Separating the effects of risk factors from type 2 diabetes on coronary and peripheral artery disease

Electronic Supplementary Material (ESM)
ESM Methods 1: Mendelian randomisation analysis assumptions

Mendelian randomisation requires three assumptions: (1) the instrument must affect the exposure (relevance), (2) the instrument must not share any common causes with the outcome (independence), and (3) the instrument must only affect the outcome through the exposure (exclusion restriction). To obtain a point estimate, we must make a further assumption, such as monotonicity. This assumption states that the exposure is a monotonic (i.e. an always increasing or always decreasing) function of the instrument (monotonicity). Mendelian randomisation performed using MR-Egger requires two alternative assumptions: the instrument strength independent of direct effect (INSIDE) assumption and the no measurement error (NOME) assumption. Finally, two-step Mendelian randomisation for mediation assumes no interaction between the exposure and the mediator.
ESM Methods 2: Non-collapsibility of odds ratio

The non-collapsibility of odds ratios can pose a problem when using summary statistics from logistic regression for binary mediators and outcomes in multivariable Mendelian randomisation. To assess whether this is likely to have impacted our results, we performed a novel GWAS of liability to type 2 diabetes using a linear mixed model. Specifically, we performed a GWAS of type 2 diabetes using 24884 cases and 437996 controls from UK Biobank. The GWAS was conducted using the Medical Research Council Integrative Epidemiology Unit GWAS pipeline. Further details regarding the pipeline can be found here: https://doi.org/10.5523/bris.pnoat8cxo0u52p6ynfackeigi. Type 2 diabetes was defined as a binary variable based on the presence of the ICD-10 code ‘E11’ as a main or secondary diagnosis in the hospital inpatient admissions data (UK Biobank data-field 41270). We used a BOLT-LMM model for the GWAS and adjusted for age, sex and chip. Individuals whose genetic sex did not match their reported gender; individuals with sex chromosome karyotypes putatively different from XX or XY; individuals who were outliers in heterozygosity and missing rates; and individuals with high levels of relatedness (3rd degree) to more than 200 other individuals in the biobank were excluded prior to the analysis. The GWAS is publicly available from the IEU OpenGWAS project. The code related to the GWAS can be found here: https://github.com/venexia/T2DLinearGWAS-UKB. We were then able to repeat our Mendelian randomisation analyses, where there was no sample overlap with UK Biobank, using this GWAS and compare the results with our main analysis to assess the impact on our results.
ESM Figure 1: Flow chart showing selection of risk factors for the analysis
ESM Figure 2: Univariate Mendelian randomisation estimates for the effect of the risk factors on liability to type 2 diabetes that meet the 5% FDR threshold (ESM Table 2)

| Risk Factor                                   | OR (95% CI)               | SNPs |
|-----------------------------------------------|---------------------------|------|
| Fasting insulin                              | 4.33 (2.95, 6.34)         | 14   |
| Overall health rating                        | 3.81 (2.89, 5.01)         | 54   |
| Waist circumference                          | 3.39 (2.96, 3.89)         | 225  |
| Glucose                                      | 3.34 (2.41, 4.63)         | 92   |
| Body fat percentage                          | 2.78 (2.32, 3.32)         | 251  |
| Body mass index                              | 2.74 (2.45, 3.08)         | 494  |
| Fasting glucose                              | 2.73 (1.58, 4.73)         | 30   |
| Waist-to-hip ratio                           | 2.52 (1.52, 4.16)         | 30   |
| Whole body fat mass                          | 2.32 (2.05, 2.63)         | 278  |
| Glycated haemoglobin                         | 2.11 (1.83, 2.44)         | 249  |
| Trunk fat percentage                         | 1.99 (1.69, 2.35)         | 236  |
| Trunk fat mass                               | 1.98 (1.73, 2.26)         | 282  |
| Weight                                       | 1.89 (1.66, 2.14)         | 333  |
| Alanine aminotransferase                     | 1.76 (1.49, 2.07)         | 174  |
| Basal metabolic rate                         | 1.53 (1.33, 1.76)         | 376  |
| Hip circumference                            | 1.52 (1.30, 1.78)         | 281  |
| Whole body fat-free mass                     | 1.30 (1.13, 1.49)         | 403  |
| Systolic blood pressure                      | 1.30 (1.16, 1.45)         | 164  |
| Whole body water mass                        | 1.30 (1.13, 1.49)         | 404  |
| Triglycerides                                | 1.27 (1.13, 1.42)         | 199  |
| Diastolic blood pressure                     | 1.25 (1.12, 1.40)         | 177  |
| High light scatter reticulocyte count        | 1.18 (1.10, 1.27)         | 256  |
| Reticulocyte count                           | 1.18 (1.09, 1.27)         | 244  |
| High light scatter reticulocyte percentage   | 1.16 (1.08, 1.25)         | 264  |
| Aspartate aminotransferase                   | 1.14 (1.04, 1.26)         | 196  |
| Reticulocyte percentage                      | 1.14 (1.06, 1.23)         | 239  |
| Lymphocyte count                             | 1.12 (1.05, 1.20)         | 279  |
| IGF-1                                        | 1.12 (1.05, 1.20)         | 294  |
| Immature reticulocyte fraction               | 1.12 (1.03, 1.21)         | 184  |
| Gamma glutamyltransferase                    | 1.11 (1.03, 1.21)         | 240  |
| Standing height                              | 0.69 (0.53, 0.95)         | 589  |
| Total cholesterol                            | 0.89 (0.82, 0.97)         | 85   |
| Apolipoprotein B                             | 0.88 (0.81, 0.97)         | 133  |
| LDL direct                                   | 0.83 (0.76, 0.92)         | 133  |
| Apolipoprotein A                             | 0.81 (0.74, 0.88)         | 209  |
| Cholesterol                                  | 0.79 (0.72, 0.88)         | 143  |
| SHBG                                         | 0.76 (0.68, 0.85)         | 229  |
| HDL cholesterol                              | 0.75 (0.68, 0.81)         | 226  |
| Impedance of whole body                      | 0.66 (0.57, 0.76)         | 352  |
| Forced vital capacity (FVC)                  | 0.66 (0.57, 0.76)         | 216  |
| Forced expiratory volume in 1-second (FEV1)  | 0.64 (0.54, 0.76)         | 156  |
| Peak expiratory flow (PEF)                   | 0.64 (0.47, 0.87)         | 81   |
| Birth weight                                 | 0.58 (0.45, 0.75)         | 84   |
| Usual walking pace                           | 0.13 (0.05, 0.33)         | 29   |

OR and 95% CI for the effect of the traits on type 2 diabetes
ESM Figure 3: Univariate Mendelian randomisation estimates for the effect of liability to type 2 diabetes on the risk factors that meet the 5% FDR threshold (ESM Table 2)
ESM Figure 4: Univariate Mendelian randomisation estimates for the effect of the risk factors on liability to coronary artery disease that meet the 5% FDR threshold (ESM Table 2)

- Overall health rating: OR 2.22 (95% CI 1.71, 2.69); 53 SNPs
- Apolipoprotein B: OR 1.80 (95% CI 1.60, 2.02); 136 SNPs
- Diastolic blood pressure: OR 1.79 (95% CI 1.54, 2.08); 172 SNPs
- LDL direct: OR 1.77 (95% CI 1.54, 2.04); 133 SNPs
- Systolic blood pressure: OR 1.76 (95% CI 1.48, 2.11); 156 SNPs
- Waist-to-hip ratio: OR 1.62 (95% CI 1.21, 2.16); 30 SNPs
- Waist circumference: OR 1.57 (95% CI 1.41, 1.75); 214 SNPs
- Cholesterol: OR 1.57 (95% CI 1.37, 1.80); 146 SNPs
- Body mass index: OR 1.52 (95% CI 1.42, 1.64); 489 SNPs
- Total cholesterol: OR 1.52 (95% CI 1.37, 1.69); 85 SNPs
- Body fat percentage: OR 1.52 (95% CI 1.33, 1.73); 245 SNPs
- Fasting insulin: OR 1.48 (95% CI 1.20, 1.82); 14 SNPs
- Whole body fat mass: OR 1.39 (95% CI 1.27, 1.51); 269 SNPs
- Trunk fat mass: OR 1.29 (95% CI 1.18, 1.42); 274 SNPs
- Alanine aminotransferase: OR 1.29 (95% CI 1.13, 1.46); 172 SNPs
- Trunk fat percentage: OR 1.27 (95% CI 1.13, 1.43); 226 SNPs
- Triglycerides: OR 1.27 (95% CI 1.17, 1.37); 205 SNPs
- Lipoprotein A: OR 1.26 (95% CI 1.21, 1.31); 14 SNPs
- Glucose: OR 1.25 (95% CI 1.11, 1.41); 95 SNPs
- Weight: OR 1.24 (95% CI 1.13, 1.36); 329 SNPs
- Glycated haemoglobin: OR 1.22 (95% CI 1.15, 1.30); 262 SNPs
- Aspartate aminotransferase: OR 1.20 (95% CI 1.07, 1.34); 199 SNPs
- Fasting glucose: OR 1.15 (95% CI 1.03, 1.29); 30 SNPs
- Hip circumference: OR 1.14 (95% CI 1.03, 1.26); 274 SNPs
- Urate: OR 1.13 (95% CI 1.05, 1.21); 222 SNPs
- High light scatter reticulocyte count: OR 1.13 (95% CI 1.05, 1.21); 266 SNPs
- Reticulocyte count: OR 1.12 (95% CI 1.04, 1.21); 246 SNPs
- Red blood cell (erythrocyte) count: OR 1.11 (95% CI 1.03, 1.19); 297 SNPs
- Immature reticulocyte fraction: OR 1.10 (95% CI 1.01, 1.20); 182 SNPs
- Reticulocyte percentage: OR 1.10 (95% CI 1.02, 1.16); 239 SNPs
- High light scatter reticulocyte percentage: OR 1.10 (95% CI 1.03, 1.17); 270 SNPs
- Gamma glutamyltransferase: OR 1.09 (95% CI 1.02, 1.17); 249 SNPs
- Cystatin C: OR 1.09 (95% CI 1.02, 1.16); 270 SNPs
- Mean corpuscular haemoglobin: OR 0.94 (95% CI 0.90, 0.99); 311 SNPs
- Mean spherated cell volume: OR 0.91 (95% CI 0.86, 0.97); 283 SNPs
- Fluid intelligence score: OR 0.91 (95% CI 0.85, 0.97); 41 SNPs
- Creatinine: OR 0.88 (95% CI 0.80, 0.98); 283 SNPs
- Trunk predicted mass: OR 0.88 (95% CI 0.80, 0.97); 405 SNPs
- Trunk fat-free mass: OR 0.88 (95% CI 0.79, 0.97); 405 SNPs
- SHBG: OR 0.88 (95% CI 0.82, 0.94); 232 SNPs
- Apolipoprotein A: OR 0.84 (95% CI 0.77, 0.91); 213 SNPs
- Impedance of whole body: OR 0.83 (95% CI 0.75, 0.91); 345 SNPs
- Sitting height: OR 0.82 (95% CI 0.76, 0.87); 426 SNPs
- Standing height: OR 0.61 (95% CI 0.57, 0.66); 574 SNPs
- HDL cholesterol: OR 0.61 (95% CI 0.75, 0.87); 239 SNPs
- Testosterone: OR 0.77 (95% CI 0.62, 0.96); 83 SNPs
- Forced expiratory volume in 1-second (FEV1): OR 0.77 (95% CI 0.66, 0.89); 153 SNPs
- Past tobacco smoking: OR 0.75 (95% CI 0.59, 0.96); 40 SNPs
- Birth weight of first child: OR 0.73 (95% CI 0.61, 0.88); 40 SNPs
- Forced vital capacity (FVC): OR 0.73 (95% CI 0.64, 0.82); 212 SNPs
- Birth weight: OR 0.69 (95% CI 0.61, 0.79); 83 SNPs
- Drive faster than motorway speed limit: OR 0.56 (95% CI 0.36, 0.85); 12 SNPs
- Usual walking pace: OR 0.55 (95% CI 0.35, 0.86); 29 SNPs
ESM Figure 5: Univariate Mendelian randomisation estimates for the effect of the risk factors on liability to peripheral artery disease that meet the 5% FDR threshold (ESM Table 2)
ESM Figure 6: Comparison plot illustrating the difference in all Mendelian randomisation estimates when using a linear GWAS model for liability to type 2 diabetes versus a logistic GWAS model for liability to type 2 diabetes.