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Response by Borges et al to Editorial Regarding Article, "Role of Adiponectin in Coronary Heart Disease Risk: A Mendelian Randomization Study"

To the Editor:

In the issue of *Circulation Research* on July 22, we published a Mendelian randomization study to assess the causal effect of higher adiponectin levels on the risk of coronary heart disease (CHD) using summary data from large-scale genome-wide association studies.[1] Overall, our findings are not supportive of the hypothesis that higher adiponectin levels protect against CHD development. In an editorial related to our paper, Turer and Scherer[2] state that "Several major issues with the present analysis suggest that the conclusions drawn are rather premature." In this letter, we discuss the points raised by the authors.

Turer and Scherer[2] point out that one important assumption of Mendelian randomization is that SNPs (single nucleotide polymorphisms) significantly influence the levels of adiponectin. Indeed, the use of weak genetic instruments cannot only reduce precision, but also introduce bias in Mendelian randomization estimates. For this reason, we selected as genetic instruments the SNPs with the strongest association with adiponectin levels from the largest genome-wide association studies available, the ADIPOGen consortium. The SNPs selected nearby the *ADIPOQ* locus, or other highly correlated SNPs, have been previously used in Mendelian randomization studies and explain ≈4% to 6% of variation in adiponectin levels.[3,4] Of note, this is a higher proportion of variation than SNPs used in Mendelian randomization studies, confirming the causal effect of systolic blood pressure (<1%) on CHD.[5] As mentioned in our article, our instrument for adiponectin gave us more than 97% power to detect an odds ratio of CHD of at least 0.80 per 2.7-fold increment in circulating adiponectin levels, indicating that we would have been able to detect even modest clinically relevant effects.

Regarding concerns over the use of different assays for adiponectin,[2] the ADIPOGen consortium included 16 cohorts that measured adiponectin using either RIA (radioimmunoassay) or ELISA (enzyme-linked immunosorbent assay) methods and found highly consistent results when analyses were stratified by type of assay.[6] As we noted in our article, there was little evidence of heterogeneity between studies for most selected SNPs, indicating that study differences, including differences in type of assay, are unlikely to have influenced our results.

Turer and Scherer[2] question whether "randomization was successful in achieving a balance of demographic (…) and clinical characteristics (…)". One of the core strengths of Mendelian randomization relates to the fact that genetic variants are not usually correlated with confounding factors as a result of the mechanisms of Mendelian inheritance. This has been demonstrated empirically[7] and is precisely why Mendelian randomization is much less vulnerable to confounding than conventional multivariable regression analysis. The only exception to this would be in the case of population stratification, where confounding could be introduced by subgroups of different genetic ancestries. As mentioned in our article, the genome-wide association studies consortia that contributed to our analyses were largely restricted to individuals of European ancestry and controlled for population stratification by undertaking double genomic control (prior and after meta-analyzing results), which is in line with good practices of genome-wide association studies. Finally, we undertook a positive control study using the same CHD data and demonstrated the expected positive causal effect of low-density lipoprotein cholesterol on CHD.

Turer and Scherer[2] are also concerned that by adjusting for some established cardiovascular risk factors, we might have over-adjusted for factors on the causal path between adiponectin and CHD. They seem to have misunderstood our methodological approach that set out specifically to explore whether these factors were potential mediators or confounders. First, we showed that SNPs nearby or in the *ADIPOQ* locus (conservative approach), which codes for adiponectin, were not related to fasting insulin, high-density lipoprotein cholesterol, triacylglycerol, waist circumference, or body mass index (Table 2 and Figure 3A). Second, we used a multiloci set of SNPs (liberal approach) and found that those SNPs outside of the *ADIPOQ* locus were associated with other CHD risk factors and that the results from MR-Egger method supported the presence of horizontal pleiotropy in the liberal approach. Together, these findings strongly suggest that adiponectin does not causally affect these risk factors and, therefore, they cannot mediate any of its causal effects on disease outcomes. In short, when we used only genetic variants in the *ADIPOQ* locus only (our conservative approach) combining 2 extremely large data sets with over 60 000 CHD cases, we find the causal odds ratio of a 1 logged unit increase in adiponectin to be 0.97 (95% confidence interval 0.84, 1.12). There were no adjustments made in these analyses because we had already shown that the variants were not related to other risk factors, and therefore, these results cannot be over adjusted.

Although animal studies suggest that adiponectin has cardioprotective effects, the picture has proven to be far more complicated in humans. Findings from observational epidemiological studies on the association between adiponectin levels and risk of CHD are conflicting[8] and probably biased by residual confounding and reverse causality. Drugs, such as peroxisome proliferator–activated receptor gamma agonists, that lead to changes in adiponectin levels also act independently on multiple other pathways that likely influence CHD, and therefore, their metabolic effects cannot be taken as evidence for causal effects of adiponectin. Mendelian randomization has successfully and increasingly been used in clinical research and can be a powerful tool to help unraveling mechanisms of disease and identifying potential drug targets, specially given the complex metabolic phenomena that commonly occur in human diseases. Our study builds on previous Mendelian randomization evidence by showing no consistent protective effect of adiponectin on cardiometabolic diseases.[3]

The editorial by Turer and Scherer concludes that our results should be treated with great caution. However, we would argue

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that conclusions based on correlational data from human studies, which they present as evidence for cardioprotection, merit the greatest caution and that preclinical evidence from animal studies lacks external validity and should not be assumed to translate to humans. Based on the multiple aspects explored in our analysis and the available evidence, we feel confident concluding that, currently, there is no consistent evidence that circulating adiponectin is more than an epiphenomenon in the context of CHD in humans.

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Disclosures

None.

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Response From Aslan Turer and Philipp Scherer

We appreciate the comments by Borges et al. However, we maintain our original concerns raised in our commentary and do not believe that there is sufficient evidence to support the categorical statements made in the original publication.

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