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1 **TO THE EDITOR:**

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3 In the issue of Circulation Research on July 22nd, we published a Mendelian
4 randomization study to assess the causal effect of higher adiponectin levels on the risk
5 of coronary heart disease (CHD) using summary data from large scale genome-wide
6 association studies (GWAS)¹. Overall, our findings are not supportive of the hypothesis
7 that higher adiponectin levels protect against CHD development. In an editorial related
8 to our paper², Turer and Scherer state that “*Several major issues with the present*
9 *analysis suggest that the conclusions drawn are rather premature*”. In this letter, we
10 discuss the points raised by the authors.

11 Turer and Scherer point out that one important assumption of Mendelian
12 randomization is that “*SNPs significantly influence the levels of adiponectin*”². Indeed,
13 the use of weak genetic instruments can not only reduce precision, but also introduce
14 bias in Mendelian randomization estimates. For this reason, we selected as genetic
15 instruments the SNPs with the strongest association with adiponectin levels from the
16 largest GWAS available, the ADIPOGen consortium. The SNPs selected nearby the
17 *ADIPOQ* locus, or other highly correlated SNPs, have been previously used in
18 Mendelian randomization studies and explain about 4%-6% of variation in adiponectin
19 levels^{3, 4}. Of note this is a higher proportion of variation than SNPs used in Mendelian
20 randomization studies confirming the causal effect of systolic blood pressure (<1%) on
21 CHD⁵. As mentioned in our paper, our instrument for adiponectin gave us more than
22 97% power to detect an odds ratio of CHD of at least 0.80 per 2.7-fold increment in
23 circulating adiponectin levels, indicating that we would have been able to detect even
24 modest clinically relevant effects.

25 Regarding concerns over the use of different assays for adiponectin². The
26 ADIPOGen consortium included 16 cohorts that measured adiponectin using either RIA
27 or ELISA methods and found highly consistent results when analyses were stratified by
28 type of assay⁶. As we noted in our paper, there was little evidence of heterogeneity
29 between studies for most selected SNPs, indicating that study differences, including
30 differences in type of assay, are unlikely to have influenced our results.

31 Turer and Scherer question whether “*randomization was successful in*
32 *achieving a balance of demographic (...) and clinical characteristics (...)*”². One of the

33 core strengths of Mendelian randomization relates to the fact that genetic variants are
34 not usually correlated with confounding factors, as a result of the mechanisms of
35 Mendelian inheritance. This has been demonstrated empirically⁷ and is precisely why
36 Mendelian randomization is much less vulnerable to confounding than conventional
37 multivariable regression analysis. The only exception to this would be in the case of
38 population stratification, where confounding could be introduced by subgroups of
39 different genetic ancestries. As mentioned in our paper, the GWAS consortia that
40 contributed to our analyses were largely restricted to individuals of European ancestry
41 and controlled for population stratification by undertaking double genomic control
42 (prior and after meta-analysing results), which is in line with good practices of GWAS.
43 Lastly, we undertook a positive control study using the same CHD data and
44 demonstrated the expected positive causal effect of LDL-c on CHD.

45 Turer and Scherer are also concerned that by adjusting for some established
46 cardiovascular risk factors we might have over-adjusted for factors on the causal path
47 between adiponectin and CHD². They seem to have misunderstood our methodological
48 approach which set out specifically to explore whether these factors were potential
49 mediators or confounders. First, we showed that SNPs nearby or in the *ADIPOQ* locus
50 (conservative approach), which codes for adiponectin, were not related to fasting
51 insulin, HDL-c, triacylglycerol, waist circumference or body mass index (Table 2 and
52 Figure 3A). Second, we used a multi-loci set of SNPs (liberal approach) and found that
53 those SNPs outside of the *ADIPOQ* locus were associated with other CHD risk factors
54 and that the results from MR-Egger method supported the presence of horizontal
55 pleiotropy in the liberal approach. Together these findings strongly suggest that
56 adiponectin does not causally affect these risk factors and therefore they cannot
57 mediate any of its causal effects on disease outcomes. In short, when we used only
58 genetic variants in the *ADIPOQ* locus only (our conservative approach) combining two
59 extremely large datasets with over 60,000 CHD cases we find the causal odds ratio of a
60 1 logged unit increase in adiponectin to be 0.97 (95% CI: 0.84, 1.12). There were no
61 adjustments made in these analyses as we had already shown that the variants were
62 not related to other risk factors and therefore these results cannot be 'over adjusted'.

63 Although animal studies suggest that adiponectin has cardio-protective effects,
64 the picture has proven to be far more complicated in humans. Findings from

65 observational epidemiological studies on the association between adiponectin levels
66 and risk of coronary heart disease (CHD) are conflicting⁸ and probably biased by
67 residual confounding and reverse causality. Drugs, such as PPAR- γ (peroxisome
68 proliferator-activated receptor gamma) agonists, that lead to changes in adiponectin
69 levels, also act independently on multiple other pathways that likely influence CHD,
70 and, therefore, their metabolic effects cannot be taken as evidence for causal effects
71 of adiponectin. Mendelian randomization has successfully and increasingly been used
72 in clinical research and can be a powerful tool to help unraveling mechanisms of
73 disease and identifying potential drug targets, specially given the complex metabolic
74 phenomena that commonly occur in human diseases. Our study builds on previous
75 Mendelian randomization evidence by showing no consistent protective effect of
76 adiponectin on cardiometabolic diseases³.

77 The editorial by Turer and Scherer concludes that our results 'should be treated
78 with great caution'. However, we would argue that conclusions based on 'correlational
79 data' from human studies, which they present as evidence for cardio-protection, merit
80 the greatest caution, and that 'preclinical evidence' from animal studies lacks external
81 validity and should not be assumed to translate to humans. Based on the multiple
82 aspects explored in our analysis and the available evidence, we feel confident
83 concluding that, currently, there is no consistent evidence that circulating adiponectin
84 is more than an epiphenomenon in the context of CHD in humans.

85

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97

98 Disclosures

99 None.

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