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Letters to the Editor

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Letter regarding article, "Associations of obesity and circulating insulin and glucose with breast cancer risk: a Mendelian randomization analysis"

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We are writing to comment on the paper 'Associations of obesity and circulating insulin and glucose with breast cancer risk: a Mendelian randomization analysis' by Shu *et al.*[1] Findings from the study suggest that genetically instrumented fasting insulin (FI) and 2-hour glucose (2hrGlu) levels were positively associated with breast cancer (BCa) risk, whereas genetically instrumented body mass index (BMI) and waist–hip ratio with adjustment of BMI (WHRadjBMI) were inversely associated with BCa risk.

Firstly, we would like to comment on the instrument choice of this study. In the methods, the authors stated that they chose single nucleotide polymorphisms (SNPs) associated with any of the traits at genome-wide significance level ($P < 5x10^{-8}$). We note that some of the SNPs for FI were weighted using BMI-adjusted betas, while others were weighted using unadjusted betas; however, this was not acknowledged. It is important to determine and report on whether any covariates have been adjusted for in the original genome wide association study (GWAS) and to take this into account when interpreting the results.[2] Use of BMI-adjusted betas has the potential to induce bias given that BMI is causally associated with glucose and insulin, and the authors assume and provide some evidence for an effect of BMI on BCa.[3] At a bare minimum, the associations should be appropriately described and interpreted (e.g. as genetically instrumented FI adjusted for BMI). The WHR variants used by the authors were also adjusted for BMI, which could have biased the mendelian randomization (MR) estimate of the effect of unadjusted WHR on BCa towards the null.[2]

Secondly, to reduce horizontal pleiotropic effects (a single locus influencing an exposure and outcome through independent pathways), Shu *et al.* excluded variants deemed to be potentially pleiotropic [e.g. BMI and WHRadjBMI-associated SNPs were excluded from instruments of 2hrGlu, fasting glucose (FG) and FI, and vice versa]. However, manual pruning of potentially pleiotropic SNPs might result in an instrument that is no longer biologically meaningful, as the SNPs that are retained might not account for the underlying genetic architecture of the trait.[4] This problem can be further exacerbated if SNPs are removed due to vertical pleiotropy (a single locus influencing an exposure and outcome through the same biological pathway).[4] For example, variants in *FTO*, known to influence BMI, are also associated with type 2 diabetes (T2D).[5] In order to evaluate whether BMI is associated with BCa independently of T2D, an MR analysis that systematically removed such variants (i.e. those BMI SNPs that also influence T2D) and kept only those that do not associate with this downstream consequence of adiposity could inadvertently enrich for horizontally pleiotropic SNPs. This is because variants not associated with T2D may reflect the presence of alternate biological pathways between the SNP and this condition (horizontal pleiotropy) balancing out the positive effect of BMI on metabolic traits (e.g. glucose and insulin measures), leading to this disease. Results of MR analyses using instruments comprised of manually pruned SNPs can therefore not only be

challenging to interpret but may also induce bias into analyses and should consequently be used with caution or with a clear justification for their use.

Lastly, for two-sample MR to be valid, the two samples should be from the same underlying population; however, this does not seem to be the case for the analyses conducted by Shu *et al*. According to the data presented in their paper (Supplementary Table 1 and in the methods), it seems that the association of the SNPs with each trait had been taken from samples that combine women and men, whereas their outcome was specifically in women. By using non-sex-specific effects, the authors are assuming that the effect of the SNPs on the risk factors is not sex-specific. However, sex-specific effects have been observed for anthropometric traits, in particular WHR[6], and the authors should have used the sex-specific beta values when available. If sex-specific instruments were not available for the exposure of interest, the authors should have considered possible biases; however, this issue was not discussed by Shu *et al*. This is despite the fact that this issue has been previously pointed out to this group of authors in a commentary[2] on a previous MR study of the effect of adiposity on cancer risk that they published in the *IJE*.[7]

Two-sample MR using publicly available data is technically easy to undertake and there has been a rapid increase in publications using this method.[8] However, the ease with which the analyses can be done should not lead to an abandoning of thorough theoretical consideration and transparent analysis. We urge academics and those appraising MR work to consider these issues carefully when undertaking and assessing two-sample MR papers.

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References

1. Shu X, Wu L, Khankari NK *et al*. Associations of obesity and circulating insulin and glucose with breast cancer risk: a Mendelian randomization analysis. *Int J Epidemiol* 2019;**48**:795–806.
2. Lawlor DA. Commentary: two-sample Mendelian randomization: opportunities and challenges. *Int J Epidemiol* 2016;**45**:908–15.
3. Aschard H, Vilhjalmsson BJ, Joshi AD *et al*. Adjusting for heritable covariates can bias effect estimates in genome-wide association studies. *Am J Hum Genet* 2015;**96**:329–39.
4. Holmes MV, Ala-Korpela M, Smith GD. Mendelian randomization in cardiometabolic disease: challenges in evaluating causality. *Nat Rev Cardiol* 2017;**14**:577–90.
5. Freathy RM, Timpson NJ, Lawlor DA *et al*. Common variation in the FTO gene alters diabetes-related metabolic traits to the extent expected given its effect on BMI. *Diabetes* 2008;**57**:1419–26.
6. Heid IM, Jackson AU, Randall JC *et al*. Meta-analysis identifies 13 new loci associated with waist-hip ratio and reveals sexual dimorphism in the genetic basis of fat distribution. *Nat Genet* 2010;**42**:949–60.
7. Gao C, Patel CJ, Michailidou K *et al*. Mendelian randomization study of adiposity-related traits and risk of breast, ovarian, prostate, lung and colorectal cancer. *Int J Epidemiol* 2016;**45**:896–908.
8. Hartwig FP, Davies NM, Hemani G *et al*. Two-sample Mendelian randomization: avoiding the downsides of a powerful, widely applicable but potentially fallible technique. *Int J Epidemiol* 2016;**45**:1717–26.