Mendelian randomisation and vitamin D: the importance of model assumptions

There are issues of concern raised by analyses presented by the Emerging Risk Factors Collaboration, EPIC-CVD, and Vitamin D Studies Collaboration in "Estimating dose-response relationships for vitamin D with cardiovascular mortality, stroke, and all-cause mortality: observational and Mendelian randomisation analyses", published in *The Lancet Diabetes & Endocrinology*.[1] The bottom panel of figure 2 of the Article (appendix) presents the estimated causal effect of 10nmol/L higher vitamin D on all-cause mortality. There is a precisely estimated null overall effect, yet in every strata of residual vitamin D concentration the effects are in a protective direction, and substantially so in the "deficient" strata.

See **Online** for appendix

Such a pattern of findings, with the overall central effect estimate lying outside of all four subgroup central effect estimates, is simply not plausible. When combining a causal interpretation of Simpson's paradox[2] with the intention of Mendelian randomisation[3] to estimate causal effects, it is not possible to envisage situations in which the key assumptions of the Mendelian randomisation analysis[3] are not violated, the assumptions regarding other causal effects are consistent with what is known, and the data are distributed as presented. The central finding of the paper—a detrimental effect of low concentrations of vitamin D—was said to have "important public health and clinical consequences" in an accompanying commentary in *The Lancet Diabetes & Endocrinology*.[4] Similar probably spurious findings have been reported in two other papers[5,6] using the same analytical approach and overlapping datasets from which impossible estimates were generated in the paper from EPIC-CVD.[1] The method is also being widely applied in many other contexts. When evaluating findings from such analyses, considerable caution should be applied.

I declare no competing interests.

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1 Emerging Risk Factors Collaboration/EPIC-CVD/Vitamin D Studies Collaboration. Estimating dose-response relationships for vitamin D with coronary heart disease, stroke, and all-cause mortality: observational and Mendelian randomisation analyses. *Lancet Diabetes Endocrinol* 2021; **9:** 837–46.
2 Hernán MA, Clayton D, Keiding N. The Simpson's paradox unraveled. *Int J Epidemiol* 2011; **40:** 780–85.
3 Sanderson E, Glymour MM, Holmes MV, et al. Mendelian randomization. *Nat Rev Methods Primers* 2022; **2:** 6.
4 Butler-Laporte G, Richards JB. Targeting of vitamin D supplementation to individuals with deficiency. *Lancet Diabetes Endocrinol* 2021; **9:** 803–04.
5 Zhou A, Selvanayagam JB, Hyppönen E. Non-linear Mendelian randomization analyses support a role for vitamin D deficiency in cardiovascular disease risk. *Eur Heart J* 2022; **43:** 1731–39.
6 Sutherland JP, Zhou A, Hyppönen E. Vitamin D deficiency increases mortality risk in the UK Biobank : A Nonlinear Mendelian Randomization Study. *Ann Intern Med* 2022; **175:** 1552–59.

In a previous issue of the journal, we had commented[1] on the use of a novel Mendelian randomisation method that tested for non-linear effects of vitamin D and its measured metabolite 25-hydroxyvitamin D (25[OH]D), on diseases, as reported by the Emerging Risk Factors Collaboration, EPIC-CVD, and Vitamin D Studies Collaboration.[2] One of their findings was an association between the genetic score and mortality outcomes in a strata of the study population with low concentrations of 25(OH)D. Mendelian randomisation is a genetic epidemiology tool that evaluates the causal effect of an exposure (here, 25[OH]D) on a disease by limiting confounding and reverse causation bias. This is important, since the role of 25(OH)D on human disease remains controversial, especially in people with low concentrations of 25(OH)D, who were under-represented in large vitamin D supplementation trials such as the VITAL study.[3] Thus, the effects of increasing 25(OH)D concentrations in people with low concentrations is not well known and could be explored using novel Mendelian randomisation methods.

Although Mendelian randomisation has generally been able to replicate, or anticipate, results from randomised trials, in this study,[2] the method makes important assumptions about the relationship between the genetic variants it uses as instrument variables, 25(OH)D concentrations, and the disease outcome of interest. In assessing the non-linear effects of 25(OH)D on disease, the method used in this study[2] also made the additional assumption that the effect of genetic variants on concentration of 25(OH)D was constant across strata of 25(OH)D concentrations.

When using a different, more robust non-linear Mendelian randomisation method, it appears that this additional assumption was not fulfilled and there was no evidence of a beneficial effect of vitamin D supplementation in people with low concentrations of 25(OH)D. Hence, our previous interpretation of these results was wrong.

Mendelian randomisation has revolutionised epidemiology, allowing for genetics to provide causal insights in human disease, with novel therapeutic development translational opportunities. However, research in Mendelian randomisation is a rapidly evolving field, with constant progress being made. It is therefore important to remember that Mendelian randomisation relies on assumptions of which consequences can take time to fully understand. We are happy to see that the authors were rapid to rectify their previous observations

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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1 Butler-Laporte G, Richards JB. Targeting of vitamin D supplementation to individuals with deficiency. *Lancet Diabetes Endocrinol* 2021; **9:** 803–04.
2 Emerging Risk Factors Collaboration/EPIC-CVD/Vitamin D Studies Collaboration. Estimating dose-response relationships for vitamin D with coronary heart disease, stroke, and all-cause mortality: observational and Mendelian randomisation analyses. *Lancet Diabetes Endocrinol* 2021; **9:** 837–46.
3 Manson JE, Cook NR, Lee I-M, et al. Vitamin D supplements and prevention of cancer and cardiovascular disease. *N Engl J Med* 2019; **380:** 33–44.

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]

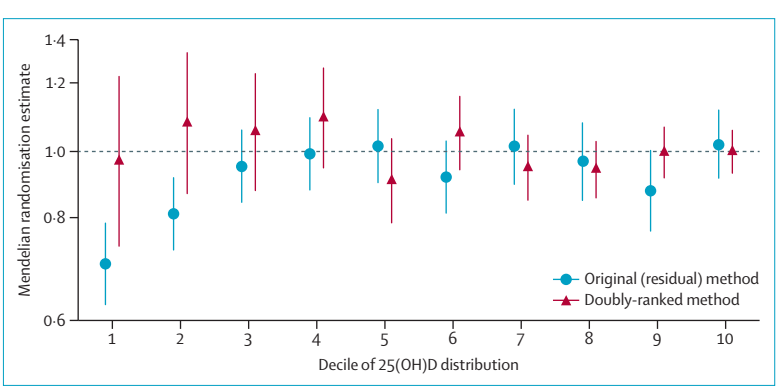

Figure: **Mendelian randomisation estimates in strata of the population defined by the residual and doubly-ranked methods**
Estimates represent odds ratios for all-cause mortality per 10 nmol/L higher genetically-predicted 25(OH)D concentration for each stratum of the study population. Error bars represent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