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Original article

Impact of vitamin D supplementation on all-cause mortality: Randomized trials revisited

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SUMMARY

Background & aims: Vitamin D insufficiency and deficiency are common worldwide and linked to adverse health outcomes, including higher all-cause mortality. Two large randomized controlled trials (VITAL and D-Health), conducted in mostly vitamin D–sufficient populations, found no mortality benefits of vitamin D supplementation. This study aims to estimate the expected effects of vitamin D supplementation in target populations with vitamin D insufficiency or deficiency..

Methods: We emulated the VITAL and D-Health trials using data from the UK Biobank cohort to estimate expected effects of the observed increases in serum 25-hydroxyvitamin-D (25(OH)D) concentrations by 30 nmol/L and 38 nmol/L. In alternative analyses, study populations meeting the trial inclusion criteria (n = 237,502 and 185,809) were either weighted to yield distributions of 25(OH)D as observed in the trials, or restricted to people with vitamin D insufficiency or deficiency. Expected effects on all-cause mortality over the mean trial follow up times (5.3 and 5.7 years) were estimated using Cox models.

Results: Emulated trials with study populations weighted to the 25(OH)D distributions of the original trials yielded null results similar to those reported (hazard ratios [HR] 0.97 [95 % CI: 0.92–1.02] and 1.02 [95%CI: 0.97–1.07]). In contrast, major mortality reduction was expected in emulated trials that were restricted to people with vitamin insufficiency (HR 0.85 [95%CI: 0.79–0.91] and 0.81 [95%CI: 0.76–0.86]) or deficiency (HR 0.79 [95%CI: 0.72–0.87] and 0.75 [95%CI: 0.69–0.81])..

Conclusions: Null effects of vitamin D supplementation were to be expected in trials conducted in vitamin D sufficient populations. Emulated trials suggest a potential for major mortality reduction in vitamin D insufficient and deficient populations..

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1. Introduction

Vitamin D deficiency and insufficiency, commonly defined by 25-hydroxy-vitamin D [25(OH)D] concentrations in blood as less than 30 nmol/L and between 30 and 50 nmol/L, respectively [1], are widespread globally and have been consistently found to be related to a range of adverse health outcomes [2–5]. In particular, numerous large-scale cohort studies have consistently reported inverse associations between vitamin D status and mortality [6–14]. Although these findings suggest a potential preventive effect of vitamin D, the causality of this association has been subject to long-standing debate [15,16].

Nevertheless, the intriguing results of the cohort studies have prompted a number of large-scale randomized controlled trials (RCTs) aimed at assessing the potential efficacy of vitamin D supplementation in reducing mortality and morbidity outcomes.

One of the largest and most well-known trials, the Vitamin D and Omega-3 Trial (VITAL) in the US, involving 25 871 participants, aimed to examine the benefits and risks of vitamin D3

Abbreviations: 25(OH)D, 25-hydroxy-vitamin D; RCT, Randomized Controlled Trial; VITAL, the Vitamin D and Omega-3 Trial; UKB, UK Biobank; RCS, Restricted Cubic Spline; HR, Hazard Ratio; SD, standard deviation; BMI, Body Mass Index.

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(cholecalciferol) supplementation at a dose of 2000 international units (IU) per day. However, mostly negative results were observed regarding its primary and secondary outcomes related to mortality and morbidity [17–19]. Similarly, in the D-Health Trial in Australia, which included 21 315 participants, monthly oral vitamin D3 supplementation with 60,000 IU did not reduce mortality [20]. These findings have been widely interpreted as evidence that vitamin D supplementation may not be effective in preventing morbidity or reducing mortality.

However, a closer examination of the data reveals a potential explanation for the apparent discrepancy between the results of the cohort studies and the RCTs. In the cohort studies, strong inverse associations between 25(OH)D concentrations and adverse health outcomes were consistently seen for individuals with vitamin D deficiency and, to a lesser extent, insufficiency, but not among those with 25(OH)D concentrations greater than 50 nmol/L [21–23]. In both the VITAL study and the D-Health Study, the mean baseline 25(OH)D concentration was as high as 77 nmol/L, and only a small minority of participants had vitamin D deficiency or insufficiency [17,20]. From a causal inference perspective, this discrepancy can also be interpreted using Hill's criteria. Cohort studies demonstrate a clear dose–response relationship, with inverse associations primarily observed among individuals with vitamin D deficiency or insufficiency. Although residual confounding and reverse causation cannot be fully excluded, biological plausibility supports a potential causal role of vitamin D [11–14], particularly in deficient status. However, RCTs conducted largely in vitamin D-replete populations may have limited ability to detect causal effects that are primarily relevant among individuals with low baseline 25(OH)D concentrations.

In this article, we demonstrate that, based on the dose-response relationship between vitamin D status and all-cause mortality, the negative results of both the VITAL study and the D-Health study were to be expected. Furthermore, we carried out emulations to assess which results would have been expected in these trials if the same interventions had been employed among participants with vitamin D deficiency or insufficiency.

2. Methods

2.1. Study population and data

Our analyses are based on data from the UK Biobank (UKB), a large-scale prospective cohort that was recruited between 2006 and 2010 and includes more than half a million participants from the UK aged 40 to 69 [24]. Large-scale biomedical data was collected from the 22 assessment centers in England, Scotland, and Wales using verbal interviews, touchscreen questionnaires, and a variety of physical and medical assessments [24]. Follow-up with respect to all-cause mortality in UKB was conducted by linkages to health care records, including the UK National Health Service (NHS) data, primary care data, cancer screening data, and disease-specific registers [25]. The study was conducted according to the guidelines of the Declaration of Helsinki and was approved by the North West Haydock Research Ethics Committee (#16/NW/0274, 13th May 2016).

2.2. Trial emulation strategy

For this analysis, we employed a number of exclusion criteria that had been used in the VITAL study [17] and the D-Health Study [20] to make the study populations as comparable as possible as outlined in Fig. 1a and b. After these exclusions, 237 502 participants and 185 809 participants were included in the comparative analyses with the VITAL study and the D-Health study, respectively. For our analyses, follow-up was censored at 5.3 years and 5.7 years, which were the median follow-up time of the VITAL study and D-Health Trial, respectively. The exposure was defined as an increase in serum 25(OH)D concentration of 30 nmol/L for VITAL and 38 nmol/L for D-Health, corresponding to the mean achieved differences in the intervention group reported in the respective trials. We then conducted emulation analyses to assess expected results of the VITAL study and the D-Health Trial based on the distribution of baseline 25(OH)D concentrations. For that purpose, observations in the UKB study population were weighted to emulate the distribution of 25(OH)D concentrations observed in the VITAL study and D-Health Trial as shown in Supplemental Tables 1a and 1b, respectively.

In both trials, limited background vitamin D supplementation was permitted. In VITAL, total supplemental vitamin D intake was restricted to ≤800 IU/day [17]. In D-Health, baseline eligibility required ≤500 IU/day, with withdrawal if non-study supplementation exceeded 2000 IU/day during follow-up [20].

2.3. Statistical analyses

Statistical analyses were performed using SAS statistical software (version 9.4, SAS Institute, Inc., Cary, NC, USA). All statistical tests were two-tailed, with a significance level of 0.05. We used multiple imputations with five imputed data sets to fill in missing covariate values after a careful check of the missing-at-random assumption [26]. Analytical results from the imputed datasets were synthesized using the SAS procedure 'PROC MIANALYZE'.

We first carried out analyses of the dose-response relationships between serum 25(OH)D concentrations and all-cause mortality in the two study populations. Knots in restricted cubic spline (RCS) regression analyses were set at the 5th, 25th, 50th, 75th, and 95th percentile of serum 25(OH)D and a concentration of 75 nmol/L was used as the reference. The dose-response analyses were conducted using Cox proportional hazards regression, with full model adjustment for all 46 covariates previously identified as determinants of vitamin D deficiency. These covariates contained demographic and socio-economic factors, biomarkers and healthcare-related factors [13].

We then conducted emulation analyses to assess expected results of the VITAL study and the D-Health Trial based on the distribution of baseline 25(OH)D concentrations and the increase in the 25(OH)D concentrations in the intervention group observed in these trials. For that purpose, observations in the UKB study population were weighted to emulate the distribution of 25(OH)D concentrations observed in the VITAL study and D-Health Trial as shown in Supplemental Tables 1a and 1b, respectively.

To emulate expected results with the baseline distributions and increases in 25(OH)D concentrations observed in the VITAL Study and the D-Health Study, Cox proportional hazards models were performed to assess the associations of 25(OH)D concentrations with all-cause mortality using the weighted data from the UKB. Hazard ratios (HRs) and their 95 % confidence intervals (CIs) were estimated for a 30 nmol/L and 38 nmol/L increase in 25(OH)D concentrations, which corresponds to the mean increase observed in the intervention group of the VITAL study [17], and the difference in 25(OH)D concentrations observed between the intervention group and the placebo group at the end of follow-up in the D-Health study [20]. In the Cox regression analyses, the serum 25(OH)D concentration was treated as a continuous variable.

Subsequently, we repeated the analyses using an unweighted Cox regression analysis by restricting the study population to participants with baseline vitamin D insufficiency (<50 nmol/L) or deficiency (<30 nmol/L), to emulate the expected outcomes of

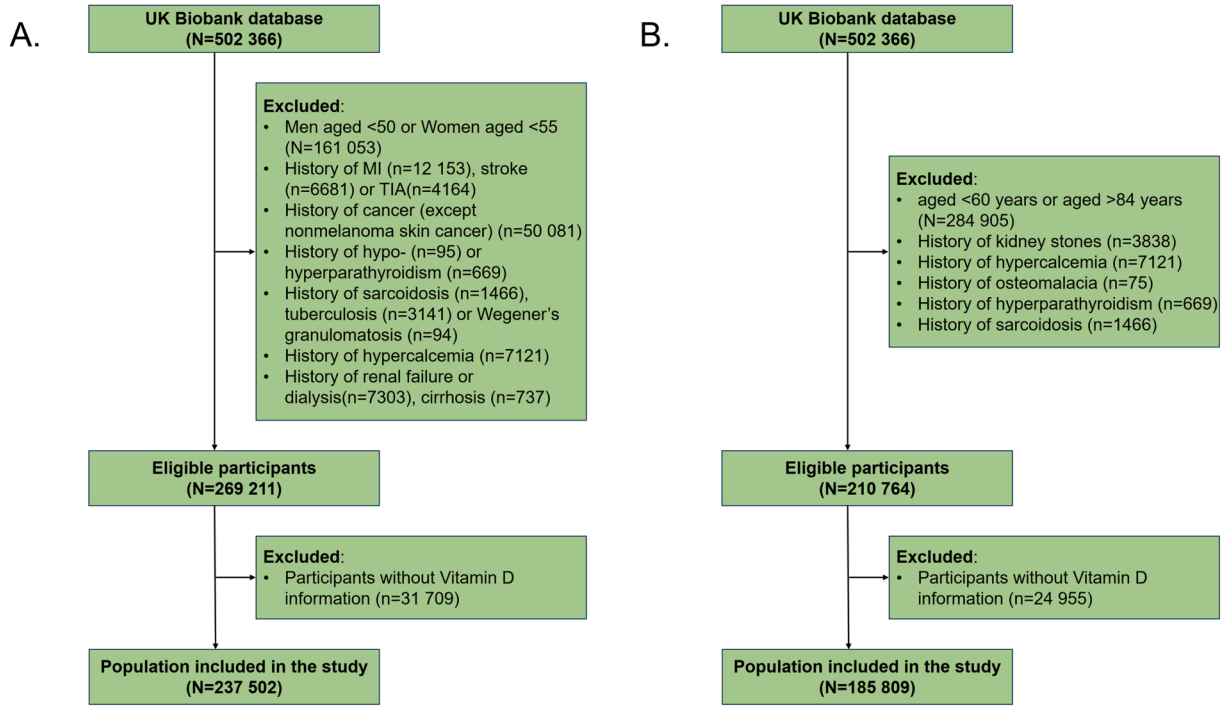

Fig. 1. Flowcharts of UK Biobank participants selected using inclusion and exclusion criteria from (A) the VITAL trial and (B) the D-Health trial.
Abbreviations: VITAL: the Vitamin D and Omega-3 Trial, MI: myocardial infarction, TIA: transient ischemic attack.

targeted interventions among individuals with low vitamin D levels, as opposed to a non-targeted intervention.

Serum 25(OH)D concentrations are reported in nmol/L; conversion to ng/mL can be obtained by dividing by 2.5 (1 ng/mL = 2.5 nmol/L).

3. Results

3.1. Characteristics of the study population

Table 1 summarizes the baseline characteristics of the UK Biobank participants that align with the inclusion and exclusion criteria of the VITAL and D-Health trials.

In the "VITAL-type cohort" (n = 237 502), the average age was 60.7 years (Standard deviation [SD]: 4.9), with 52.7 % being male. A total of 4156 participants died during follow-up. Most participants had at least 12 years of education (45.8 %). Regarding the body mass index (BMI), the majority were in the 25 to <30 kg/m^2 range (44.8 %) or the 18.5 to <25 kg/m^2 range (30.6 %). The majority were non-smokers, accounting for 53.2 %, and most had low alcohol consumption, comprising 42.0 %. Diabetes had been diagnosed in 5.3 % of the population. In terms of vitamin D intake, 75.3 % reported not using vitamin D supplements. The average 25(OH)D concentration was 49.8 nmol/L (SD: 20.7) in total, and 46.6 nmol/L (SD: 21.4) in those who died. A majority of participants had vitamin D insufficiency (34.1 %) or deficiency (18.6 %). Among those who died, prevalence of vitamin D deficiency was 25.9 %.

In the D-Health-type cohort (n = 185 809), the participants were older on average (mean age, 64.1 years [SD: 2.9]) and had a slightly higher proportion of females (51.2 %). A total of 6123 participants died during follow-up. Other demographic characteristics were similar to those of the VITAL-type cohort. The average 25(OH)D concentration and the prevalence of vitamin D deficiency were 50.9 nmol/L (SD: 20.7) and 17.0 % in the entire D-Health-type cohort, and 47.4 nmol/L (SD: 21.7) and 24.3 % in those who died. Among participants who died during follow-up, cancer was the most common cause of death in both cohorts. In the VITAL-type cohort, 57.6 % of deaths were attributed to cancer, 22.3 % to cardiovascular causes, and 20.1 % to other causes. Similarly, in the D-Health-type cohort, cancer accounted for 59.8 % of deaths, followed by cardiovascular deaths (21.7 %) and other causes (18.5 %).

The complete list of baseline characteristics of the study population is presented in Supplemental Table 2.

3.2. Association of serum 25(OH)D concentrations with all-cause mortality

Fig. 2 shows the adjusted dose-response relationship between serum 25(OH)D concentrations and all-cause mortality over follow-up periods of approximately 5.3 and 5.7 years, respectively. In the VITAL-type cohort (n = 237 502; Fig. 2a), an L-shaped relationship was observed, with a significantly increased risk of all-cause mortality at 25(OH)D concentrations <30 nmol/L. A similar pattern was seen in the D-Health-type cohort (n = 185 809; Fig. 2b). Additionally, a modestly increased mortality risk was noted for 25(OH)D concentrations between 30 and 60 nmol/L compared to higher concentrations.

3.3. Comparison of 25(OH)D distribution

Fig. 3 compares 25(OH)D distributions across study populations. Fig. 3a shows that the VITAL trial included a much higher proportion of participants with 25(OH)D concentrations ≥50 nmol/L (85.1 %), in particular participants with 25(OH)D concentrations ≥80 nmol/L (41.5 %), and much lower proportions of participants with vitamin D insufficiency or deficiency (14.9 %) than the VITAL-type UKB cohort (52.7 %). Similarly, Fig. 3b demonstrates that the D-Health Trial participants likewise included a much higher proportion of participants with baseline 25(OH)D concentrations ≥50 nmol/L (86.0 %), and much lower proportions

Table 1
Characteristics of the UK Biobank study population by trial criteria.

Characteristic	VITAL-type cohort		D-Health-type cohort	
	All (n = 237 502)	Died (n = 4156)	All (n = 185 809)	Died (n = 6123)
Sex, No. (%)				
Female	112 258 (47.3)	1439 (34.6)	95 068 (51.2)	2161 (35.3)
Male	125 244 (52.7)	2717 (65.4)	90 741 (48.8)	3962 (64.7)
Age (years), median (IQR)	61 (57; 65)	63 (60; 67)	64 (62; 66)	65 (63; 68)
School education, No. (%)				
≤ 9 years	65 914 (27.8)	1575 (37.9)	64 267 (34.6)	2647 (43.2)
10–11 years	62 773 (26.4)	1018 (24.5)	47 782 (25.7)	1431 (23.4)
≥ 12 years	108 815 (45.8)	1563 (37.6)	73 760 (39.7)	2045 (33.4)
BMI, No. (%)				
<18.5 kg/m^2	1039 (0.4)	50 (1.2)	794 (0.4)	61 (1.0)
18.5 - <25 kg/m^2	72 376 (30.6)	1130 (27.5)	54 543 (29.5)	1587 (26.2)
25 - < 30 kg/m^2	105 896 (44.8)	1761 (42.8)	83 584 (45.2)	2607 (43.1)
≥30 kg/m^2	57 349 (24.2)	1176 (28.6)	46 171 (24.9)	1796 (29.7)
Smoking, No. (%)				
Never	126 190 (53.2)	1531 (36.9)	92 749 (49.9)	2150 (35.1)
Former	89 827 (37.8)	1741 (41.9)	77 837 (41.9)	2933 (47.9)
Current	21 422 (9.0)	879 (21.2)	15 174 (8.2)	1035 (16.9)
Alcohol consumption, No. (%)				
Abstainer	68 299 (28.8)	1323 (31.8)	57 280 (30.8)	2137 (34.9)
Women 0–19.99, men 0–39.99 g/d	99 688 (42.0)	1594 (38.4)	76 239 (41.0)	2382 (38.9)
Women 20–39.99, men 40–59.99 g/d	40 129 (16.9)	636 (15.3)	30 770 (16.6)	821 (13.4)
Women≥40 g/d, men≥60 g/d	29 386 (12.4)	603 (14.5)	21 520 (11.6)	783 (12.8)
Diabetes, No. (%)				
No	224 765 (94.7)	3723 (89.6)	173 035 (93.1)	5285 (86.3)
Yes	12 682(5.3)	431 (10.4)	12 739 (6.9)	836 (13.7)
Vitamin D intake, No. (%)				
No	178 919 (75.3)	3229 (77.7)	139 570 (75.1)	4760 (77.7)
Multivitamins ± minerals	47 753 (20.1)	761 (18.3)	36 492 (19.6)	1078 (17.6)
Vitamin D	10 830 (4.6)	166 (4.0)	9747(5.3)	285 (4.7)
25(OH)D (nmol/L), median (IQR)	48.4 (34.0; 63.3)	44.9 (29.4; 60.5)	49.7 (35.2; 64.5)	45.6 (30.3; 61.4)
25(OH)D categories, No. (%)				
<30 nmol/L	44 214 (18.6)	1076 (25.9)	31 510 (17.0)	1490 (24.3)
30 - < 50 nmol/L	81 052 (34.1)	1342 (32.3)	62 182 (33.5)	2042 (33.4)
≥50 nmol/L	112 236 (47.3)	1738 (41.8)	92 117 (49.6)	2591 (42.3)
Cause of death, No. (%)				
Cardiovascular deaths	–	925 (22.3)	–	1331 (21.7)
Cancer deaths	–	2394 (57.6)	–	3660 (59.8)
Others	–	837 (20.1)	–	1132 (18.5)

VITAL = the Vitamin D and Omega-3 Trial; SD=Standard Deviation; BMI=Body Mass Index; 25(OH)D = 25-hydroxy-vitamin D.
Note: Numbers may not add up to total due to missing values.

of participants with vitamin D insufficiency or deficiency (14.0 %) than the D-Health-type UKB cohort (50.4 %).

3.4. Emulation analyses: expected impact of increases in 25(OH)D achieved in the trials

Table 2 presents the expected impact of a 30 nmol/L or 38 nmol/L increase in 25(OH)D concentrations (the increases achieved in the trials) on all-cause mortality in the emulated trials conducted in the VITAL-type cohort or D-Health-type cohort, respectively.

For the UK Biobank population weighted to match the 25(OH)D distribution of the VITAL study, a 30 nmol/L increase in 25(OH)D had no significant effect on the risk of all-cause mortality (HR 0.97 [95%CI: 0.92–1.02]), consistent with the HR observed in the VITAL study (HR 0.99 [95%CI: 0.87–1.12]). Similarly, for the UK Biobank population weighted to match the 25(OH)D distribution of the D-Health Trial, the 38 nmol/L increase did not significantly impact all-cause mortality risk (HR 1.03 [95%CI: 0.98–1.08]), aligning closely with the HR observed in the D-Health Trial itself (HR 1.04 [95%CI: 0.93–1.18]).

In contrast, when the emulated trials were restricted to participants with vitamin D insufficiency (baseline 25(OH)D < 50 nmol/L) or deficiency (<30 nmol/L), substantial reductions in mortality risk were observed. In the VITAL-type emulation, the hazard ratios were 0.85 (95 % CI: 0.79–0.91) and 0.79 (95 % CI: 0.72–0.87) for the insufficient and deficient groups, respectively. Corresponding estimates in the D-Health-type emulation were 0.81 (95 % CI: 0.76–0.86) and 0.75 (95 % CI: 0.69–0.81).

4. Discussion

4.1. Summary of the main findings

In this study, we explored the expected effect of vitamin D supplementation on all-cause mortality in populations with different vitamin D levels by emulating clinical trials achieving analogous increases in 25(OH)D concentrations as the VITAL trial and the D-Health trial. Our analyses were based on subcohorts of the UK Biobank meeting in- and exclusion criteria of the trials. The prevalence of vitamin D deficiency or insufficiency (25(OH)D < 50 nmol/L) was much higher in the UKB subcohorts than in the VITAL trial (53 % versus 15 %) and the D-Health trial (50 % versus 14 %). An inverse association between 25(OH)D and mortality was essentially restricted to those with vitamin D deficiency or insufficiency. In emulated trials, expected effects on mortality were almost identical to the observed ones if participants were weighted to yield baseline 25(OH)D distributions as observed in the trials. By contrast, strong mortality reduction would have been expected with the baseline distribution of 25(OH)D observed in the UK Biobank subcohorts. Expected effects

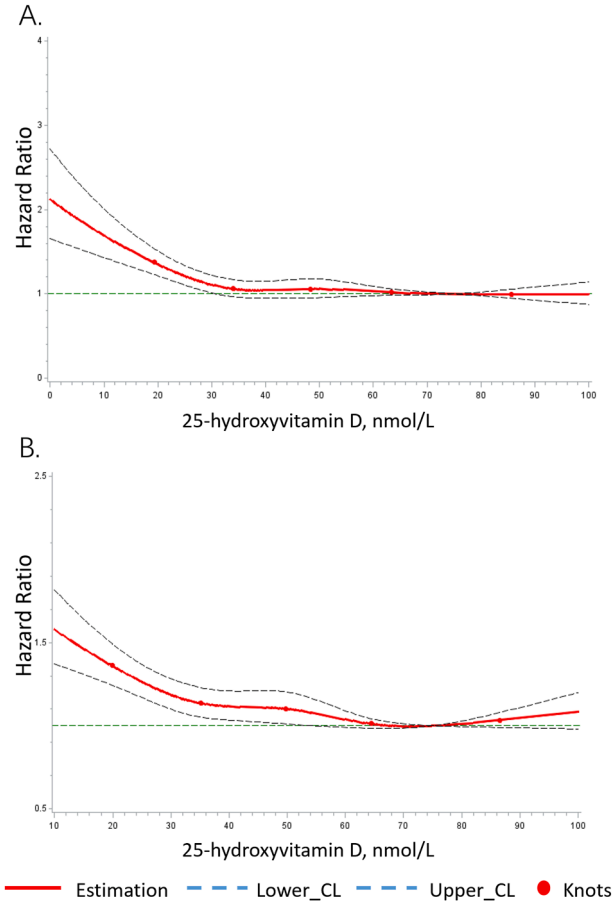

Fig. 2. Adjusted dose-response relationships between serum 25-hydroxyvitamin D concentrations and all-cause mortality over (A) 5.3 years in the VITAL-type cohort and (B) 5.7 years in the D-Health-type cohort.
Note: 5 knots were used and located at the 5th, 25th, 50th, 75th, and 95th serum 25-hydroxyvitamin D percentile and the 75 nmol/L was used as the reference. Horizontal lines represent the hazard ratio of 1. Solid lines are estimates of hazard ratios and dashed lines are their 95 % confidence intervals. Knots are represented by dots.
The models are adjusted for age, sex, skin colour, latitude of study center and calendar month of attending the assessment center, socio-economic factors (education, deprivation index at recruitment, no of individuals in household, and household income), life-style factors (smoking, alcohol consumption, physical activity, frequency of visiting friends/family and consumption of oily fish, cereal, processed meat, milk, bread and spread), and vitamin D specific factors (time spend outdoors in summer and winter, ease of skin tanning, use of sun screen/UV protection, and solarium/sunlamp use), weight variables (body mass index and waist circumference), diseases and disease symptoms (diabetes, chronic obstructive pulmonary disease, osteoporosis, arthritis, gout, Parkinson, depressed mood, and tiredness/lethargy), biomarkers (estimated glomerular filtration rate, HbA1c, HDL cholesterol, systolic blood pressure, diastolic blood pressure, C-reactive protein, forced expiratory volume in 1-s, and hand grip strength), and general health status (no. of drugs, no. of chronic diseases, disability, and general self-rated health). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

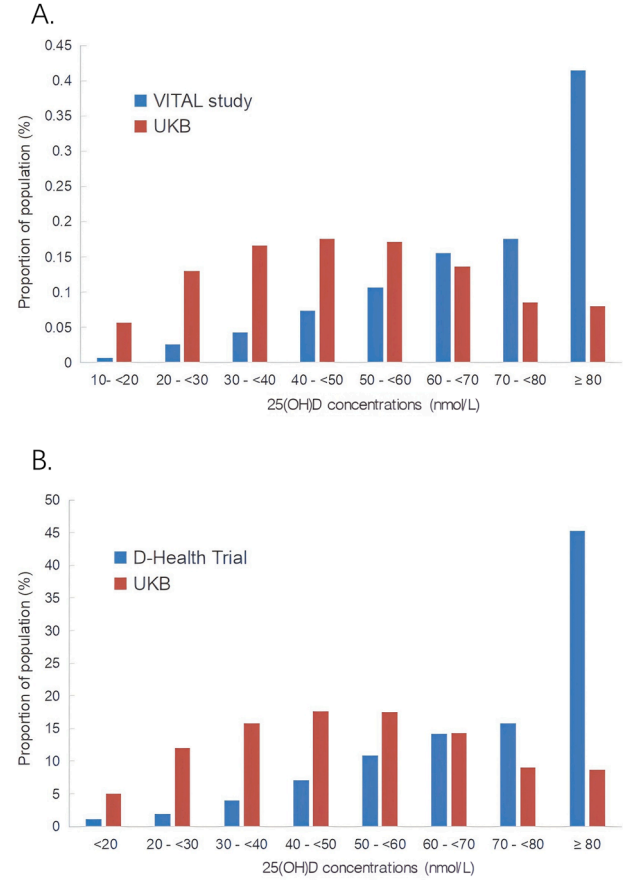

Fig. 3. Comparison of baseline 25(OH)D distributions between (A) the VITAL trial and matched UK Biobank cohort and (B) the D-Health trial and matched cohort.
Note: The data of VITAL study were provided by personal communication of the corresponding author of the main VITAL study publication.
The data of the D-Health Trial were derived from the mean (77 nmol/L) and standard deviation (25 nmol/L) in the placebo group reported by Neale et al., assuming normal distribution of 25(OH)D and using the "pnorm()" function in R software.

would have been particularly strong when restricting study participants to those with vitamin D insufficiency or deficiency.

4.2. Evidence from epidemiology studies

Our findings demonstrate a "L-shaped" dose–response relationship between serum 25(OH)D concentrations and all-cause mortality, with the strongest inverse associations observed at concentrations below 50 nmol/L. This pattern aligns with previous large-scale cohort analyses: Fan et al. reported a 17 % lower all-cause mortality for people with 25(OH) concentrations above 60 nmol/L compared to those with lower concentrations in the UKB study [10], while Sha et al. observed a 10 % mortality reduction among supplement users in real-world data [13]. Earlier, Zittermann et al.'s meta-analysis of prospective cohorts demonstrated a nonlinear decrease in mortality risk as 25(OH)D rose toward 75–87.5 nmol/L [6]. The protective mechanisms of adequate vitamin D may relate to its roles in bone health, immune modulation, and cardiovascular function [13,16,27–29].

Despite consistent epidemiological associations, the key question remains whether these relationships are causal [30,31], and if so, what threshold of serum 25(OH)D confers benefit [32,33]. Our emulation approach helps to explain the apparent inconsistencies between the trial results and the results of the large-scale observational studies and suggests that differences in these baseline concentrations rather than residual confounding in the observational studies are the primary source of these inconsistencies. Our results should help to inform future supplementation trials and supplementation strategies which should focus on populations with vitamin D insufficiency or deficiency.

4.3. Comparison with results from previous RCTs

RCTs are essential for establishing causality. The VITAL trial (n = 25 871) administering 2000 IU/day of vitamin D_3 over 5.3

Table 2
Observed and emulated trial results for serum 25(OH)D increase.

Trial	Baseline 25(OH)D		25(OH)D Increase	Hazard ratio (95 % CI)
	Inclusion criteria	Mean		
VITAL, reported	Unrestricted	77 nmol/L	30 nmol/L	0.99 (0.87, 1.12)
VITAL, emulated	Unrestricted, weighted[a]	77 nmol/L	30 nmol/L	0.97 (0.92, 1.02)
	<50 nmol/L	34 nmol/L	30 nmol/L	0.85 (0.79, 0.91)
	<30 nmol/L	22 nmol/L	30 nmol/L	0.79 (0.72, 0.87)
D-Health, reported	Unrestricted	77 nmol/L	38 nmol/L	1.04 (0.93, 1.18)
D-Health, emulated	Unrestricted, weighted[b]	77 nmol/L	38 nmol/L	1.02 (0.97, 1.07)
	<50 nmol/L	34 nmol/L	38 nmol/L	0.81 (0.76, 0.86)
	<30 nmol/L	23 nmol/L	38 nmol/L	0.75 (0.69, 0.81)

[a] Weighted to yield the same 25(OH)D distribution as observed in the VITAL trial.
[b] Weighted to yield the same 25(OH)D distribution as observed in the D-Health trial.

years reported an HR of 0.99 (CI, 0.87 to 1.12) for all-cause mortality [17], and the D-Health Trial (n = 21 315) using monthly 60 000 IU doses over 5.7 years observed an HR of 1.04 (95%CI: 0.93–1.18) [20]. In both trials, fewer than 20 % of participants had baseline 25(OH)D < 50 nmol/L, limiting power to detect benefits in deficient subgroups [32,34]. Our emulations show that, when UKB subcohorts are reweighted to the trials' higher baseline distributions, expected mortality effects mirror these null RCT results. Conversely, using the lower 25(OH)D distribution of the UKB, substantial mortality reductions emerged, particularly among those with 25(OH)D < 50 nmol/L, emphasizing the importance of enrolling participants with vitamin D insufficiency or deficiency. A meta-analysis of 80 RCTs by Ruiz-García et al. reported a modest 5 % reduction in all-cause mortality ($P = 0.013$) with vitamin D supplementation [35], smaller than the 11–13 % reductions we observed for 30 or 38 nmol/L increases in our unadjusted UKB analysis. This disparity underscores how population baseline status influences effect size and may explain why large, predominantly vitamin D replete cohorts fail to demonstrate benefit.

4.4. Clinical and public health implication

Our results suggest a major potential of mortality reduction by vitamin D supplementation in vitamin D less sufficient populations, despite the negative results of the large trials, which were conducted in mostly vitamin D sufficient US and Australian populations. Future RCTs should prioritize recruitment of individuals with baseline 25(OH)D below commonly cited sufficiency thresholds (e.g., <50 nmol/L) to maximize statistical power and detect clinically meaningful effects. Such targeted designs would align supplementation resources with those most likely to benefit, improving cost-effectiveness and public health impact. In routine practice, baseline assessment of vitamin D status should guide supplementation, avoiding unnecessary treatment in sufficient individuals and focusing efforts on those with deficiency or insufficiency.

Prediabetes, type 2 diabetes, overweight, and obesity are highly prevalent in older adults, particularly in the United States [36,37]. These complications frequently coexist with advanced age and polypharmacy [38]. Although our analyses did not explicitly model these conditions, their frequent clustering may further modify responses to vitamin D supplementation. Therefore, future studies specifically addressing these overlapping conditions are warranted.

In our study, vitamin D deficiency and insufficiency were defined using commonly applied epidemiological cut-offs for serum 25(OH)D concentrations (<30 nmol/L and 30–50 nmol/L, respectively) [2–5]. These thresholds are used as operational definitions to facilitate comparisons across studies and to characterize baseline vitamin D status distributions, rather than as normative clinical targets. The Endocrine Society (2024) recommends against screening serum 25(OH)D concentrations in adults aged 18–74 years [39], emphasizing absence of supportive clinical trial evidence. Our emulated trial analyses results provide suggestive evidence for the importance of baseline vitamin D status, with greater expected benefits expected among individuals with low baseline 25(OH)D concentrations. These findings support a risk-stratified approach in which vitamin D supplementation may be most relevant for populations with low vitamin D status, rather than a universal supplementation strategy.

Given the modest effect sizes observed in vitamin D-replete populations, substantially larger sample sizes than those of VITAL and D-Health would have been required to achieve adequate statistical power to detect clinically meaningful reductions in all-cause mortality. In contrast, the larger effect sizes observed among participants with vitamin D insufficiency or deficiency suggest that trials enriched for low baseline 25(OH)D concentrations would be more efficient and would require substantially smaller sample sizes to detect comparable relative risk reductions. These considerations highlight the importance of baseline vitamin D status in trial design and may partly explain the null findings of recent large trials conducted predominantly in vitamin D–replete populations.

Although our analysis focused on all-cause mortality as the primary endpoint of the VITAL and D-Health trial following a prespecified analysis protocol, similar dose-response relationships between 25(OH)D concentrations and other important health outcomes observed in observational studies call for further emulated trial analyses with such outcomes. Our study may serve as a model to design such emulated trial analyses.

4.5. Strengths and limitations

Our study has several strengths. Firstly, this study pioneers the concurrent emulation of two major RCT designs within a single observational cohort, providing a framework to examine how differences in baseline exposure distributions may contribute to discrepancies between clinical trials and real-world evidence within broadly comparable western populations. Secondly, the use of a large dataset from the UKB cohort enhances the statistical power of the study, allowing for robust estimates of the association between vitamin D levels and all-cause mortality. Thirdly, the UKB contains a wealth of data on a wide range of covariates, which allows for detailed adjustments and control for potential confounding factors in the analysis. Lastly, this study was conducted in a population with prevalence of vitamin D insufficiency and deficiency in an order of magnitude encountered among older adults in many countries around the globe, in which vitamin D food

fortification or supplementation is much less common than in the US and Australia, where the large supplementation trials were conducted.

However, our study also has limitations. First, it relies on emulations rather than actual clinical trials, which restricts the ability to make definitive causal inferences. While emulations are valuable for generating hypotheses and informing future research, they may not fully capture the complexity of real-world scenarios. Second, the data were drawn from the UKB, which may not be entirely comparable to the general UK population and to populations in the VITAL and D-Health trials. The UK population differs from those in the US and Australia not only in baseline vitamin D but also in lifestyle, healthcare systems, and environmental factors—all of which can influence mortality outcomes. Additionally, although efforts were made to match the age and sex distribution, slight differences in these factors could still affect the comparability of the results across studies. Despite these limitations, our findings highlight the need to target vitamin D supplementation at individuals with deficiency or insufficiency. Future RCTs evaluating vitamin D's impact on mortality should prioritize these populations to increase statistical power and better capture potential benefits.

5. Conclusions

By emulating the VITAL and D-Health trial designs within the UK Biobank, we demonstrated that baseline vitamin D status critically determines the observable benefit of supplementation on all-cause mortality. Whereas trial-analogous increases in 25(OH)D produced null effects when the UKB population was weighted to reflect the higher baseline distributions of VITAL and D-Health, substantial mortality reductions were observed with the UKB participants' lower baseline 25(OH)D concentrations. These reductions were concentrated among individuals with vitamin D insufficiency or deficiency and grew more pronounced as baseline levels decreased. Our findings suggest that the lack of efficacy seen in large RCTs likely reflects recruitment of predominantly vitamin D–sufficient populations rather than an absence of true benefit in deficient groups. Future trials and clinical guidelines should therefore prioritize individuals with vitamin D insufficiency or deficiency.

Study registration and permissions

This study utilized data from the UK Biobank Resource, obtained under application number "89329". Publicly available data is accessible to researchers via an open application on https://www.ukbiobank.ac.uk/register-apply/.

Author contributions

Youqing Wang: Data curation, Methodology, Formal statistical analysis, Writing - original draft. Sha Sha: Methodology, Writing - review & editing. Tafirenyika Gwenzi: Writing - review & editing. Ben Schöttker: Methodology, Project administration, Communication with the corresponding author of the main VITAL study publication, Writing - review & editing. Hermann Brenner: Conceptualization, Methodology, Project administration, Supervision, Writing - review & editing. All individuals who meet the criteria for authorship have been included in the author list, and no one eligible for authorship has been omitted.

Ethics approval

The UK Biobank was approved by the North West Multi-center Research Ethics Committee (MREC) as a Research Tissue Bank (RTB) approval (2021: 21/NW/0157) and all participants provided written informed consent.

Declaration of generative AI and AI-assisted technologies in the writing process

No generative AI or AI-assisted technologies were used in the writing process of this manuscript.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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