with a more robust method and are eager to see if this method can lead to additional insights into other diseases. In our view, this incorrect use of Mendelian randomisation represents an excellent example of the self-correcting nature of science and its ability to continually improve the insights that it affords to society.

We declare no competing interests.

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Authors' reply

As noted by George Davey Smith and Guillaume Butler-Laporte and J Brent Richards, our recent paper exploring genetic evidence for an effect of vitamin D supplementation on major health outcomes showed associations between a genetic score that predicts 25-hydroxyvitamin D (25[OH]D) concentrations and mortality outcomes in a strata of the study population with low concentrations of 25(OH)D.[1] Under the assumptions of non-linear Mendelian randomisation, this finding indicates a potential inverse causal effect of vitamin D in people with low vitamin D status.

The statistical oddity in the results from our paper pointed out by Davey Smith is a sign of potential bias. For cardiovascular mortality, the overall estimate is positive, whereas the stratum-specific estimates are all negative (although all estimates have wide CIs that overlap the null, except for the estimate in the deficient stratum). Although this difference in signs is logically possible for a conventional observational analysis, it is not possible if all estimates have a causal interpretation, as we claimed in our original paper.[1]

An assumption of the stratification method used in the analysis is that the effect of the genetic score on the exposure (here, 25[OH]D concentration) is constant in the population.[2,3] We have since developed a non-parametric stratification method (the doubly-ranked method) that is less sensitive to violation of this constant genetic effect assumption than our previous stratification method and also allows the validity of this assumption to be assessed.[4]

As part of academic due diligence, we applied our new method to the vitamin D dataset.[5] We found strong evidence that the constant genetic effect assumption was not satisfied for 25(OH)D, and no evidence of an association between the genetic score and mortality outcomes at any concentration of 25(OH)D in the study population using the doubly-ranked method. The genetic effect on 25(OH)D was around 5 times stronger in the highest decile group compared with the lowest decile group. Although the original stratification method is robust to some variation in the genetic effect on the exposure,[2] this degree of difference can lead to substantial bias due to a residual association between the genetic score and confounders within strata that arises from conditioning on a collider.[4]

Although estimates from the doubly-ranked method are not directly comparable with those from the method in the original publication, as the doubly-ranked method cannot stratify on the basis of a threshold 25(OH)D value, the Mendelian randomisation estimate in the lowest decile group of UK Biobank from the doubly-ranked method had an odds ratio of 0·97 (95% CI 0·75–1·25) per 10 nmol/L higher genetically-predicted 25(OH)D. The corresponding estimate from the original method is 0·71 (95% CI 0·63–0·81), indicating substantial disagreement between the methods (figure). Due to evidence of differences in the genetic effect on the exposure between strata, and due to the evidence of bias pointed out by Davey Smith, we have more confidence in the null estimate from the doubly-ranked method. Similar attenuation was observed using the doubly-ranked method for all outcomes considered in the original paper.[5]

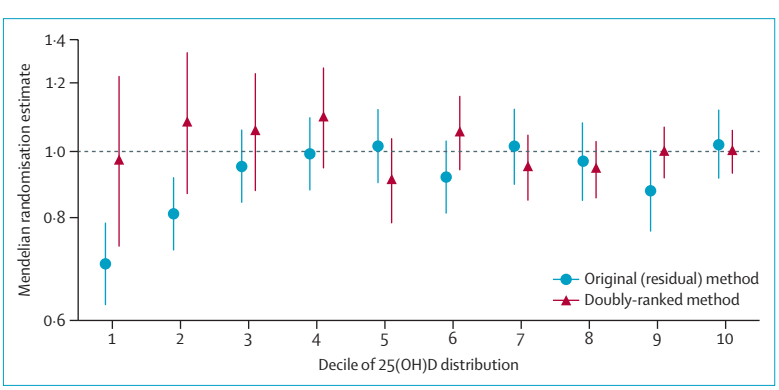

Figure: **Mendelian randomisation estimates in strata of the population defined by the residual and doubly-ranked methods**
Estimates represent odds ratios for all-cause mortality per 10 nmol/L higher genetically-predicted 25(OH)D concentration for each stratum of the study population. Error bars represent 95% CIs. 25(OH)D=25-hydroxyvitamin D.

As highlighted by Butler-Laporte and Richards, all statistical models have inherent assumptions, with varying degrees of tolerance to violation of these assumptions. The evolution of statistical methods is an ongoing process, allowing ever deeper and increasingly reliable analyses. We regret that the constant genetic effect assumption made by the stratification method we used in the original publication appears to be strongly violated in this case, and so non-linear Mendelian randomisation results from that paper are unreliable in the lowest strata. We conclude that evidential support from human genetics for a potential causal effect of vitamin D on mortality at any concentration of vitamin D is at best inconsistent.

We declare no competing interests.

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