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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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To the Editor:

We are writing to comment on the paper “Associations of obesity and circulating insulin and glucose with breast cancer risk: a Mendelian randomization analysis”¹ by Shu et al. Findings from the study suggest that genetically-instrumented fasting insulin (FI) and 2-hr 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 ($p < 5 \times 10^{-8}$). 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 GWAS and to take this into account when interpreting the results². 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 BCa³. 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 MR estimate of the effect of unadjusted WHR on BCa towards the null².

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 trait⁴. 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 the paper (Supplementary Table 1 and in their 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 WHR⁶, 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 commentary² on a previous MR study of the effect of adiposity on cancer risk that they published in the IJE⁷.

Two-sample MR using publicly available data is technically easy to undertake and there has been a rapid increase in publications using this method⁸. 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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