Annals of Internal Medicine ORIGINAL RESEARCH

Vitamin D Deficiency Increases Mortality Risk in the UK Biobank

A Nonlinear Mendelian Randomization Study

Joshua P. Sutherland, BHSc Nut Med (Hons); Ang Zhou, PhD; and Elina Hyppönen, PhD

Background: Low vitamin D status is associated with increased mortality, but randomized trials on severely deficient participants are lacking.

Objective: To assess genetic evidence for the causal role of low vitamin D status in mortality.

Design: Nonlinear Mendelian randomization analyses.

Setting: UK Biobank, a large-scale, prospective cohort from England, Scotland, and Wales with participants recruited between March 2006 and July 2010.

Participants: 307 601 unrelated UK Biobank participants of White European ancestry (aged 37 to 73 years at recruitment) with available measurements of 25-hydroxyvitamin D (25-(OH)D) and genetic data.

Measurements: Genetically predicted 25-(OH)D was estimated using 35 confirmed variants of 25-(OH)D. All-cause and cause-specific mortality (cardiovascular disease [CVD], cancer, and respiratory) were recorded up to June 2020.

Results: There were 18 700 deaths during the 14 years of follow-up. The association of genetically predicted 25-(OH)D with all-cause mortality was L-shaped (P for nonlinearity < 0.001), and risk for death decreased steeply with increasing concentrations until 50 nmol/L. Evidence for an association was also seen in analyses of mortality from cancer, CVD, and respiratory diseases ($P \leq 0.033$ for all outcomes). Odds of all-cause mortality in the genetic analysis were estimated to increase by 25% (odds ratio, 1.25 [95% CI, 1.16 to 1.35]) for participants with a measured 25-(OH)D concentration of 25 nmol/L compared with 50 nmol/L.

Limitations: Analyses were restricted to a White European population. A genetic approach is best suited to providing proof of principle on causality, whereas the strength of the association is approximate.

Conclusion: Our study supports a causal relationship between vitamin D deficiency and mortality. Additional research needs to identify strategies that meet the National Academy of Medicine's guideline of greater than 50 nmol/L and that reduce the premature risk for death associated with low vitamin D levels.

Primary Funding Source: National Health and Medical Research Council.

Ann Intern Med. doi:10.7326/M21-3324 **Annals.org**
For author, article, and disclosure information, see end of text.
This article was published at Annals.org on 25 October 2022.

The effects of vitamin D supplementation on mortality remain largely unexplored in the context of deficiency. This is because randomized controlled trials (RCTs) often do not recruit people with vitamin D deficiency or, because of ethical reasons, are prevented from doing so (1, 2). This leaves studies to investigate the effects of intakes that may be in surplus to the actual nutritional requirement.

Observational analyses and RCTs have considered the effects of vitamin D supplementation on a range of pathologies (3), with ongoing debate about the true health implications arising from deficiency. Wide-ranging health effects might be expected because vitamin D is a nutrient and prohormone that is mostly derived from exposure to type B ultraviolet radiation from the sun, and vitamin D receptors are found in most major organs and human tissues (4). More than 70 RCTs have looked at the effects of vitamin D supplementation on mortality (5–8). Some modest associations with all-cause mortality have been shown (6, 7); however, meta-analyses of RCTs have typically supported survival benefits only for specific groups, such as elderly persons and those with cancer (5, 6). A key problem with RCTs of vitamin D supplementation is the failure to include participants with vitamin D deficiency, and further limitations relate to heterogeneous dosing methods, inadequate participant diversity, and short follow-up periods (9).

See also:

Web-Only
Supplement

Mendelian randomization is a genetic approach that uses genetic variants that approximate the exposure as an instrument or "proxy indicator"; it can be used to provide causal evidence for exposures where RCTs are either unethical or infeasible (10). Mendelian randomization allows us to overcome key challenges, such as reverse causation and confounding, of other observational approaches. The Mendelian randomization approach has been recently expanded to allow for nonlinear associations (11), making it possible to explore threshold effects. This is particularly helpful in the context of nutritional exposures because it provides a novel, noninvasive approach to seek evidence on benefits that might only be seen in the context of rectifying an existing deficiency.

Until recently, Mendelian randomization studies investigating the effects of 25-hydroxyvitamin D (25-(OH)D), a marker of nutritional vitamin D status, have used linear Mendelian randomization analyses, with mixed findings for effects on mortality (12–15). Although some studies have inferred benefits by higher genetically predicted concentrations of 25-(OH)D, other large studies on mortality from all causes (12), cancer (12, 13), and cardiovascular disease (CVD) (12, 14) have not provided

evidence for a causal relationship. However, recent Mendelian randomization findings (16) provide evidence for a threshold effect between 25-(OH)D and mortality. In this study, we used an expanded genetic instrument, in conjunction with information from up to 307 601 UK Biobank participants, to examine evidence for a nonlinear causal role of 25-(OH)D in all-cause and cause-specific mortality (cancer and CVD), while providing novel insight into respiratory mortality and applicability across ethnic groups. Where appropriate, we used a nonlinear approach.

METHODS

The UK Biobank is a large-scale, prospective cohort that includes 502 316 participants from England, Scotland, and Wales who were aged 37 to 73 years at recruitment between March 2006 and July 2010 (17). Participants were invited to take survey questionnaires, have physical assessments, and provide biological samples. Our primary analysis was limited to unrelated participants of White European ancestry with measurements of serum 25-(OH)D concentration (n = 307 601) (**Supplement Figure 1**, available at Annals.org). Mortality data (n = 18 700) were obtained from NHS Digital and the NHS Central Register (18). The latest recorded death in our study occurred in June 2020. Primary causes of death were defined using the International Classification of Diseases, 10th Revision, codes for cancer (C00 to D48), CVD (I00 to I89), and respiratory diseases (J09 to J18, J20 to J22, and J40 to J47) (19). The LIAISON XL 25-(OH)D assay (DiaSorin) was used to determine measured baseline concentrations of serum 25-(OH)D, as described in the **Supplement** (available at Annals.org). All covariates were derived at baseline from self-reported, touchscreen questionaries, aside from location and socioeconomic status, which were ascertained from assessment center and residential data, respectively. The Townsend deprivation index was used to establish socioeconomic status (20). Body mass index was assessed at baseline from height and weight measurements (calculated as body weight in kilograms divided by height in meters squared). We constructed a weighted vitamin D genetic score by collating 35 common autosomal single-nucleotide polymorphisms that were discovered in the UK Biobank from a genome-wide association analysis on measured 25-(OH)D concentrations (21) and replicated in the SUNLIGHT (Study of Underlying Genetic Determinants of Vitamin D and Highly Related Traits) consortium (22). Selection of variants and construction of the vitamin D genetic score are described in the **Supplement**. Ethics approval for the UK Biobank was granted by the National Information Governance Board for Health and Social Care and the North West Multi-centre Research Ethics Committee (11/NW/0382). The present analysis operates under UK Biobank application 20175.

Statistical Analysis

The phenotypic analyses were done using logistic regression, with all models adjusted for age, sex, assessment center, education, Townsend index, body mass index, physical activity, alcohol, smoking, and nuisance factors that could affect the measured 25-(OH)D concentrations (blood sampling month, fasting time before sample acquisition, and aliquot). We used a likelihood ratio test to compare model fit between the best-fitting fractional polynomial model and the linear model, taking P less than 0.05 to indicate a nonlinear association (23).

We used genetically predicted concentrations of 25-(OH)D and did linear and nonlinear Mendelian randomization analyses on mortality, with the latter capable of assessing evidence for curvature (pages 4 to 6 of the **Supplement** and **Supplement Figures 2** and **3**, available at Annals.org). We present nonlinear Mendelian randomization analyses as the primary findings if evidence for curvature was observed; otherwise, results from linear Mendelian randomization analyses are considered to be the primary findings. As a secondary analysis, we did stratified Mendelian randomization analysis (page 6 of the **Supplement** and **Supplement Figure 3**) where we segmented the cohort into 4 strata of residual measured 25-(OH)D concentrations—less than 25 nmol/L, 25 to 49.9 nmol/L, 50 to 74.9 nmol/L, and 75 nmol/L or higher—and examined evidence of a linear association within each stratum. Residual measured concentrations of 25-(OH)D for each participant were determined as the measured 25-(OH)D minus the difference in concentrations attributed to genetic variants (correlation between residual measured and measured 25-(OH)D; r = 0.986). Further sensitivity analyses were done restricting the population to non-White ethnic groups (**Supplement Table 1**, available at Annals.org). Linear, nonlinear, and stratified Mendelian randomization analyses are detailed in the **Supplement** Methods.

Valid causal inference from the Mendelian randomization analysis relies on 3 key assumptions (24) (**Supplement Figure 2**), which in the context of our study can be described as follows: vitamin D genetic score associates with measured 25-(OH)D concentrations, vitamin D genetic score has no direct effect on mortality, and vitamin D genetic score does not associate with confounders of measured 25-(OH)D and mortality. To assess the first Mendelian randomization assumption, we examined the association of the vitamin D genetic score with measured 25-(OH)D concentrations in the UK Biobank. To assess the second and third assumptions, we examined horizontal pleiotropy. Horizontal pleiotropy describes the situation when the genetic instrument associates with outcomes via pathways other than exposure of interest; it will violate the second or third Mendelian randomization assumption and consequently biases the Mendelian randomization analysis. To gauge potential horizontal pleiotropy, we examined the association of the vitamin D genetic score with several potential confounders in the UK Biobank, including body mass index, smoking, alcohol intake, physical activity, education, and Townsend deprivation index. We also did an analysis in which we left out specific blocks of potentially pleiotropic variants (in particular, pleiotropy by lipid traits) to examine if our primary finding is driven by any particular block (25). Functional blocks were based on the traits that variants were associated with in the PhenoScanner search (26)

(page 9 of the Supplement and Supplement Table 2, available at Annals.org). We identified 4 functional blocks, including blocks for "blood," "lipids/metabolic," and "renal" traits; variants whose associated traits did not fall into 1 of these 3 blocks were grouped together as the "unclassified" block (Supplement Table 3, available at Annals.org). Further, in the stratified Mendelian randomization analysis where inclusion of pleiotropy-robust methods based on single-nucleotide polymorphisms is possible, we used 5 methods with largely independent assumptions on pleiotropy to compute stratum-specific Mendelian randomization estimates. These 5 Mendelian randomization methods are inverse variance–weighted Mendelian randomization, Mendelian randomization–Egger, weighted median Mendelian randomization, weighted mode Mendelian randomization, and Mendelian randomization pleiotropy residual sum and outlier; the Supplement (pages 7 to 9) details these methods. In strata where associations were evident, we also computed E-values to evaluate the sensitivity of the observed associations to unmeasured confounding (Supplement). The nonlinear Mendelian randomization analysis additionally assumes that the association between vitamin D genetic score and measured 25-(OH)D concentration is constant over the entire distribution of measured 25-(OH)D (page 5 of the Supplement). To assess this assumption, we examined the heterogeneity of the associations between vitamin D genetic score and measured 25-(OH)D concentration across 100 strata of residual measured 25-(OH)D (11) (page 5 of the Supplement and Supplement Figure 4, available at Annals.org). Supplement Table 1 outlines the ethnic sensitivity analysis. We used R, version 3.6.1 (R Foundation), for nonlinear Mendelian randomization (NLMR package) and linear Mendelian randomization (TwoSampleMR and MRPRESSO packages) sensitivity analyses and Stata, version 14.1 (StataCorp), for all other analyses.

Role of the Funding Source

Neither the National Health and Medical Research Council nor the Australian Research Training Program Scholarship had any role in this study's design, conduct, or reporting.

RESULTS

The Table shows baseline participant characteristics and the distribution of measured 25-(OH)D concentrations and vitamin D deficiency (measured 25-(OH)D concentration <25 nmol/L). The average measured concentration of 25-(OH)D was 45.2 nmol/L, and 11.71% of participants (n = 36 009) had concentrations between 10.0 and 24.9 nmol/L. Higher average measured concentrations were seen in participants living in southern areas; nonsmokers; and those with higher physical activity, less socioeconomic deprivation, and lower body mass index. During follow-up, 6.08% (n = 18 700) of participants died, with overrepresentation among those with no declared educational status and those who smoked at the time of the baseline survey (mortality of 11% and 12%, respectively) (Supplement Table 4, available at Annals.org).

Phenotypic Analyses

Nonlinear inverse relationships with measured 25-(OH)D were similar among all-cause mortality and the various cause-specific mortality rates (Figure 1). Crude and fully adjusted models followed similar patterns, with some attenuation by adjustment. For all 4 outcomes, odds ratios (ORs) were highest for concentrations lower than 25 nmol/L and adjusted associations seemed to plateau between 50 and 75 nmol/L, with little to no further reduction in mortality with measured 25-(OH)D values of 75 to 125 nmol/L. The fully adjusted odds of all-cause mortality were 36% higher for participants at 25 nmol/L compared with 50 nmol/L (OR, 1.36 [95% CI, 1.33 to 1.40]).

Mendelian Randomization

As shown in Figure 2, genetically predicted 25-(OH)D had an L-shaped association with all-cause (P for nonlinearity < 0.001), cancer, and CVD (P for nonlinearity ≤ 0.033) mortality when displayed across the measured 25-(OH)D concentrations shown on the x-axis. Genetically predicted 25-(OH)D had the strongest association with these outcomes in participants with measured 25-(OH)D concentrations below 25 nmol/L, and the associations plateaued by 50 nmol/L. For respiratory diseases, there was no significant curvature, but we observed evidence for a linear association (OR, 0.81 [CI, 0.68 to 0.96] per 10-nmol/L increase in genetically predicted 25-(OH)D concentration) (Figure 2, D). However, in the stratified analyses, we observed no evidence for an association between genetically predicted 25-(OH)D and respiratory disease mortality in the stratum including participants with the highest measured 25-(OH)D concentrations (>75 nmol/L: OR, 1.04 [CI, 0.52 to 2.09]; 69 cases) (Supplement Table 5, available at Annals.org). Compared with a measured 25-(OH)D concentration of 50 nmol/L, we estimated that the genetically predicted odds of all-cause mortality would increase by 6-fold (OR, 6.00 [CI, 3.22 to 11.17]) for participants at 10 nmol/L and by 25% (OR, 1.25 [CI, 1.16 to 1.35]) for those at 25 nmol/L (Figure 2). Compared with a measured 25-(OH)D concentration of 50 nmol/L, participants at 10 nmol/L had genetically predicted ORs of 5.98 (CI, 1.73 to 20.59) for CVD mortality, 3.37 (CI, 1.37 to 8.28) for cancer mortality, and 12.44 (CI, 4.32 to 35.85) for respiratory mortality. For the comparison of measured 25-(OH)D concentrations of 25 nmol/L versus 50 nmol/L, these outcomes had ORs of 1.25 (CI, 1.07 to 1.46), 1.16 (CI, 1.04 to 1.30), and 1.96 (CI, 1.88 to 4.67), respectively.

Sensitivity Analyses

Sensitivity analyses excluding the first and 100th strata of residual measured 25-(OH)D (Supplement Figure 5, available at Annals.org) and using the version of the vitamin D genetic score with 122 single-nucleotide polymorphisms (Supplement Figure 6, available at Annals.org) provided similar results. Findings were also similar in analyses in which blocks were left out (Supplement Tables 2 and 3) and in stratified Mendelian randomization analyses using multiple Mendelian randomization approaches, with the Egger intercept test detecting no pleiotropy (Supplement Table 6, available at Annals.org). Finally, analyses using information on the subsample of participants who were of

non-White ethnic origin (n = 20 837) also provided consistent results supportive of a causal effect of genetically predicted 25-(OH)D on all-cause mortality in those with low measured 25-(OH)D concentrations (**Supplement Table 1**).

DISCUSSION

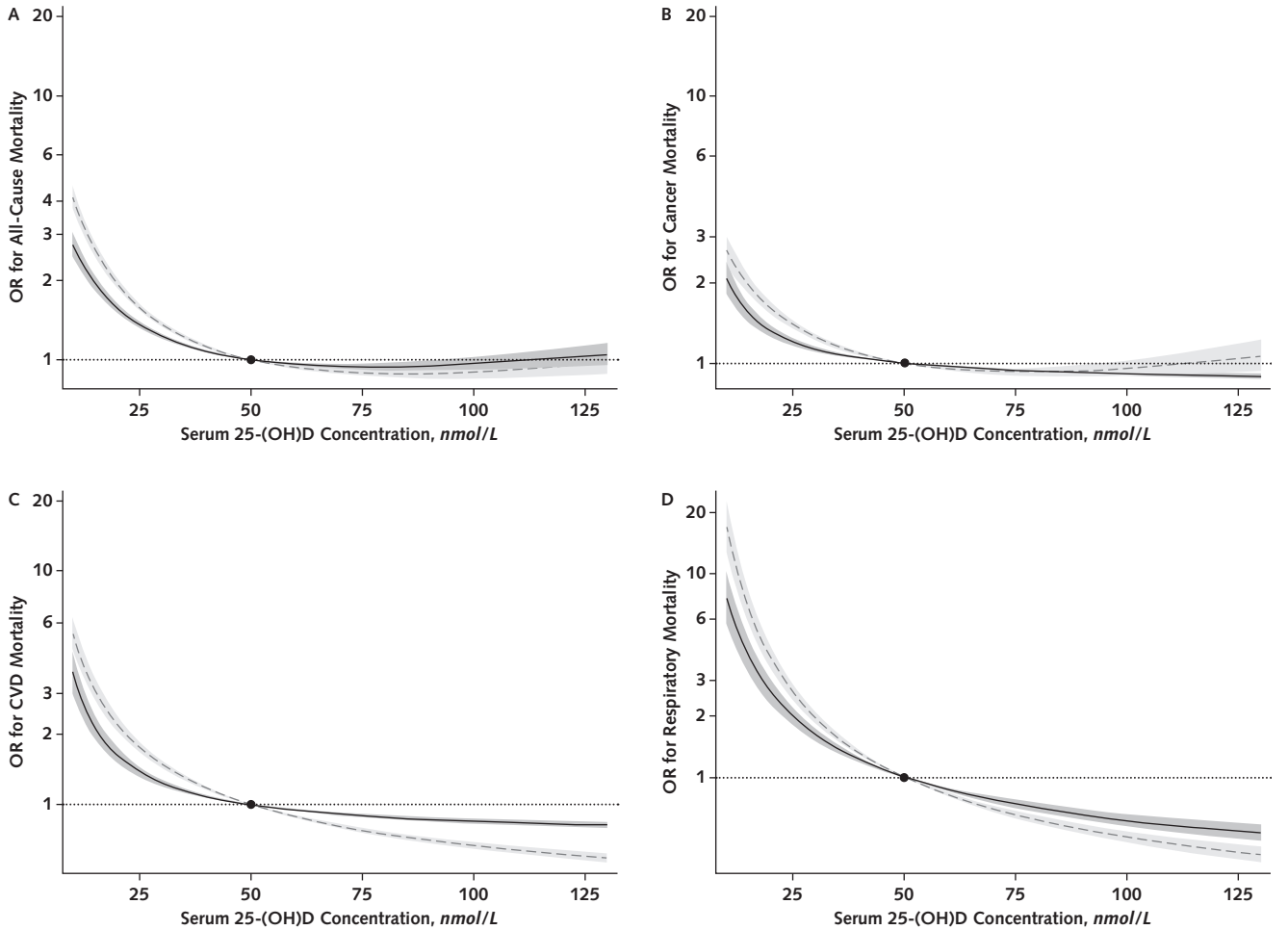
The causal effects of low vitamin D status in a general population are challenging to establish, with RCTs either failing to recruit people with severe deficiency or, because of ethical reasons, being prevented from doing so (1, 2). We examined the association of genetically predicted 25-(OH)D with mortality, observing evidence for a causal relationship across all included mortality outcomes. Of note, the genetic evidence supporting a relationship between higher 25-(OH)D concentrations and mortality was largely restricted to persons with measured concentrations below 50 nmol/L, which reflects the cutoff

Table. Demographic Characteristics of UK Biobank Participants

Characteristic	Participants, *n (%)*	Geometric Mean 25-(OH)D Concentration (95% CI) (*n* = 307 601), *nmol/L*	25-(OH)D Concentration <25 nmol/L (*n* = 36 009), %
All	307 601 (100)	45.2 (45.1–45.3)	11.71
Sex			
Men	144 680 (47.0)	45.2 (45.1–45.4)	11.60
Women	162 921 (53.0)	45.2 (45.1–45.3)	11.80
Age			
<60 y	169 756 (55.2)	43.7 (43.6–43.8)	13.46
≥60 y	137 845 (44.8)	47.2 (47.1–47.3)	9.54
BMI			
Lowest 25% (12.1–24.1 kg/m^2)	76 640 (24.9)	48.1 (48.0–48.3)	10.19
Middle 50% (24.1–29.8 kg/m^2)	153 321 (49.8)	46.5 (46.4–46.6)	10.05
Highest 25% (29.8–74.7 kg/m^2)	76 675 (24.9)	40.2 (40.1–40.3)	16.40
Missing	965 (0.3)	37.6 (36.4–38.9)	22.90
Location			
South (≤51° latitude)	102 335 (33.3)	47.1 (47.0–47.2)	9.29
Middle (52°–53° latitude)	144 654 (47.0)	45.4 (45.3–45.5)	11.36
North (≥54° latitude)	60 612 (19.7)	41.8 (41.6–41.9)	16.62
Smoking			
Nonsmokers	167 703 (54.5)	45.6 (45.5–45.7)	10.92
Former smokers	108 118 (35.2)	46.2 (46.1–46.3)	10.67
Current smokers	30 719 (10.0)	40.0 (39.7–40.2)	19.63
Missing	1061 (0.3)	45.1 (43.8–46.4)	12.54
Alcohol			
Daily	65 542 (21.3)	46.5 (46.6–46.3)	11.03
1–4 times/wk	155 608 (50.6)	46.4 (46.5–46.3)	10.20
1–3 times/mo	34 098 (11.1)	43.7 (43.9–43.5)	12.91
Special occasion	32 179 (10.5)	41.6 (41.3–41.8)	15.76
Never	19 963 (6.5)	41.0 (40.8–41.3)	17.06
Missing	211 (0.07)	40.1 (42.9–37.5)	16.59
Physical activity			
Low	92 012 (29.9)	41.8 (41.7–41.9)	15.18
Moderate	149 205 (48.5)	46.1 (46.0–46.2)	10.50
High	59 561 (19.4)	49.5 (49.3–49.7)	8.11
Missing	6823 (2.2)	38.2 (37.7–38.6)	22.51
Education			
None	52 193 (17.0)	45.6 (45.4–45.8)	11.90
NVQ/CSE/A-levels	109 099 (35.5)	45.9 (45.8–46.0)	11.23
Degree/professional	143 735 (46.7)	44.6 (44.5–44.7)	12.00
Missing	2574 (0.84)	45.7 (44.9–46.5)	11.58
Townsend index			
Quartile 1 (lowest deprivation)	76 793 (25.0)	47.6 (47.4–47.7)	9.16
Quartile 2	76 821 (25.0)	47.1 (47.0–47.3)	9.35
Quartile 3	76 815 (25.0)	45.4 (45.3–45.5)	11.20
Quartile 4 (highest deprivation)	76 811 (25.0)	41.1 (40.9–41.2)	17.11
Missing	361 (0.1)	45.6 (43.5–47.7)	11.63

25-(OH)D = 25-hydroxyvitamin D; A-levels = advanced levels; BMI = body mass index; CSE = Certificate of Secondary Education; NVQ = National Vocational Qualification.

Figure 1. Phenotypic association of measured 25-(OH)D serum concentrations with all-cause (*A*), cancer (*B*), CVD (*C*), and respiratory (*D*) mortality in the UK Biobank.



Shaded areas reflect 95% CIs. The dot represents the reference point of a measured 25-(OH)D serum concentration of 50 nmol/L. Simple models (light gray) were adjusted for sex, age, assessment center, and nuisance factors that could affect 25-(OH)D serum measurements, including month in which blood sample was taken, fasting time before blood sample was taken, and sample aliquots for measurement. Full models (dark gray) were additionally adjusted for educational status, Townsend deprivation index, body mass index, physical activity, alcohol, and smoking. 25-(OH)D = 25-hydroxyvitamin D; CVD = cardiovascular disease; OR = odds ratio.

endorsed by the U.S. National Academy of Medicine (27). The strongest effects were seen for persons with measured 25-(OH)D concentrations in the severe deficiency range (<25 nmol/L). Although the past decade has seen benefits in some settings through increases in food fortification and updates on policy guidelines (28, 29), recent estimates for the prevalence of severe deficiency range from 5% to 50%, with rates varying by geographic location and population characteristics (28, 30–32). Therefore, our study affirms the potential for a notable effect on premature death and the continued need for efforts to abolish vitamin D deficiency.

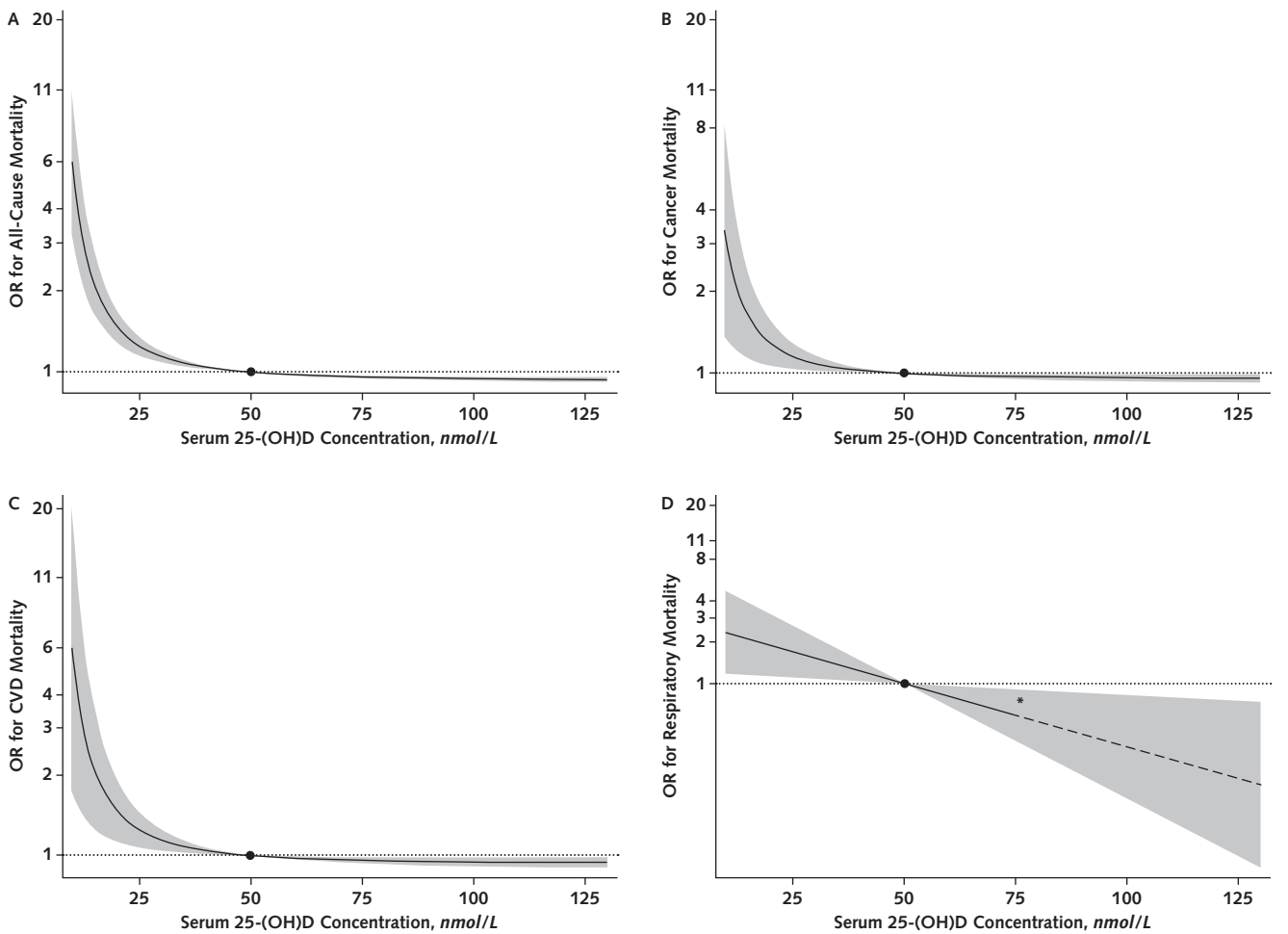
Prior data are limited on the efficacy of vitamin D supplementation in preventing death in persons with vitamin D deficiency. In fact, a recent meta-analysis that included 9 trials of vitamin D supplementation in critically ill persons (33)–which did not provide evidence for benefit–contained only 1 RCT that was restricted to vitamin D–deficient participants (34) and another study with a dedicated subgroup with severe deficiency (35). These 2 studies, independent of the meta-analysis, were among those to show some positive effect of vitamin D supplementation on mortality. All studies also used large one-off (or "bolus") doses, although evidence suggests that this type of dosing may interfere with the synthesis and breakdown of enzymes regulating vitamin D activity (36). Our data suggest that although remediation of vitamin D deficiency is essential, supplementation is unlikely to have notable benefits for preventing death when given in surplus to the nutritional requirement.

The human genome contains at least 13 000 known receptor-binding sites for vitamin D (37), and most tissues and cell types are responsive to 1,25-$(OH)_2D_3$ (the active vitamin D hormone), suggesting universal action

(4). In line with this, we observed effects on all major causes of death investigated. The association of vitamin D deficiency with CVD, respiratory illness, and cancer is biologically plausible. Of note for CVD, 1,25-$(OH)_2D_3$ is a demonstrated homeostatic regulator of the renin–angiotensin–aldosterone system, which is related to blood pressure, whereas impaired vitamin D metabolism has an adverse effect on macrophage differentiation, potentially increasing vascular cholesterol retention (38). For the respiratory tract, epithelial cells have been shown to convert 25-(OH)D from serum into 1,25-$(OH)_2D_3$ and subsequently produce antimicrobial peptides—one possible mechanism through which vitamin D reduces risk for respiratory tract infection (39). Effects of 1,25-$(OH)_2D_3$ on cancer may be mediated through established influences on cellular proliferation, differentiation, and apoptosis (40, 41). However, in our analyses done in the context of vitamin D deficiency, the genetically predicted effects of increasing 25-(OH)D concentrations on mortality were unspecific. Although many factors may influence mortality, our results may also suggest that general frailty—such as that acquired at life-threatening stages of chronic diseases—is related to vitamin D, with higher 25-(OH)D concentrations promoting the ability to retain at least the minimal physiologic reserves required to sustain life.

A primary strength of our study is its genetic approach, which has allowed us to safely explore the effects of raising 25-(OH)D in persons with very low concentrations, in contrast with RCTs, where participants would be subjected to potential harm if left deficient. Our study has been made possible by the availability of measured 25-(OH)D concentrations and genotyping in 307 601 participants, with

Figure 2. Nonlinear (*A*, *B*, and *C*) and linear (*D*) Mendelian randomization analyses for the association of genetically predicted 25-(OH)D with all-cause (*A*), cancer (*B*), CVD (*C*), and respiratory (*D*) mortality in the UK Biobank, projected on the measured 25-(OH)D scale.



The *x*-axis refers to the measured 25-(OH)D scale on which the genetically predicted analysis has been mapped; the dot represents the reference point of 50 nmol/L. Shaded areas reflect 95% CIs. Adjusted for age, sex, assessment center, birth location, single-nucleotide polymorphism array, top 40 genetic principals, and nuisance factors that could affect serum 25-(OH)D measurements, including month in which blood sample was taken, fasting time before blood sample was taken, and sample aliquots for measurement. 25-(OH)D = 25-hydroxyvitamin D; CVD = cardiovascular disease; OR = odds ratio.

* Dashed line denotes the beginning of data that cannot be interpreted because of insufficient statistical power from there onward.

enough cases to allow analyses on all-cause, CVD, cancer, and respiratory mortality. This is the first study to our knowledge to use the nonlinear Mendelian randomization approach, and we were powered to include more specific causes of death than prior stratified Mendelian randomization work undertaken in this same population (17). We also included sensitivity analyses on non-White ethnic groups, which provides proof of principle for external validity outside White ethnic populations. Although the Mendelian randomization approach can provide support for a causal association, estimates can be biased by horizontal pleiotropy, which occurs when variants influence outcomes through pathways other than via the exposure (10). We restricted our instrument to variants with replicated evidence for an association with measured 25-(OH)D concentrations and replicated our analyses using several pleiotropy-robust approaches and other sensitivity analyses, confirming the stability of our findings. In addition, we found no evidence for an association between the vitamin D genetic score and potential confounders across the cohort or within the strata of residual measured concentrations of 25-(OH)D. A reasonably large E-value for the association between vitamin D genetic score and mortality seen in the stratum with residual measured 25-(OH)D concentrations less than 25 nmol/L (page 10 of the **Supplement**) provides further assurance that the observed adverse effects for vitamin D deficiency are unlikely to be explained by residual confounding. With all Mendelian randomization analyses, genetic instruments approximate average effects over the life course; therefore, the true shape and strength of association may be more complex than presented here. The UK Biobank is not representative of the general population of the United Kingdom (5% response rate) (42). However, earlier publications from the UK Biobank have replicated expected associations between exposure and disease (43), and studies using the Mendelian randomization approach have been shown to be less affected by selection bias, suggesting limited influence on our findings (43).

In conclusion, our study supports a causal relationship between vitamin D deficiency and mortality. Additional research needs to identify strategies that meet the National Academy of Medicine's guideline concentration of greater than 50 nmol/L and that reduce the premature risk for death associated with low vitamin D levels.

From Australian Centre for Precision Health, Unit of Clinical and Health Sciences, University of South Australia, Adelaide, South Australia, Australia (J.P.S.); and Australian Centre for Precision Health, Unit of Clinical and Health Sciences, University of South Australia, and South Australian Health and Medical Research Institute, Adelaide, South Australia, Australia (A.Z., E.H.).

Acknowledgment: The authors thank all UK Biobank participants involved, as well as Dr. Anwar Mulugeta (University of South Australia) for support in data management.

Financial Support: By grant 11123603 from the National Health and Medical Research Council. Mr. Sutherland's studentship is funded by an Australian Research Training Program Scholarship.

Disclosures: Disclosures can be viewed at www.acponline.org/authors/icmje/ConflictOfInterestForms.do?msNum=M21-3324.

Reproducible Research Statement: *Study protocol:* Not available. *Data set and statistical code:* This research has been conducted using the UK Biobank resource under application 20175. All data and code will be available to approved users on application to the UK Biobank.

Corresponding Author: Elina Hyppönen, PhD, Australian Centre for Precision Health, University of South Australia, c/o South Australian Health and Medical Research Institute, GPO Box 2471, Adelaide, SA 5001, Australia; e-mail, Elina.Hypponen@unisa.edu.au.

Author contributions are available at Annals.org.

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Author Contributions: Conception and design: E. Hyppönen.
Analysis and interpretation of the data: E. Hyppönen, J.P. Sutherland, A. Zhou.
Drafting of the article: E. Hyppönen, J.P. Sutherland, A. Zhou.
Critical revision for important intellectual content: E. Hyppönen, A. Zhou.
Final approval of the article: E. Hyppönen, J.P. Sutherland, A. Zhou.
Provision of study materials or patients: E. Hyppönen.
Statistical expertise: E. Hyppönen, A. Zhou.
Obtaining of funding: E. Hyppönen.
Administrative, technical, or logistic support: E. Hyppönen, J.P. Sutherland.
Collection and assembly of data: J.P. Sutherland, A. Zhou.