



Vitamin D and risk of death from vascular and non-vascular causes in the Whitehall study and meta-analyses of 12 000 deaths

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Aims

To examine the independent relevance of plasma concentrations of 25-hydroxyvitamin D [25(OH)D] for vascular and non-vascular mortality.

Methods and results

We examined associations of plasma concentrations of 25(OH)D and cause-specific mortality in a prospective study of older men living in the UK and included findings in meta-analyses of similar studies identified by a systematic search reporting on vascular and all-cause mortality. In a 13-year follow-up of 5409 men (mean baseline age 77 years), 1358 died from vascular and 1857 from non-vascular causes. Median season-adjusted baseline 25(OH)D concentration was 56 (interquartile range: 45–67) nmol/L. After adjustment for age and seasonality, higher concentrations of 25(OH)D were inversely and approximately linearly (log–log scale) associated with vascular and non-vascular mortality throughout the range 40–90 nmol/L. After additional adjustment for prior disease and cardiovascular risk factors, a doubling in 25(OH)D concentration was associated with 20% [95% confidence interval (CI): 9–30%] lower vascular and 23% (95% CI: 14–31%) lower non-vascular mortality. In meta-analyses of prospective studies, individuals in the top vs. bottom quarter of 25(OH)D concentrations had 21% (95% CI: 13–28%) lower vascular and 28% (95% CI: 24–32%) lower all-cause mortality.

Conclusions

Despite strong inverse and apparently independent associations of 25(OH)D with vascular and non-vascular mortality, causality remains uncertain. Large-scale randomized trials, using high doses of vitamin D, are required to assess the clinical relevance of these associations.

Keywords

Vitamin D • Cardiovascular disease • Mortality

Introduction

Prospective observational studies have reported that low circulating concentrations of 25-hydroxyvitamin D [25(OH)D] are associated with higher risks of cardiovascular disease,^{1–7} cancer,^{6,8–10} and all-cause mortality.^{5,7,11–13} However, as low 25(OH)D concentrations are correlated with several known vascular risk factors, it is possible that any such associations with disease may reflect confounding by these risk factors. Alternatively, these associations with 25(OH)D may be due to reverse causation, as individuals with vascular disease or cancer, who may be frail or unwell,

may be more likely to stay indoors, and have low plasma 25(OH)D concentrations due to inadequate sunlight exposure. Previous meta-analyses of prospective studies reported a significant inverse association of 25(OH)D with all-cause mortality,^{14,15} but did not distinguish vascular from non-vascular causes of death.

The relevance of measurements of circulating 25(OH)D levels in the general population, including those with vascular disease, is uncertain. No large randomized trials of vitamin D have yet been completed with vascular disease or cancer as the primary outcome. Previous meta-analyses of randomized trials of vitamin D reported only borderline statistically significant effects of

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vitamin D treatment on all-cause mortality,^{16,17} but were unable to detect significant effects on vascular outcomes.¹⁸ These trials typically used daily doses of 400–800 IU of vitamin D₃, which may not be sufficient to optimize plasma 25(OH)D concentrations throughout the year.¹⁹ If the inverse associations of 25(OH)D with vascular disease and other outcomes are causal and reversible by treatment, then this could have important implications for public health, particularly for countries in the Northern hemisphere²⁰ where low vitamin D levels are highly prevalent.

We examined the associations of plasma concentrations of 25(OH)D with vascular and non-vascular causes of mortality in a 13-year follow-up of a prospective study of 5409 older men living in the UK in 1997, and compared the results in meta-analyses of similar prospective studies of 25(OH)D and vascular and all-cause mortality. The aims of the present study were: (i) to examine cross-sectional associations of 25(OH)D with other vascular risk factors; (ii) to assess the shape and strength of the associations of plasma 25(OH)D concentrations with vascular and non-vascular causes of death, overall and separately in those with and without any pre-existing disease; and (iii) to compare these results in meta-analyses of prospective studies of 25(OH)D and vascular and all-cause mortality.

Methods

Study population

The Whitehall study is a prospective study of 19 019 male civil servants who were working in London at the time of recruitment in 1967–70.^{21,22} Following a successful pilot study in 1995, a resurvey was conducted of all surviving 8448 participants in this cohort during 1997–98, after approval by the relevant ethics committees of the participating institutions. Data were collected for the resurvey using mailed questionnaires seeking details of previous medical history (diagnoses of heart attack, angina, stroke, cancer, and diabetes), self-reported health status, medications taken in the past month, lifestyle characteristics (e.g. smoking status and alcohol consumption), and last known civil service employment grade. All 7044 (83%) respondents were subsequently sent a blood collection kit and asked to attend their local surgery to have a sample collected and measurements of blood pressure, height, and weight recorded. Complete data on questionnaires and relevant blood results were available from 5409 (77% of respondents) men for the present report. Compared with responders, non-responders were older and had a lower previous employment grade, but response rates were not associated with total cholesterol levels recorded from 1967 to 70.²³

Laboratory methods

Whole-blood samples (~10 mL) were returned by mail in sealed tubes (Vacutainer; Becton Dickson, Franklin Lakes, NJ, USA) containing potassium ethylenediaminetetraacetic acid with 0.34 mmol/L of aprotinin. These samples were mailed at room temperature to the CTSU Wolfson Laboratory in Oxford, with 78% arriving within 24 h and 96% arriving within 48 h of blood collection [mean time in post: 1.3 days (range 0–7 days)]. On arrival in the laboratory, the blood was centrifuged, and plasma was aliquoted and stored at –40°C. Plasma 25(OH)D concentrations were measured using an automated immunoassay on the IDS-iSYS analyser (Immunodiagnostic systems, Boldon, UK). In addition, 187 of these men had plasma 25(OH)D

concentrations measured on blood samples collected for the pilot study for this resurvey, on average 1.5 years prior to the main resurvey. The IDS-iSYS 25(OH)D assay standardization is traceable to ultraviolet quantification and calibration was verified by comparison with an isotope dilution liquid chromatography-tandem mass spectrometry method. Over the testing period, proficiency testing with vitamin D external quality assessment scheme samples indicated that the IDS-iSYS assay was systematically overestimating concentrations compared with the mean obtained for all 25(OH)D assays. Hence, a linear correction, multiplying by 0.757, was applied to all measured 25(OH)D concentrations. Plasma concentrations of total cholesterol, LDL cholesterol, HDL cholesterol, apolipoprotein A₁ (Apo A₁), and apolipoprotein B (Apo B) as well as biomarkers of inflammation [C-reactive protein (CRP), fibrinogen, and albumin] and cystatin C were measured using standard methods.²² Estimated glomerular filtration rate (eGFR, in mL/min/1.73 m²) was calculated from cystatin C concentration using the formula $eGFR = 80.35/\text{cystatin C (in mg/L)} - 4.32$.²⁴

Cause-specific mortality follow-up

Participants were flagged for mortality at the Office for National Statistics (UK), which provided the date and cause [including International Classification of Disease (ICD) codes] of all deaths occurring until end of August 2010. The mean follow-up period among survivors was 13.1 years. Cause-specific mortality was coded using ICD-9 up to August 2002 and ICD-10 subsequently. Vascular deaths (heart disease, stroke, and other vascular disease) were defined if coded as ICD-9 codes 390–459, 798 or ICD-10 codes I00–I99, R96, with all other causes of death being defined as non-vascular. Deaths from ischaemic heart disease (IHD: ICD-9 codes 410–414; ICD-10 codes I20–I25), stroke (ICD-9 codes 430–438; ICD-10 codes I60–I69), cancer (ICD-9 codes 140–208; ICD-10 codes C0–C97), and respiratory disease (ICD-9 codes 460–519; ICD-10 codes J00–J99) were also analysed separately.

Statistical methods

Plasma 25(OH)D concentrations were analysed on the logarithmic scale. Since concentrations of plasma 25(OH)D varied throughout the year, the values were adjusted for month of blood sampling. This was done by adding to each participant's log 25(OH)D measurement the difference between the overall mean log 25(OH)D concentration (seen in all men) and the mean observed just among men sampled in the same month. Since the majority of men were examined in the summer of 1997, when 25(OH)D concentrations tended to be highest (see Supplementary material online, Figure S2), this adjustment tended to shift values towards those seen in summer rather than winter months. Men were subsequently classified into five equal sized groups on the basis of their season-adjusted 25(OH)D concentration, and the means and prevalences of other baseline characteristics, adjusted for age, were estimated [with tests for linear trend between log 25(OH)D and the risk factor performed using, respectively, linear or logistic regression adjusted for age]. To assess the shape of the association between 25(OH)D and mortality, men in the top and bottom fifths were further divided in half for the purpose of the plotting of dose–response relationships to better characterize any relationships at more extreme concentrations. Relative risks (RRs) (estimated by hazard ratios in Cox models) were estimated for each group relative to the lowest and are shown as 'floating absolute risks' [which does not alter their values but merely ascribes a 95% confidence interval (CI) to the RR in every group].²⁵ The average RR corresponding to a doubling in 25(OH)D concentration (approximately a

2 SD increase on the log scale) was also estimated. The proportionality assumption of the Cox model was assessed using the method described by Grambsch and Therneau.²⁶ Analyses were done before and after adjustment for age, prior history of disease (myocardial infarction, angina, stroke, cancer, or diabetes), self-reported health status (on a four point scale), ability to perform particular activities of daily living (based on a 15-point questionnaire), smoking status (current smoker, ex-smoker, and never smoker), alcohol consumption, last known employment grade, blood pressure (both at entry to Whitehall study in 1967–70 and at resurvey in 1997–98, as well as treatment for hypertension at resurvey), body mass index, blood lipids, and apolipoproteins (LDL-C, HDL-C, Apo A₁, and Apo B), markers of inflammation (CRP, albumin, and fibrinogen), and eGFR. To further assess the effects of reverse causality, analyses were repeated separately in men with and without a prior history of disease (defined as above), while further analyses excluded deaths during the first 5 years of follow-up.

Meta-analyses of prospective studies

Data from the Whitehall study were included in meta-analyses of all published reports of prospective studies that reported associations of circulating concentrations of 25(OH)D with either vascular mortality or all-cause mortality before January 2012. Eligible population-based studies (see Supplementary material online, Table S1), based on prespecified selection criteria, were identified by electronic literature searches (PubMed, Embase, and Cochrane databases) using a systematic search strategy (see Supplementary material online, Figure S1). Studies were to be included if they (i) involved a prospective (or nested case-control) study design; (ii) included more than 200 adult participants recruited from the general population; and (iii) had data on 25(OH)D concentrations and risk of death from cardiovascular or all-causes. Studies were excluded if they were selected on the basis of (i) diagnosis of prior disease (cancer, vascular, or renal disease); (ii) risk factors (diabetes and hypertension); (iii) nursing home residents; (iv) participants in clinical trials; or (v) meta-analyses of previous studies. Data were abstracted from each report on RRs (commonly hazard ratios from Cox regression models) and their 95% CI for vascular and all-cause mortality, and verified by two authors working independently. For each study, the most fully adjusted RR and its 95% CI were extracted. Any RR models that had been adjusted for calcium and phosphate were not included (as these were considered to be on the causal pathway). Where necessary, the RRs were recalibrated to correspond to the top vs. bottom quarter of the 25(OH)D distribution (most common approach taken in individual studies).²⁷ This was done by estimating the number of SDs that each published RR would have corresponded to [on some normal transformation of the underlying 25(OH)D distribution] before recalibrating the log RR (and its standard error) to correspond to a 2.54 SD difference (since 2.54 is the difference in mean values between the top and bottom quarters of a normal distribution). Principal investigators were contacted and asked to provide additional data on the SD of 25(OH)D concentration to facilitate standardized comparisons. Overall summary estimates of the effect were calculated using the Mantel–Haenszel inverse-variance weighted method for meta-analysis. In forest plots, studies were ordered according to the amount of statistical information they contributed to the overall result [and, for display only, were grouped as being: ‘small’ (providing <1% of the total information provided by all the studies); ‘medium’ (1 to <10%); or ‘large’ (at least 10%)]. All *P* values were two sided and *P* values <0.05 were deemed

conventionally significant. Analyses were done using SAS version 9.1 (SAS Institute, Cary, NC, USA) and R version 2.11.1 (www.r-project.org).

Results

Baseline characteristics

Selected characteristics of the 5409 men included in the analyses are summarized in Table 1. The mean age of participants at resurvey was 76.9 (SD 4.9) years, and about one-third (1841 men) had a history of prior vascular disease, cancer, or diabetes at resurvey. The majority (87%) were non-smokers, while 78% were self-reported ‘current’ alcohol drinkers.

Distribution of 25-hydroxyvitamin D concentrations at baseline

Plasma concentrations of 25(OH)D varied substantially by month of blood collection, and, even after adjustment for month of blood collection, concentrations had a log-normal distribution (see Supplementary material online, Figure S2). Median 25(OH)D concentration (standardized for month of blood collection) was 56 nmol/L (interquartile range 45–67 nmol/L) (Table 1). In a sample of 187 men with repeated measurements taken 1.5 years apart, the self-correlation in log 25(OH)D was 0.64. At any given age, men with higher 25(OH)D concentrations were less likely to have a history of vascular disease, cancer, or diabetes, and less likely to have been diagnosed with hypertension or taking treatment for hypertension, than men with lower concentrations. Measured systolic blood pressure at resurvey in 1997 was only weakly related with 25(OH)D concentrations, and blood pressure at the initial examination for the Whitehall study in 1967–70 was unrelated with 25(OH)D concentrations. Men with higher 25(OH)D also had lower mean body mass index than men with lower 25(OH)D and were less likely to have been of manual/clerical grade at retirement. In contrast, men with higher plasma 25(OH)D concentrations had higher mean LDL-C, HDL-C, ApoA₁, and albumin concentrations, and lower mean CRP and fibrinogen concentrations, than men with lower 25(OH)D concentrations.

Association of 25-hydroxyvitamin D with vascular and non-vascular mortality

Overall among the 5409 participants, 3215 men died during over 50 000 person years of follow-up (overall death rate: 6.4% per year; mean follow-up among survivors 13 years), including 1358 deaths (2.7% per year) from vascular causes and 1857 deaths (3.7% per year) from non-vascular causes (Table 2). Among the 3568 men without a history of vascular disease, cancer, or diabetes, there were 727 deaths (2.0% per year) from vascular causes and 1124 deaths (3.1% per year) from non-vascular causes. After classifying men into seven groups based on season-adjusted 25(OH)D concentration, higher concentrations of 25(OH)D were inversely and, on the log–log scale, approximately linearly related to the risk of vascular and, at least throughout the range 40–90 nmol/L, of non-vascular mortality in

Table 1 Study characteristics, overall and by prior disease, and baseline 25-hydroxyvitamin D concentration

Baseline characteristics	All men	No prior disease	Prior disease	Fifth of baseline 25(OH)D ^a					p ^{†b}
				I	II	III	IV	V	
Number of men	5409	3568	1841	1081	1082	1082	1082	1082	
Age (years)	76.9 (4.9)	76.5 (4.8)	77.6 (5.0)	78.9 (5.2)	77.1 (5.1)	76.7 (4.6)	76.1 (4.5)	75.5 (4.3)	
25(OH)D (nmol/L)	56 (45–67)	57 (47–68)	54 (43–65)	36 (5)	48 (3)	56 (2)	65 (3)	83 (18)	
Medical history (%)									
IHD	19.9	0.0	58.4	22.0	20.5	20.3	19.8	16.5	0.002
Stroke	7.2	0.0	21.1	9.3	6.6	6.7	6.7	5.9	0.001
CVD	24.9	0.0	73.3	28.1	25.4	25.4	24.6	20.7	<0.001
Diabetes	5.9	0.0	17.4	8.8	6.7	5.8	2.8	4.0	<0.001
Cancer (not skin)	7.9	0.0	23.2	10.1	8.1	7.4	6.8	6.5	0.006
Self-reported health good/excellent	77.4	85.3	62.0	67.1	75.8	79.9	80.5	85.5	<0.001
Manual/clerical socio-economic grade at baseline	17.4	17.7	16.8	20.2	19.4	16.2	15.8	14.9	<0.001
Lifestyle (%)									
Current tobacco smoker	12.7	14.1	10.2	16.8	12.3	12.7	10.1	11.4	<0.001
Current alcohol drinker	77.9	79.2	75.4	73.3	75.8	80.5	79.5	80.8	<0.001
Blood pressure and body mass									
Diagnosis of hypertension/use of blood	42.0	33.1	59.3	45.0	43.1	41.4	40.2	40.5	0.003
Systolic blood pressure (mm Hg)	144.8 (20.1)	145.4 (19.8)	143.7 (20.7)	144.0 (20.5)	144.6 (20.1)	145.2 (20.1)	144.9 (20.2)	145.5 (20.3)	0.05
Diastolic blood pressure (mm Hg)	80.2 (10.8)	80.9 (10.7)	78.8 (10.9)	79.6 (11.0)	80.4 (10.8)	80.1 (10.8)	80.1 (10.8)	80.5 (10.8)	0.10
Body mass index (kg/m ²)	25.2 (3.2)	25.1 (3.2)	25.4 (3.3)	25.5 (3.3)	25.4 (3.2)	25.4 (3.2)	25.1 (3.2)	24.8 (3.2)	<0.001
Blood pressure measured in 1967–70 (~30 years earlier; mm Hg)									
Systolic blood pressure	130.8 (17.6)	129.5 (17.2)	133.3 (18.2)	130.5 (17.9)	130.9 (17.5)	131.3 (17.5)	130.5 (17.6)	130.5 (17.7)	0.57
Diastolic blood pressure	80.3 (12.1)	79.4 (11.7)	82.0 (12.6)	80.1 (12.3)	80.6 (12.1)	80.8 (12.1)	80.1 (12.1)	79.9 (12.2)	0.35
Laboratory measurements									
LDL-C (mmol/L)	3.37 (0.79)	3.40 (0.78)	3.31 (0.80)	3.28 (0.79)	3.36 (0.78)	3.42 (0.78)	3.42 (0.78)	3.36 (0.78)	<0.001
HDL-C (mmol/L)	1.09 (0.38)	1.12 (0.37)	1.04 (0.38)	1.06 (0.38)	1.06 (0.38)	1.08 (0.38)	1.11 (0.38)	1.17 (0.38)	<0.001
Apolipoprotein A ₁ (g/L)	0.95 (0.15)	0.96 (0.14)	0.93 (0.15)	0.93 (0.15)	0.94 (0.14)	0.95 (0.14)	0.96 (0.14)	0.98 (0.15)	<0.001
Apolipoprotein B (g/L)	0.87 (0.23)	0.87 (0.23)	0.87 (0.24)	0.85 (0.23)	0.87 (0.23)	0.88 (0.23)	0.88 (0.23)	0.85 (0.23)	0.38
C-reactive protein (mg/L)	3.7 (7.7)	3.4 (7.2)	4.4 (8.5)	4.3 (7.8)	3.7 (7.7)	3.6 (7.7)	3.5 (7.7)	3.5 (7.7)	<0.001
Albumin (g/L)	39.7 (3.0)	39.8 (2.8)	39.3 (3.2)	39.3 (2.9)	39.6 (2.9)	39.7 (2.9)	39.9 (2.9)	39.9 (2.9)	<0.001
Fibrinogen (μmol/L)	3.5 (0.8)	3.5 (0.8)	3.6 (0.9)	3.6 (0.9)	3.6 (0.8)	3.5 (0.8)	3.5 (0.8)	3.5 (0.9)	0.02
Renal function									
eGFR (mL/min/1.73 m ²)	72.2 (15.3)	74.0 (14.4)	68.6 (16.4)	69.8 (14.7)	71.7 (14.4)	72.9 (14.4)	73.0 (14.4)	73.5 (14.5)	<0.001

Mean (SD), median (interquartile range), or *n* (%) shown.

25(OH)D, 25-hydroxyvitamin D; IHD, ischaemic heart disease (recall of diagnosis of myocardial infarction or angina); eGFR, estimated glomerular filtration rate; CVD, cardiovascular disease.

^aWith the exception of age and vitamin D, estimates are adjusted for age differences across vitamin D groups.

^{†b}Test of linear trend between log[25(OH)D] concentration and baseline characteristics after adjustment for age.

age-adjusted models (Figure 1). The shape of these associations were broadly similar for both vascular and non-vascular mortality [albeit with some attenuation of risk for non-vascular mortality with 25(OH)D concentrations above 80 nmol/L], and, for both outcomes, associations were consistent among men with and without a prior history of vascular disease, cancer, or diabetes.

Effect of adjustment for potential confounders

Given age, a doubling in 25(OH)D concentration [corresponding to a ln(2) absolute difference—~2 SDs—on the log-scale] was, on average, associated with a 34% lower risk of vascular mortality (RR 0.66, 95% CI: 0.58–0.75) and a 36% lower risk of non-vascular mortality (RR 0.64, 95% CI: 0.58–0.72; Figure 2). After adjustment for prior diseases (including self-reported measures of health and frailty), established vascular risk factors, markers of inflammation and renal function, this was reduced to a 20% lower risk of vascular mortality (RR 0.80; 95% CI: 0.70–0.91) and a 23% lower risk of non-vascular mortality (RR 0.77; 95% CI: 0.69–0.86). The substantial change in the χ^2 statistics associated with 25(OH)D concentration with these adjustments (from 41.1 to 11.5 for vascular death and 63.3–21.4 for non-vascular death) suggest that a large part of

Table 2 Cause-specific mortality (annual death rate: % per year), overall and by prior disease

	All men	No prior disease	Prior disease
Number of men	5409	3568	1841
Total follow-up (years)	50 388	35 937	14 452
Cause of death			
IHD	659 (1.3)	297 (0.8)	362 (2.5)
Stroke	378 (0.8)	221 (0.6)	157 (1.1)
Other vascular	321 (0.6)	209 (0.6)	112 (0.8)
Subtotal: any vascular cause	1358 (2.7)	727 (2.0)	631 (4.4)
Cancer	809 (1.6)	460 (1.3)	349 (2.4)
Respiratory	497 (1.0)	321 (0.9)	176 (1.2)
Other non-vascular	551 (1.1)	343 (1.0)	208 (1.4)
Subtotal: any non-vascular cause	1857 (3.7)	1124 (3.1)	733 (5.1)
Total: any cause	3215 (6.4)	1851 (5.2)	1364 (9.4)

IHD: ischaemic heart disease.

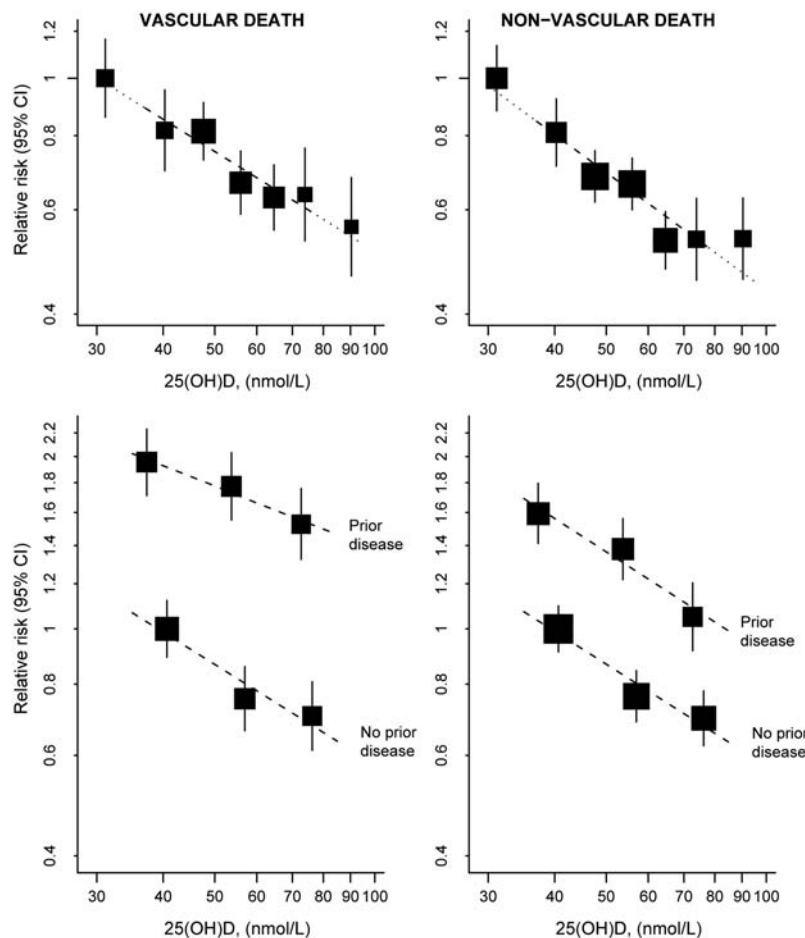


Figure 1 Age-adjusted relevance of measured 25-hydroxyvitamin D for vascular and non-vascular mortality in old age, overall, and separately in men with and without prior disease. Prior disease is defined as cardiovascular disease (recall of a diagnosis of myocardial infarction, angina, or stroke), diabetes, or cancer. In the lower panels, only three risk groups are shown for each disease category to increase the statistical reliability of such subgroup analyses. To convert 25-hydroxyvitamin D from nmol/L to ng/mL, divide by 2.496.

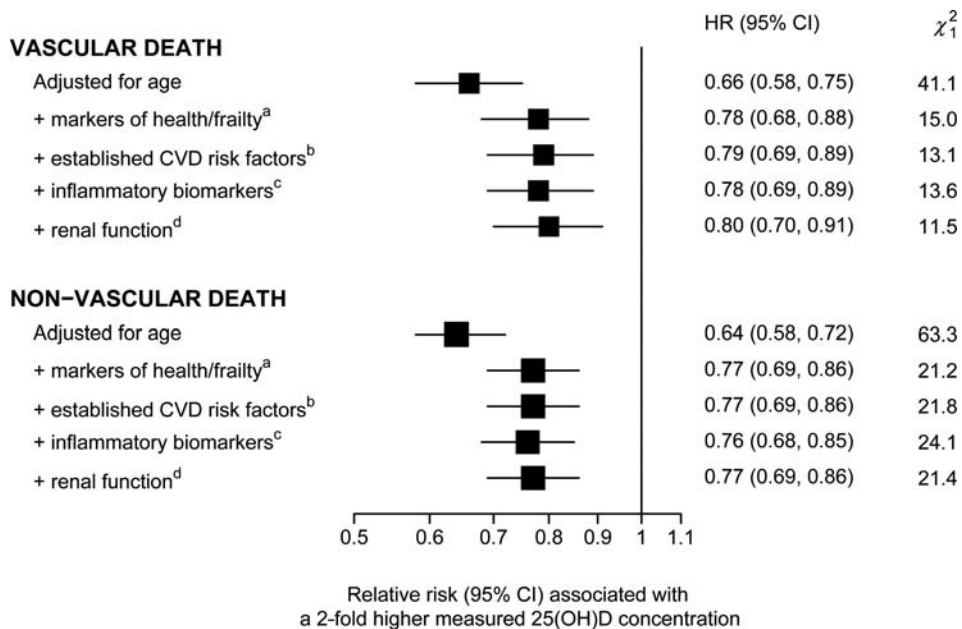


Figure 2 Effect of adjustment for known risk factors on the association between measured 25-hydroxyvitamin D and vascular and non-vascular mortality. (A) Recall of a diagnosis of ischaemic heart disease, stroke, cancer, or diabetes, plus self-reported health/frailty; (B) smoking status (current/ex/never); drinking status (current/non); grade of employment; LDL-C, HDL-C, apolipoprotein A₁, apolipoprotein B, body mass index, and blood pressure [recall (in 1997) of a diagnosis of hypertension, treatment (in 1997) for hypertension and measured systolic and diastolic blood pressure in both 1997 and in ~1970]; (C) albumin, fibrinogen, and C-reactive protein, and (D) estimated glomerular filtration rate.

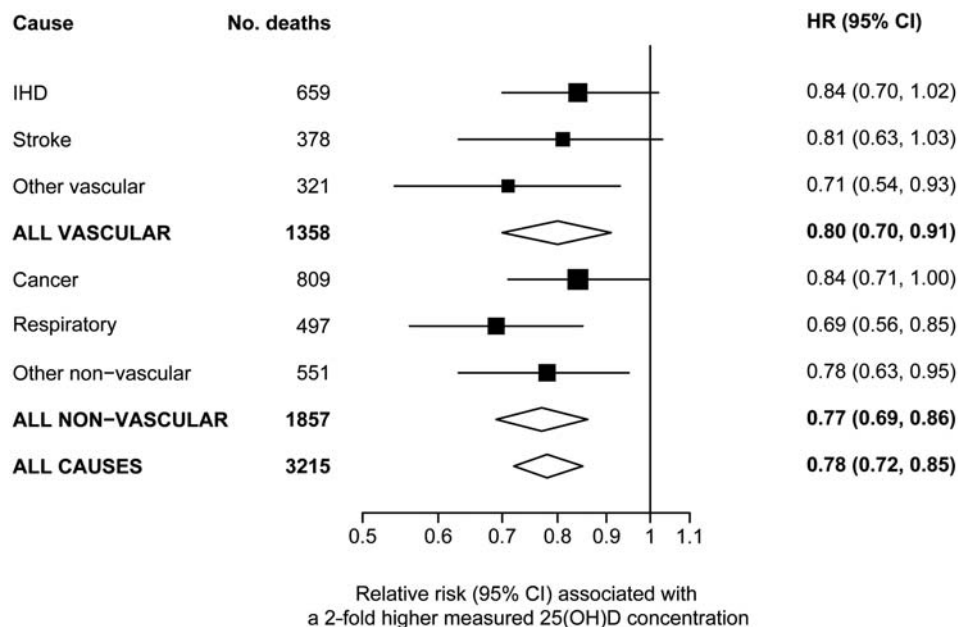


Figure 3 Association between measured 25-hydroxyvitamin D and cause-specific mortality after adjustment for measured confounders. IHD, ischaemic heart disease. Analyses are adjusted for smoking status (current/ex/never), drinking status (current/non), recall of a diagnosis of ischaemic heart disease, stroke, cancer, or diabetes, self-reported health/frailty, employment grade, LDL-C, HDL-C, apolipoprotein A₁, apolipoprotein B, body mass index, markers of inflammation (albumin, fibrinogen, and C-reactive protein), recall (in 1997) of a diagnosis of hypertension, treatment (in 1997) for hypertension and measured systolic and diastolic blood pressure in both 1997 and in ~1970, and estimated glomerular filtration rate.

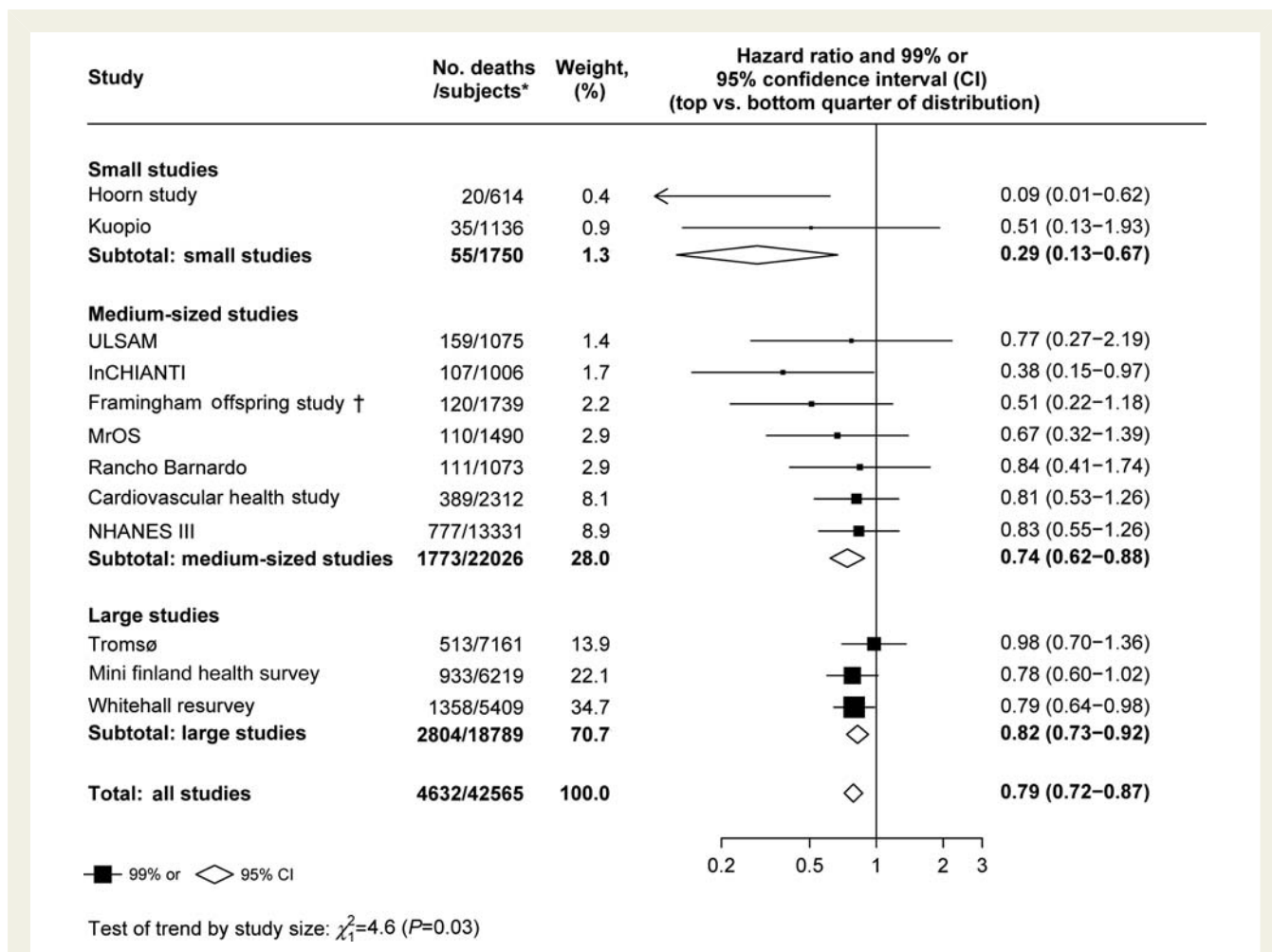


Figure 4 Meta-analysis of the relationship between 25-hydroxyvitamin D concentration and vascular mortality. *Numbers of deaths/people in the whole study are reported for each study, but only half of these deaths would be expected to contribute to analyses of top vs. bottom quarter. †This study included both fatal and non-fatal vascular events.

these associations was due to confounding, principally by prior disease. There was no evidence that the RRs associated with a doubling in baseline 25(OH)D concentration varied during follow-up (P values for test of proportionality assumption: $P = 0.48$ for vascular mortality and $P = 0.13$ for non-vascular mortality). Associations with particular types of vascular and non-vascular death (e.g. IHD, stroke, cancer, and respiratory death), after adjustment for measured confounders, were broadly similar to the overall RRs seen for vascular and non-vascular mortality (Figure 3). The findings for participants with no prior disease at resurvey were similar to those of the overall study population (see Supplementary material online, Figure S3 and S4). Results were also broadly similar after the exclusion of deaths within the first 5 years of follow-up (to further reduce the possible effect of reverse causality; see Supplementary material online, Figure S5) and were similar when the original 25(OH)D concentrations (i.e. before correction for seasonality) were used in analyses instead of season-adjusted concentrations (see Supplementary material online, Figure S6).

Meta-analysis of studies of 25-hydroxyvitamin D and vascular and all-cause mortality

The meta-analyses (which included results from the current study) included 12 prospective studies with 4632 vascular deaths and 18 prospective studies with 11 734 deaths from all causes. Participants with a 25(OH)D concentration in the top vs. bottom quarter of distribution had on average, 21% (95% CI: 13–28%) lower vascular mortality (Figure 4) and 28% (95% CI: 24–32%) lower total mortality (Figure 5). Observed RRs varied inversely with the amount of statistical information provided by each study (i.e. study size), with more extreme estimates being seen among smaller studies for both vascular and all-cause mortality.

Discussion

The Whitehall study, involving 3215 deaths, is one of the largest and longest prospective studies reporting associations of

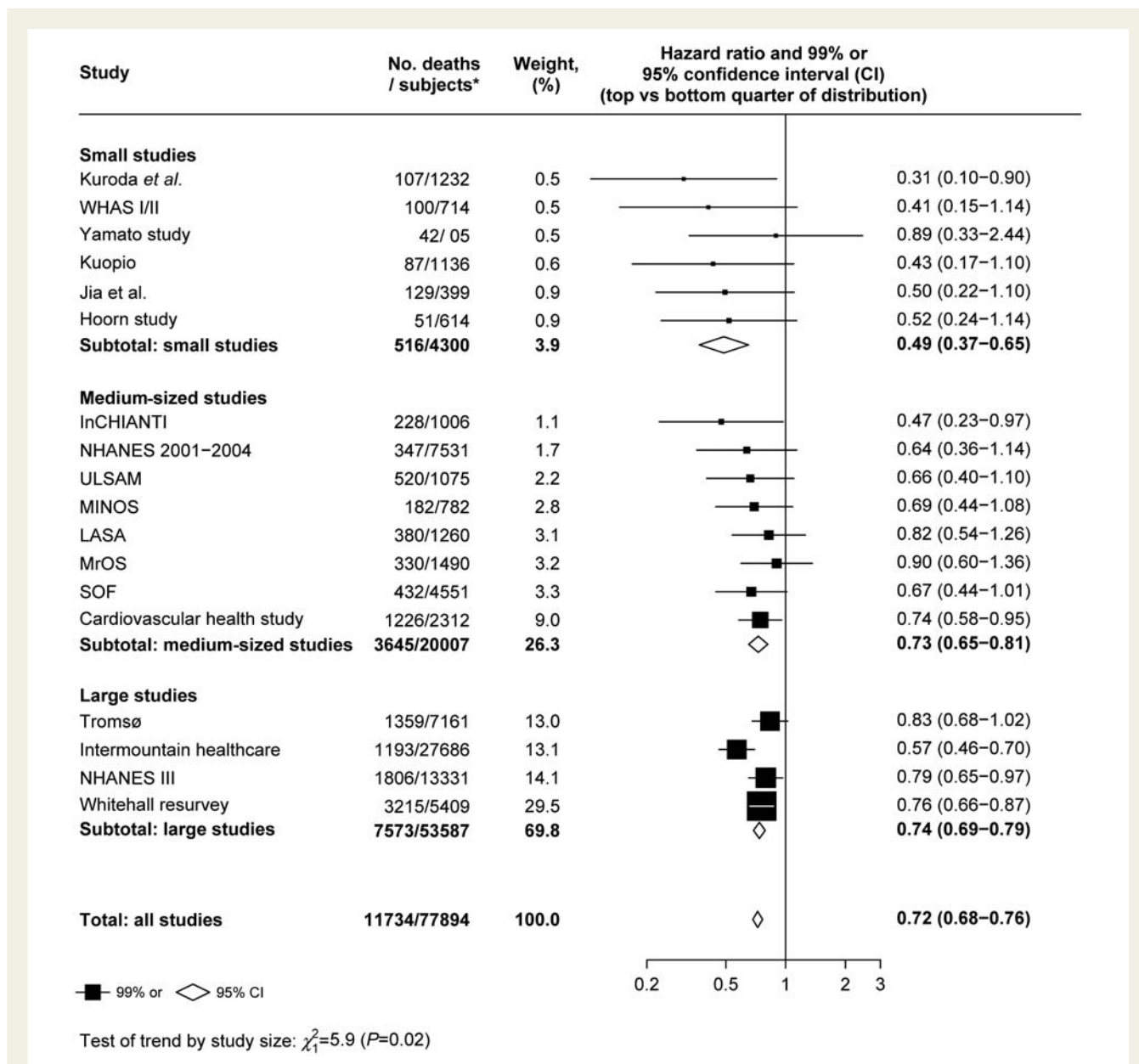


Figure 5 Meta-analysis of the relationship between 25-hydroxyvitamin D concentration and all-cause mortality. *Number of deaths/people in the whole study are reported for each study, but only half of these deaths would be expected to contribute to analyses of top vs. bottom quarter.

25(OH)D with cause-specific mortality, and the most informative study in the meta-analyses. This study showed an approximately linear (on the log–log scale) inverse association of plasma 25(OH)D concentration with both vascular and non-vascular mortality, at least within the range 30–90 nmol/L. After adjustment for age, seasonality, prior disease, markers of health and frailty, and other cardiovascular risk factors, a two-fold higher plasma concentration of 25(OH)D, achievable by supplementation with high doses of vitamin D, was associated with one-fifth lower risk of mortality (20% lower vascular mortality and 23% lower non-vascular mortality). The shape and strength of these associations

were broadly similar among men with and without prior vascular disease, cancer, or diabetes and persisted even after excluding deaths during the first 5 years of follow-up.

In contrast to previous meta-analyses that only reported on associations with all-cause mortality, the present analyses of prospective studies demonstrated a consistent trend in associations of 25(OH)D with vascular and all-cause mortality. Our analyses also showed a significant trend in effect size when the studies were ordered by size consistent with publication bias (where smaller studies are more likely to be published if their findings are strikingly positive than if they are negative or null). With

over 11 700 deaths, the current meta-analysis provides greater statistical precision than the recent meta-analysis involving 5562 deaths.²⁸

The similarities in the shape and strength of the associations of 25(OH)D with vascular and non-vascular causes of death observed in the present study may argue against a causal relationship with cardiovascular disease. It is possible that these associations may reflect incomplete adjustment for known risk factors that were measured imprecisely, or for unknown risk factors that may not have been measured. The possibility that associations could still reflect some reverse causality (despite the exclusion of deaths occurring within 5 years of blood collection in the present study) also cannot be entirely excluded.

Alternatively, the effects of vitamin D on vascular disease may be mediated by mechanisms that are independent of known cardiovascular risk factors. Increased vascular stiffness or vascular calcification may be one such mechanism, given the role vitamin D plays in calcium metabolism.²⁹ Moreover, as vitamin D receptors are found in a wide range of tissues, vitamin D may possibly influence diverse causes of death by some fundamental mechanism that is not yet fully understood. For example, vitamin D is believed to modulate the immune response which could influence deaths from cardiovascular and non-vascular causes, including cancer and infection.³⁰

Among the limitations of this study, data on prior disease and health status at resurvey were self-reported. Hence, while the strength of the associations were attenuated substantially after adjustment for these measures, it is possible that residual confounding by poor health status may persist. Formal assessments of physical activity were not made at baseline, but participants reported their self-rated health and ability to undertake activities of daily living (based on a 15-point questionnaire) and analyses were adjusted for these responses. Any non-response bias (by preferentially excluding frail older people) should have minimized rather than accentuated the effects of reverse causality.

Causes of deaths were supplied by the Office of National Statistics which may also have resulted in some misclassification of the causes of death in this population, which would tend to dilute any real differences between the different causes of death.³¹ Most men in this study were still living in and around the Greater London area. Although not representative of the wider population, this should reduce the likelihood of confounding by possible geographical factors associated with both hours of sunshine and mortality risk. Moreover, the results were broadly consistent with other studies included in the meta-analyses. Since individuals were classified on the basis of a single measurement of plasma 25(OH)D, it is possible that the true strength of the mortality associations observed with long-term average or usual 25(OH)D concentrations may be substantially steeper.³² The correlation between repeated measurements of log 25(OH)D recorded in 187 men over a 1.5 year period was 0.64. Hence, the mortality associations with long-term usual levels of 25(OH)D may be expected to be ~50% more extreme than those classified on the basis of single baseline measures.³²

Although our analyses of the observational studies have included strategies to minimize the effects of confounding and of reverse causality (within the limits of the study design), such studies are

unable to assess the causal relevance of 25(OH)D with vascular or non-vascular mortality. As yet, randomized trials have not been able to confirm or refute a causal role for vitamin D supplementation for either cardiovascular disease or cancer prevention. In a meta-analysis of 18 randomized-controlled trials, involving 57 311 participants, allocation to vitamin D for ~5.7 years was associated with a modest 7% lower overall mortality (RR 0.93, 95% CI: 0.87–0.99).¹⁶ In a recent Cochrane meta-analysis, involving nearly 11 000 deaths, allocation to vitamin D supplements did not significantly reduce mortality (RR 0.97, 95% CI: 0.94–1.00). Similarly, no beneficial effects of vitamin D supplements on risk of coronary heart disease or stroke were reported in the Women's Health Initiative (WHI) trial, in which 36 282 postmenopausal women were randomized to 400 IU vitamin D₃ daily vs. placebo.³³ Furthermore, in the RECORD trial of 5292 older people randomized to 800 IU vitamin D₃ daily vs. placebo, there was no evidence of any beneficial effects on mortality (RR 0.93; 95% CI: 0.85–1.02), or vascular disease (0.91; 95% CI: 0.79–1.05).³⁴

In the Whitehall study, the optimal concentration of 25(OH)D appeared to be ~80–90 nmol/L. While a recent meta-analysis reported an increased risk of mortality with concentrations above 87.5 nmol/L,²⁸ there were too few individuals with 25(OH)D levels greater than this in the present study to have sufficient statistical power to confirm or refute such an association. However, it is likely that larger doses of vitamin D, than those tested in previous trials, will be required to maintain concentrations of 25(OH)D > 80 nmol/L associated with the lowest risk in the observational studies.¹⁹ For example, among men in the lowest fifth of the 25(OH)D concentration in the present study, doses >2000 IU of vitamin D₃ daily may be required to double plasma 25(OH)D concentrations. Large trials are currently assessing whether daily supplementation with 2000–3000 IU of vitamin D₃ can reduce the risk of vascular disease, cancer, and other outcomes,³⁵ but it is unclear if even higher doses of vitamin D may be required to maintain blood concentrations of 25(OH)D > 80 nmol/L throughout the year.

The reported inverse associations of 25(OH)D with higher risks of all-cause mortality, and now with vascular mortality, are of considerable public health interest, because low 25(OH)D concentrations are common in the population and may be easily reversed by supplements. However, the lack of specificity of the associations of 25(OH)D with particular causes of death in the present study casts doubt on the causal relevance of these associations. Large-scale trials using high doses of vitamin D supplements are required to determine whether such observed associations are causal and reversible or have other beneficial effects. Hence, it would be prudent to remain cautious about altering public health strategies to increase population mean levels of 25(OH)D pending the results of these trials.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

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