**Supplemental file**

**Instrument strength and validity in multivariable Mendelian randomization analysis**

*Instrument strength*

We calculated instrument strength of the multivariable Mendelian randomization based on equation #12 from Sanderson and coauthors (1). Since equation #12 rely on knowing the sampling covariances between the effect of each SNP on BMI and the effect of each SNP on insulin (1), we estimated these sampling covariances using the intercept from the genetic correlation analysis of BMI and insulin; σ12j=int ×SE(𝜋 ̂\_1𝑗)SE(𝜋 ̂\_2𝑗), as suggested by Wu and coauthors in 2021 (2).

R script:

Qx1 <- sum(((exp1\_ES - (IvW\_ES\_exp1\*exp2\_ES))^2)/(((exp1\_SE)^2) + ((IvW\_ES\_exp1^2)\*(exp2\_SE)^2))-((2\*IvW\_ES\_exp1)\*(int\*(exp1\_SE\*exp2\_SE))))

Qx2 <- sum(((exp2\_ES - (IvW\_ES\_exp2\*exp1\_ES))^2)/(((exp2\_SE)^2) + ((IvW\_ES\_exp2^2)\*(exp1\_SE)^2))-((2\*IvW\_ES\_exp2)\*(int\*(exp1\_SE\*exp2\_SE))))

pQx1 <- pchisq(Qx1, df=n-1, lower.tail=FALSE)

pQx2 <- pchisq(Qx2, df=n-1, lower.tail=FALSE)

IVW\_ES\_exp1 = causal effect of exposure 2 (exp2) on exposure 1 (exp1) (IVW effect size) (-0.28638)

IVW\_ES\_exp2 = causal effect of exp1 on exp2 (IVW effect size) (-0.01544783)

int = Genetic covariance intercept of exp1 and exp2 from genetic correlation analysis (gcov\_int =

-0.0821)

exp1\_ES = Effect size from GWAS summary data of exp1 for all SNPs used in the multivariable Mendelian randomization analysis (betaBMI, Supplementary Table 6)

exp2\_ES <- Effect size from GWAS summary data of exp2 for all SNPs used in the multivariable Mendelian randomization analysis (betaINS, Supplementary Table 6)

exp1\_SE = Standard error from GWAS summary data of exp1 for all SNPs used in the multivariable Mendelian randomization analysis (seBMI, Supplementary Table 6)

exp2\_SE <- Standard error from GWAS summary data of exp2 for all SNPs used in the multivariable Mendelian randomization analysis (seINS, Supplementary Table 6)

*Instrument validity*

We calculated instrument validity of the multivariable Mendelian randomization based on equation #13 from Sanderson and coauthors (1), using the same modified term to estimate sampling covariances.

R script:

QA <- sum(((o\_ES –((MVMR\_ES\_eksp1\_o\*exp1\_ES)+(MVMR\_ES\_eksp2\_o\*exp2\_ES)))^2) /((o\_SE^2)+((MVMR\_ES\_exp1\_o^2)\*(exp1\_SE^2))+((MVMR\_ES\_exp2\_o^2)\*(exp2\_SE^2))+(2\*MVMR\_ES\_exp1\_o\*MVMR\_ES\_exp2\_o\*(int\*(exp1\_SE\*exp2\_SE)))))

pQA <- pchisq(Qx1, df=207, lower.tail=FALSE)

o\_ES = Effect size from GWAS summary data of outcome for all SNPs used in the multivariable Mendelian randomization analysis

o\_SE <- Standard error from GWAS summary data of outcome for all SNPs used in the multivariable Mendelian randomization analysis (se.outcome, Supplementary Table 6)

MVMR\_ES\_exp1\_o = causal effect of exp1 on the outcome from MVMR (IVW -0.21)

MVMR\_ES\_exp2\_o = causal effect of exp2 on the outcome from MVMR (IVW -1.60)

int = Genetic covariance intercept (gcov\_int) from genetic correlation analysis of exp1 and exp (2-0.0821)

exp1\_ES = Effect size from GWAS summary data of exp1 for all SNPs used in the multivariable Mendelian randomization analysis (betaBMI, Supplementary Table 6)

exp2\_ES <- Effect size from GWAS summary data of exp2 for all SNPs used in the multivariable Mendelian randomization analysis (betaINS, Supplementary Table 6)

exp1\_SE = Standard error from GWAS summary data of exp1 for all SNPs used in the multivariable Mendelian randomization analysis (seBMI, Supplementary Table 6)

exp2\_SE <- Standard error from GWAS summary data of exp2 for all SNPs used in the multivariable Mendelian randomization analysis (seINS, Supplementary Table 6)

**References**

1. Sanderson E, Davey Smith G, Windmeijer F, Bowden J. An examination of multivariable Mendelian randomization in the single-sample and two-sample summary data settings. Int J Epidemiol2019; 48:713-727

2. Wu Y, Zhong X, Lin Y, Zhao Z, Chen J, Zheng B, Li JJ, Fletcher JM, Lu Q. Estimating genetic nurture with summary statistics of multigenerational genome-wide association studies. Proc Natl Acad Sci U S A2021; 118