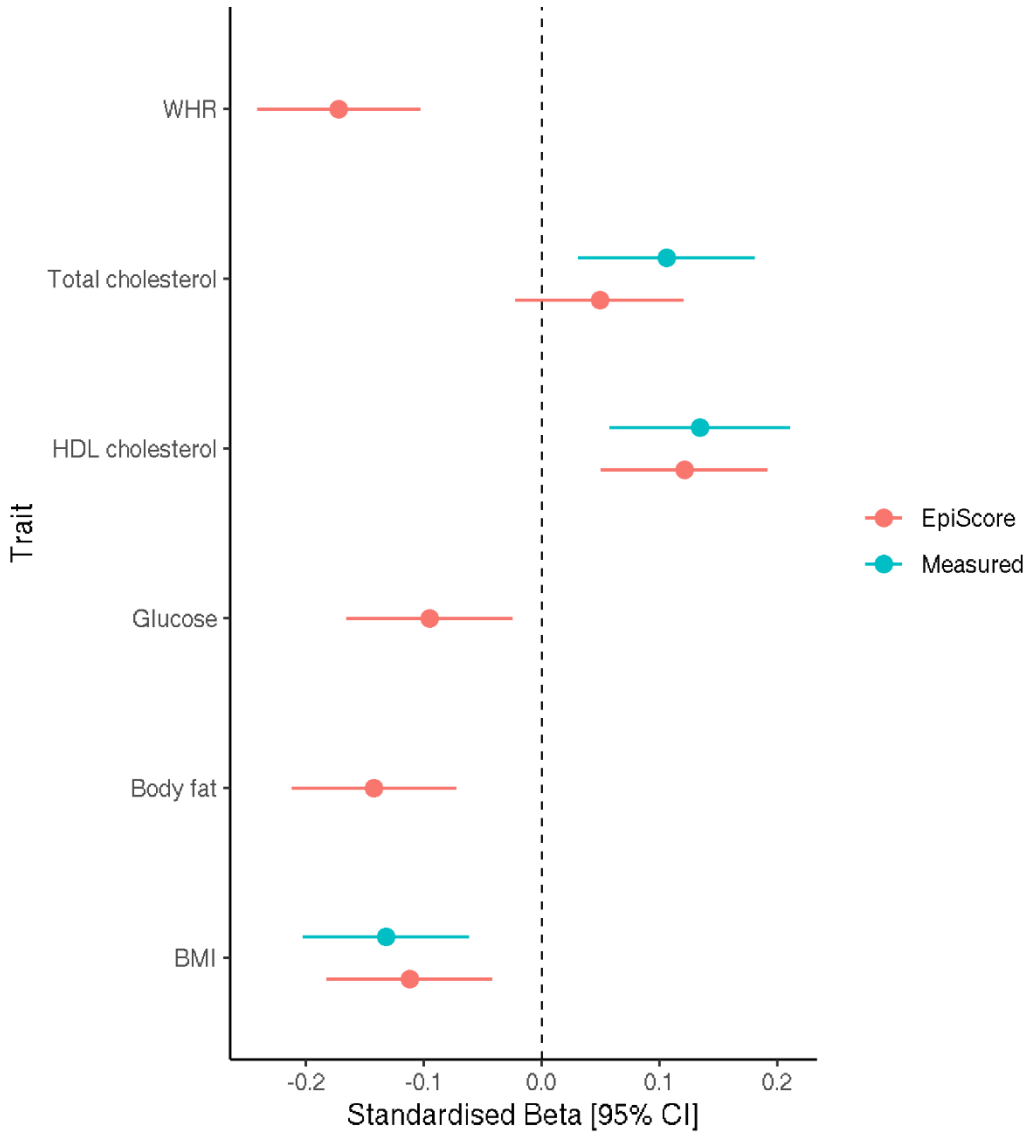


Figure S12: EpiScore and measured metabolic trait associations with general cognitive function level in the Lothian Birth Cohort (LBC1936). The figure shows associations between measured metabolic traits (BMI in kg/m^2; HDL cholesterol and total cholesterol in mmol/L) or EpiScores with general cognitive function level in models with basic adjustments (age and sex). Standardised betas are shown and error bars represent 95% confidence intervals. BMI = body mass index; WHR = waist-hip ratio; HDL cholesterol = high-density lipoprotein cholesterol.

Supplemental Methods

Metabolic measures in Generation Scotland (GS), Lothian Birth Cohort 1936 (LBC1936) and the Health for Life in Singapore (HELIOS) study

This study investigated six metabolic measures including body mass index (BMI), waist-hip ratio (WHR), body fat percentage, high-density lipoprotein (HDL) cholesterol, total cholesterol and glucose. The Generation Scotland (GS) study had all six traits available for analysis. The HELIOS study had all traits available for analysis except glucose. The LBC1936 had only BMI, HDL cholesterol and total cholesterol available for analysis. Outlier removal strategies were chosen on a cohort-by-cohort basis in line with previous approaches. In GS, BMI, body fat percentage and WHR outliers were removed by visual inspection after bivariate plots of all pairwise combinations ($n_{removed}$ = 426). Outliers > 4 standard deviations from the mean were removed for glucose, HDL cholesterol and total cholesterol ($n_{removed}$ = 173, 26, 15, respectively). In LBC1936, measured metabolic trait data were visually inspected and no outliers were removed. In HELIOS, data points were considered outliers if they were beyond 3.5 standard deviations from the mean. BMI (weight in kg /height in m^2), WHR (waist/hip circumference) and body fat percentage were measured in the clinic. In HELIOS, whole-body DEXA scans were used to quantify body fat [1]. In GS, body fat percentage is quantified with bioimpedance. HDL cholesterol, total cholesterol and glucose from blood samples were measured in mmol/L.

Bayesian EWAS

BayesR+ is a software implemented in C++ for performing Bayesian penalised regression on complex continuous traits [2]. A prior distribution is assumed as a mixture of Gaussian distributions, which correspond to groups of probes with different effect sizes. A discrete spike at zero is included, which removes probes that have a negligible effect on the trait. Informed by data from a previous analysis of BMI, prior mixture variances of probes were set to 0.0001, 0.001, 0.01 [2]. Pre-

corrected phenotype and DNA methylation data were scaled to mean zero and unit variance. Gibbs sampling was used to sample over the posterior distribution and consisted of 10,000 samples with 5,000 as burn-in. A thinning of 5 samples was applied to reduce autocorrelation. Four chains were used and the final 250 samples per chain (after thinning) were combined to form the final set of 1,000 iterations from which variance and effect size estimates were obtained. Probes with a posterior inclusion probability (PIP) ≥ 95% were deemed to be significant.

Cognitive measures in the Lothian Birth Cohort 1936 (LBC1936)

Cognitive measures in the LBC1936 have been described previously [3-6]. Cognitive testing was repeated for 5 waves at ages 70, 73, 76, 79, and 82. Thirteen cognitive measures for all five waves were available. Several cognitive domains were measured including visuospatial ability (tests: Block Design, Matrix Reasoning (WAIS-III^{UK}) and Spatial span (WMS-III^{UK})), memory (tests: Verbal Paired Associates, Logical Memory – a combination of immediate and delayed memory (WMS-III^{UK}) and Digit-span backwards (WAIS-III^{UK})), and verbal ability (tests: National Adult Reading Test, Wechsler Adult Reading Test and Verbal Fluency Test (using letters V, F and L)). Processing speed was evaluated using the Digit-symbol, Symbol Search (WAIS-III^{UK}), Choice Reaction Time and Inspection Data availability and descriptive statistics for each measure can be found in **Table S3**.

General cognitive function level and change in LBC1936

Latent measures of general cognitive function and change were generated using confirmatory factor analysis in a structural equation modelling (SEM) framework using the *Lavaan* (version 0.6-12) R package [7]. Intercepts and slopes of each cognitive test were used to indicate a latent intercept and slope (level and change) of general

cognitive function (**Table S4**). Levels and changes in cognitive functioning were modelled with a latent growth curve model (LGCM) using a Factor of Curves specification [8]. A first-order hierarchical structure was specified, and residual covariance between tests in the same cognitive domain was included, in line with a previously established correlational structure of cognitive domains (speed, memory, verbal ability and visuospatial [9]). Residual covariance between intercept and slope for individual tests was also modelled. Within-wave residual covariance between the National Adult Reading Test and the Wechsler Adult Reading Test were modelled as these tests were highly correlated. The marker method was used to scale according to the first variable to aid model convergence. Negative latent residual variances were fixed to zero. Full information maximum likelihood was used to include all data available. Confirmatory factor index (CFI), Tucker-Lewis index (TLI), root mean squared error approximation (RMSEA) and the standardised root mean squared residual (SRMR) fit measures are reported (**Table S5**). Linear regression models were run in Lavaan to test associations between general cognitive function level and change, and the metabolic traits/EpiScores with basic- and full-adjustments as follows:

Basic model: Latent G factor (intercept or slope) ~ measured trait/EpiScore + Age at baseline + Sex

Full model: Latent G factor (intercept or slope) ~ measured trait/EpiScore + Age at baseline + Sex + Scottish Index of Multiple Deprivation (SIMD) + Epigenetic smoking score (EpiSmokEr) + Alcohol units per week

Descriptive statistics for each covariate in LBC1936 can be found in **Table S6**.

Acknowledgements

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