# RESEARCH

# Vitamin D and risk of cause specific death: systematic review and meta-analysis of observational cohort and randomised intervention studies

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## Abstract

**Objective** To evaluate the extent to which circulating biomarker and supplements of vitamin D are associated with mortality from cardiovascular, cancer, or other conditions, under various circumstances.

**Design** Systematic review and meta-analysis of observational studies and randomised controlled trials.

**Data sources** Medline, Embase, Cochrane Library, and reference lists of relevant studies to August 2013; correspondence with investigators.

**Study selection** Observational cohort studies and randomised controlled trials in adults, which reported associations between vitamin D (measured as circulating 25-hydroxyvitamin D concentration or vitamin D supplement given singly) and cause specific mortality outcomes.

**Data extraction** Data were extracted by two independent investigators, and a consensus was reached with involvement of a third. Study specific relative risks from 73 cohort studies (849 412 participants) and 22 randomised controlled trials (vitamin D given alone versus placebo or no treatment; 30 716 participants) were meta-analysed using random effects models and were grouped by study and population characteristics.

**Results** In the primary prevention observational studies, comparing bottom versus top thirds of baseline circulating 25-hydroxyvitamin D distribution, pooled relative risks were 1.35 (95% confidence interval 1.13 to 1.61) for death from cardiovascular disease, 1.14 (1.01 to 1.29) for death from cancer, 1.30 (1.07 to 1.59) for non-vascular, non-cancer death, and 1.35 (1.22 to 1.49) for all cause mortality. Subgroup analyses in the observational studies indicated that risk of mortality was significantly higher in studies with lower baseline use of vitamin D supplements. In randomised controlled trials, relative risks for all cause mortality were 0.89 (0.80 to 0.99) for vitamin $D_3$ supplementation and 1.04 (0.97 to 1.11) for vitamin $D_2$ supplementation. The effects observed for vitamin $D_3$ supplementation remained unchanged when grouped by various characteristics. However, for vitamin $D_2$ supplementation, increased risks of mortality were observed in studies with lower intervention doses and shorter average intervention periods.

**Conclusions** Evidence from observational studies indicates inverse associations of circulating 25-hydroxyvitamin D with risks of death due to cardiovascular disease, cancer, and other causes. Supplementation with vitamin $D_3$ significantly reduces overall mortality among older adults; however, before any widespread supplementation, further investigations will be required to establish the optimal dose and duration and whether vitamin $D_3$ and $D_2$ have different effects on mortality risk.

## Introduction

Vitamin D is a group of fat soluble vitamins responsible for intestinal absorption of calcium and phosphate.[1] Two major forms of vitamin D exist. Vitamin $D_2$ (ergocalciferol), found in plants, is produced by ultraviolet B irradiation of ergosterol and can be consumed as a supplement or in fortified foods.[2] Vitamin

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$D_3$ (cholecalciferol), on the other hand, a product of ultraviolet B irradiation of 7-dehydrocholesterol, is synthesised in the human epidermis or consumed in the form of natural (for example, fish) or fortified food sources or as a supplement.[2] Supplementation with vitamin D has been shown to benefit skeletal conditions such as rickets, fractures, and falls,[3-5] although a similar effect on bone mineral density was not evident in a recent review of trials.[6] A growing body of evidence indicates that vitamin D may reduce risks of a wide range of diseases including multiple sclerosis, autoimmune disorders, infections, and cardiometabolic and cancer outcomes,[7-12] indicating a possible pleiotropic effect across extraskeletal systems. However, the evidence for vitamin D reducing the risk of non-skeletal diseases is still being debated.[13]

Suboptimal concentrations of vitamin D have also been implicated as a potential determinant of mortality because of its wide ranging anti-inflammatory and immune modulating effects.[2 14 15] However, available observational studies examining this intriguing link are yet to be rigorously reviewed, and the extent to which vitamin D deficiency confers risk of death from cardiovascular disease, cancer, or other conditions remains uncertain. Although several individual reports and reviews have been published on the topic,[16-21] they vary greatly and lack sufficient detail (for example, associations for diverse causes of death or primary versus secondary prevention settings). Additionally, interpretation of the earlier quantitative reviews of randomised trials is difficult,[18 21] as they typically include studies with mixed interventions (for example, combined with calcium intake, which has been associated with cardiovascular risk[22]) and lack detailed assessments to distinguish the effects across important characteristics (such as geographical location, intervention dosage and duration, and follow-up time). A need exists, therefore, for an adequately powered, comprehensive assessment of associations of vitamin D concentrations with the risk of mortality across primary and secondary prevention settings and from a broad range of causes. This is of particular importance because estimates of mortality risk remain a cornerstone in formulating health policies to prevent or reduce premature deaths and improve quality of life, and in this sense vitamin D might play a key role.

In this study, we have aimed to summarise the available observational and interventional evidence in one updated systematic review and meta-analysis to (a) determine the associations of 25-hydroxyvitamin concentrations with the risk of cause specific mortality outcomes in observational cohort studies; (b) quantify the effects of vitamin D supplementation (overall and by subtypes), when given alone compared with placebo or no treatment, on mortality outcomes in the randomised controlled trials; and (c) examine all associations under a wide range of study level characteristics.

## Methods

### Data sources, search strategy, and eligibility criteria

We did this review according to a predefined protocol and in accordance with the PRISMA and MOOSE guidelines (eAppendix 1 and 2).[23 24] Two independent authors, in duplication, sought studies published before 1 August 2013 (date last searched) using Medline, Embase, and Cochrane databases. The computer based searches combined terms related to the exposure (such as vitamin D, 25-hydroxyvitamin D) and outcomes (such as mortality, all cause mortality, death), without any language restriction. Details of the search strategy are provided in eAppendix 3. We sought studies that had reported on associations of circulating vitamin D (measured as 25-hydroxyvitamin D) or vitamin D supplements with all cause mortality (defined as deaths from any causes) or cause specific mortality (defined as deaths due to cardiovascular disease, cancer, and other causes), in which fatal outcomes were registered according to the primary cause (or, in its absence, the underlying cause), on the basis of coding from the international classification of diseases or according to study defined classifications; ascertainment was based on death certificates.

### Study selection

Observational cohort studies were eligible for inclusion if they assessed association of circulating 25-hydroxyvitamin D concentration with cause specific or all cause deaths in adults, and recruited participants from either of the following categories: general populations—that is, participants not selected on the basis of pre-existing chronic disease, including cardiovascular, metabolic, malignant, or renal disorders (that is, primary prevention cohorts); or people with pre-existing baseline conditions mentioned above (that is, secondary prevention cohorts). Intervention studies were eligible if they were randomised; assessed effects of vitamin D supplements singly (that is, randomised controlled trials with a "vitamin D alone" intervention group) in adults compared with a placebo or no treatment; and collected cause specific or all cause mortality endpoints (as defined before). Two independent reviewers worked together to screen the titles and abstracts of all initially identified studies according to the selection criteria. Full texts were retrieved for studies that satisfied all selection criteria. We searched reference lists of selected studies and relevant systematic reviews on the topic for additional publications.

### Data extraction

Two independent authors extracted data, and a consensus was reached with involvement of a third. We used a predesigned data abstraction form to extract relevant information. This included questions on study size, study design, baseline population, location, age at baseline, duration of follow-up, reported degree of adjustment (defined as "+" when relative risks were adjusted for established cardiovascular risk factors such as age, sex, smoking status, lipids, hypertension, history of cardiometabolic disease; "++" when adjusted for other potential risk factors such as physical activity, body mass index, social status; and "+++" when additionally adjusted for other additional variables such as bone minerals), type and numbers of mortality outcomes, and reported relative risks. Where appropriate, we extracted information on subtypes of vitamin D supplement, number of participants in the supplement and control groups, baseline circulating vitamin D concentration, assay method, blinding status, and composition of supplement or placebo. If risk estimates were unavailable from a published report, we collected relevant data by corresponding with the authors,[25-27] abstracting from other published reviews,[19 21 28] or hand calculating on the basis of the available information from the paper,[29-31] where appropriate. Additionally, in the case of multiple publications, we included the most up to date or comprehensive information.[32 33]

### Assessment of risk of bias

For observational cohort studies, we used the Newcastle-Ottawa Scale to assess the risk of bias.[34] This scale uses a star system (with a maximum of nine stars) to evaluate a study in three domains: selection of participants, comparability of study groups, and the ascertainment of outcomes of interest. We

judged studies that received a score of nine stars to be at low risk of bias, studies that scored seven or eight stars to be at medium risk, and those that scored six or less to be at high risk of bias. Similarly, for the randomised trials, we used the Cochrane Collaboration's tool for assessing the risk of bias.[35] This tool evaluates seven possible sources of bias: random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting, and other bias. For each individual domain, we classified studies into low, unclear, and high risk of bias.

## Data synthesis and analysis

To enable a consistent approach to meta-analysis and interpretation of findings in this review, we used previously described methods to transform relative risk estimates for association of circulating 25-hydroxyvitamin D and mortality outcomes,[36] which were often differently reported by each observational cohort study (for example, per unit change, per one standard deviation change, or comparing fifths, quarters, thirds, and other groupings), to consistently correspond to comparison of the bottom versus the top third of the baseline distribution of 25-hydroxyvitamin D concentrations in each study. Briefly, we transformed log risk ratios by assuming a normal distribution, with the comparison between extreme thirds being equivalent to 2.18 times the log risk ratio for one standard deviation increase (or equivalently as 2.18/2.54 times the log risk ratio for a comparison of extreme quarters). We calculated standard errors of the log risk ratios by using published confidence limits and standardised them in the same way. We assumed hazard ratios and odds ratios to approximate the same measure of risk ratios. We combined study specific risk ratios by using a random effects model that included between study heterogeneity (and additionally using fixed effect models). Where studies reported risk ratios with varying degrees of adjustments, we used the maximally adjusted estimate. We based subsidiary assessments involving circulating 25-hydroxyvitamin D cut-offs (defined as 21-29, 10-20, and <10 ng/mL)[2 37 38] compared with the reference category (≥30 ng/mL) on combining comparable risk ratio estimates across studies, using random effects meta-analyses (and additionally using fixed effect models).[39 40]

For randomised intervention trials we used reported adjusted risk ratios, or calculated study specific unadjusted risk ratios based on event rates if these were unavailable, for overall vitamin D supplementation (and individually by supplements of vitamins $D_3$ and $D_2$ subtypes). We calculated summary risk ratios by pooling the study specific estimates with a random effects model that included between study heterogeneity (parallel analyses used fixed effect models). We assessed consistency of findings across studies with standard $\chi^2$ tests and the $I^2$ statistic.[41] We used random effects meta-regression to quantify heterogeneity by comparing results from studies grouped according to study level characteristics. Additionally, we did univariate meta-regression analyses to investigate the effect of study level characteristics such as daily intervention dose of supplement and duration of intervention or follow-up on the size of the effect estimates for both supplementation trials and observational cohort studies. We used the natural logarithm of the risk ratio as the dependent variable and the study level characteristic as the explanatory factor. We assessed evidence of publication bias by using funnel plots and the Egger test.[42] We calculated the population attributable risk with the following equation: $PAR\% = 100 \times Pe(RR-1)/(Pe(RR-1)+1)$,[43] where Pe is the prevalence of the exposure (eAppendix 5). All statistical tests were two sided and used a significance level of P<0.05. We used Stata release-12 for all statistical analyses.

## Results

The search strategy identified 2704 unique citations. After initial screening based on titles and abstracts, 320 articles remained for further evaluation. Of these articles, 225 were excluded in the subsequent detailed assessments for reasons shown in eFigure 1. The remaining 95 unique study reports met our inclusion criteria and were included in the meta-analysis (eAppendix 6). In aggregate, these included studies comprised 880 128 unique participants and 71 625 mortality outcomes (including 10 777 deaths from cardiovascular disease and 6911 deaths from cancer) (table⇓; eTables 1-3).

## Association of circulating 25-hydroxyvitamin D concentration with cause specific mortality

Circulating 25-hydroxyvitamin D concentration in relation to subsequent risk of death was reported in 73 observational cohort studies, involving 849 412 participants and 66 511 mortality events recorded during an average follow-up ranging from 0.3 to 29 years (table⇓; eTable 1). Of these observational cohort studies, 38 involved participants from Europe, 26 from North America, 8 from the Asia-Pacific region, and 1 from South America. The median age of all included participants was 63 (interquartile range 59-71) years. We judged eight studies to be at low risk of bias, 41 to be at medium risk, and 24 studies to be at high risk of bias (eTable 1). Of the medium quality studies, all showed a potential bias in the selection of participants. The median baseline concentration of 25-hydroxyvitamin D in these studies was 20.7 (interquartile range 17.5-24.3) ng/mL. For the primary prevention cohorts, pooled risk ratios in comparisons of people in the bottom versus top thirds of the population distribution of baseline circulating 25-hydroxyvitamin D concentration, adjusted for several potential risk factors, were 1.35 (95% confidence interval 1.13 to 1.61) for death from cardiovascular disease (6416 events), 1.14 (1.01 to 1.29) for death from cancer (5003 events), 1.30 (1.07 to 1.59) for other non-vascular, non-cancer death (1444 events), and 1.35 (1.22 to 1.49) for all cause mortality (48 488 events) (fig 1⇓ and eFigure 2). The corresponding pooled risk ratios were broadly similar in the secondary prevention cohorts. Additional analyses by various cut-off values of circulating 25-hydroxyvitamin D concentration showed a significant inverse association with all cause mortality (P<0.05; fig 2⇓). Assuming linearity, each 10 ng/mL decline of 25-hydroxyvitamin D concentration was associated with a 16% (95% confidence interval 8% to 23%) increased risk of all cause mortality (fig 2⇓).

In subsidiary analyses, we found significant inverse associations for various cause specific mortality outcomes, including deaths due to coronary disease, lymphoma, upper digestive tract cancer, and respiratory diseases (eFigure 3). We observed a moderate level of heterogeneity in observational studies, which was only partially explained by between study differences in various subgroups (figures 3⇓, 4⇓, and 5⇓). Specifically, the risk of all mortality outcomes for low baseline circulating 25-hydroxyvitamin D concentration was significantly higher in studies in which less than 10% of the population used vitamin D supplements (P for meta-regression<0.05; figures 3⇓, 4⇓, and 5⇓). Additionally, risk of all cause mortality in participants with low 25-hydroxyvitamin D concentrations was significantly higher in studies with less than five years' average follow-up (P for meta-regression=0.001; fig 5⇓). The overall associations observed, however, were similar across other subgroups such

as latitude of study location, sex, study quality, levels of multivariate adjustment, assay methods, adjustments for seasonality or socioeconomic status, and geographical location (figures 3⇓, 4⇓, and 5⇓ and eFigure 4). Results from subsidiary univariate meta-regression analyses showed no evidence of associations of the duration of follow-up with risk of death from cardiovascular disease, death from cancer, other non-vascular, non-cancer death, and all cause mortality (P>0.05 for all) (eFigure 5).

## Effects of vitamin D supplementation on all cause mortality

Twenty two randomised controlled trials reported effects of vitamin D supplementation in isolation on mortality outcomes, including a total of 30 716 participants in the supplement and control groups combined (table⇓; eTable 3). Fourteen of these trials assessed the effect of vitamin $D_3$, and eight reported effects of vitamin $D_2$. Thirteen trials involved participants from Europe, five from North America, and four from the Asia-Pacific region. The average age of participants included in these trials ranged from 56 to 85 years. Eleven trials included participants from community based registers (six from the general population and five from care or residential homes), and the rest recruited participants from clinical registers. The risk of bias assessment in each trial is reported in eAppendix 4. Most of the trials had a low risk of bias for random sequence generation, allocation concealment, participants' blinding, and selective reporting. Seven trials had a high risk of bias for blinding of outcome assessment, and eight had a high bias in outcome data completion. Among the vitamin $D_3$ studies, participants in the intervention arm received vitamin $D_3$ supplementation ranging from 10 to 6000 IU/day, and oral tablets were the principle form of supplementation. The corresponding range was 208 to 4500 IU/day for vitamin $D_2$. After an average follow-up ranging from 0.38 to 6.8 years, a total of 2527 all cause mortality events occurred among participants in the intervention group compared with 2587 events in the control group, with a combined risk ratio of 0.98 (0.94 to 1.02) in all studies. The corresponding risk ratios according to type of vitamin D supplementation was 0.89 (0.80 to 0.99) for vitamin $D_3$ and 1.04 (0.97 to 1.11) for vitamin $D_2$ (fig 6⇓).

We found no evidence of heterogeneity across vitamin $D_3$ (eFigure 6a; P for heterogeneity=0.34) or vitamin $D_2$ trials (eFigure 6b; P for heterogeneity=0.38). For vitamin $D_3$ the overall effect did not vary significantly across location, sex, population source, daily dose, and duration of intervention or follow-up (P for metaregression>0.05; fig 7⇓). The effects, however, differed importantly for vitamin $D_2$ supplementation, for which we observed an increased risk of mortality in the randomised controlled trials that used an intervention dose of 600 to 2000 IU/day, had shorter average intervention period (<1.5 *v* ≥1.5 years), and had high risk of bias (P for metaregression≤0.05 for all; fig 7⇓). Similarly, results from univariate meta-regression analysis showed no evidence of an association of daily intervention dose or average intervention period with treatment effect for vitamin $D_3$ supplementation (P=0.47 and 0.50, respectively). The evidence did, however, suggest associations of daily intervention dose and average intervention period with the treatment effect for vitamin $D_2$ supplementation, although these were not statistically significant (P=0.06 and 0.07, respectively) (eFigure 7). We had insufficient data to meaningfully combine the effects of vitamin D supplementation alone on cause specific mortality outcomes. We found no evidence of publication bias across all included

studies in this review (P for Egger's asymmetry>0.05 for all) (eFigure 8).

## Prevalence of vitamin D deficiency and estimated absolute risk

In supplementary analyses, based on data from available cohorts, the prevalence of vitamin D insufficiency (defined as 25-hydroxyvitamin D concentration <30 ng/mL) was 69.5% (95% confidence interval 62.1% to 77.7%) for the United States and 86.4% (78.4% to 95.2%) for Europe. Furthermore, using 25-hydroxyvitamin D concentrations less than 10 ng/mL as the criterion, 4% and 15% of the general population were severely deficient in the Europe and United States, respectively (eFigure 9). Additionally, using the most recent mortality statistics for the United States and Europe,[44 45] the estimated absolute risk differences for all cause mortality associated with vitamin D deficiency were 75.4 events in Europe and 96.6 events in the United States, per 100 000 population, per year (eAppendix 5). Using the population prevalence estimates of vitamin D deficiency from this study, 9.4% of all deaths in Europe and 12.8% of those in the United States could be attributed to vitamin D deficiency.

## Discussion

The findings of this review indicate that a moderate, but significant, inverse association exists between circulating vitamin D concentrations and the risk of all cause mortality in the primary prevention cohort studies. The inverse association was evident generally for all broad causes of death and more specifically for deaths due to coronary disease, lymphoma, upper digestive cancer, and respiratory disorders. In all randomised controlled trials combined, vitamin D supplementation, when given alone, did not reduce overall mortality significantly among older adults. However, when stratified by type of supplementation, vitamin $D_3$, given singly, reduced all cause mortality significantly by 11%. By contrast, supplementation with vitamin $D_2$ alone had no overall effect on mortality.

## Possible explanations for findings

The inverse association between vitamin D and mortality can be explained by several different mechanisms. Firstly, activated vitamin D may influence a range of biological responses involved in cellular growth, proliferation, and apoptosis and immune system functions.[2 8] Vitamin D receptors and the enzyme required for its activation are present in most human cells and tissues, indicating a major role for vitamin D in "non-skeletal" physiological processes. Secondly, approximately 3000 binding sites for the vitamin D receptor have been found throughout the human genome,[46] indicating regulation of a very large number of genes (estimated to be about 3% of the human genome[2]) either directly or indirectly responsive to vitamin D receptors. This, along with the potential adverse consequences of low 25-hydroxyvitamin D concentrations, such as coronary heart disease, cancer, and death,[47] found in people with 25-hydroxyvitamin D related genetic variants, reinforces the importance of an endocrine system beyond extracellular calcium and phosphate homeostasis.[46] Thirdly, the positive association between vitamin D concentrations and longer leucocyte telomere length, a potential determinant of age related disorders and overall longevity,[48] emphasises the possible beneficial effects of vitamin D on healthy ageing and associated outcomes. Fourthly, as primary causes of vitamin D deficiency include insufficient exposure to sunlight, poor diet, increased adiposity, and reduced synthesis or absorption,[49] a poor vitamin D status

might essentially reflect suboptimal lifestyle and socioeconomic circumstances. These individual level factors may, in turn, influence risk for their potential roles on several established determinants of morbidity and mortality such as smoking, blood pressure, body mass index, and use of supplements.[50] Although most of the studies included in this review controlled for these characteristics, and our pooled estimates were largely unchanged when they were further stratified by adjustment for standard socioeconomic factors, potential residual and unmeasured confounding by differences in diet, lifestyle, and socioeconomic status remains a concern. Such unaccounted confounding could partly explain the discrepancy of findings observed earlier between observational and interventional studies of other dietary factors.[51] Finally, our study indicates that vitamin D is inversely and moderately associated with risk of death from coronary disease, lymphoma, cancers of the upper digestive tract, and respiratory disease. Although these associations require better characterisation in future larger studies, local expression of vitamin D receptors and systemic immunomodulatory roles of vitamin D have been proposed to explain them.[52-55]

Subgroup analyses among observational studies indicated that the inverse associations of circulating 25-hydroxyvitamin D concentration with all cause and cancer specific mortality were significantly stronger in the populations with a low prevalence of vitamin D supplement use. This suggests that the effect of vitamin D may be dependent on baseline vitamin D status. Given that baseline circulating 25-hydroxyvitamin D concentrations in a population with low prevalent vitamin D supplement use is likely to be low,[56] and that the risk of mortality outcomes is known to be greater at the lower concentrations of 25-hydroxyvitamin D,[27] these findings are not unexpected. In addition, a previous study has suggested a threshold effect in 25-hydroxyvitamin D concentrations up to 112 nmol/L, which can be achieved by daily use of 600 IU of vitamin $D_3$.[57] Additionally, we found a significantly higher mortality risk of low 25-hydroxyvitamin D concentrations in studies with a follow-up of less than five years. This may be attributed to reverse causality, in which people have underlying diseases that are associated with low 25-hydroxyvitamin D concentrations, such as cardiometabolic diseases.

Our meta-analysis of all available randomised controlled trials of vitamin D supplements, given singly among principally older adults, suggests that this nutrient may not significantly reduce mortality outcomes. However, when we considered the effects of specific vitamin D metabolites, supplementation in the form of vitamin $D_3$ (animal derived, known as cholecalciferol) but not vitamin $D_2$ (plant based, known as ergocalciferol) was associated with reduced mortality. Earlier evidence described ergocalciferol as being potentially less potent, unit for unit, in maintaining 25-hydroxyvitamin D concentrations in the circulation,[58] so the expected effect of vitamin $D_3$ on mortality could be greater. Additionally, previous reviews reported that in the absence of concomitant use of calcium supplements, compared with vitamin $D_3$, vitamin $D_2$ was associated with a significantly lower overall increase in serum 25-hydroxyvitamin D concentration.[59 60] Interestingly, concomitant use of vitamin D with calcium at baseline was associated with lower increases in 25-hydroxyvitamin D concentrations.[59] Subgroup analyses showed that vitamin $D_2$ supplementation increased the aggregate risk of mortality in trials that had shorter average intervention periods. Similar higher risks were reported for trials using lower intervention doses. The discrepant findings in our meta-analysis could also be explained by insufficient power (average follow-up duration in the vitamin $D_2$ trials was about a year less than for $D_3$ trials) or, importantly, factors other than supplements

themselves in these studies (such as diversity in population characteristics); therefore, further randomised controlled trials are needed to reinforce these findings.

## Comparison with previous work

Findings of this updated meta-analysis generally concur with and further extend the previous reviews in several important ways. Firstly, this study had enhanced power to examine the associations in greater detail. For example, our meta-analysis of the primary prevention cohort studies involved about 10 times as many participants and three times as many mortality outcomes as previous reviews on this topic combined,[10 19 20] and included about 10 recent, large scale observational cohort studies. Secondly, in contrast to the earlier reviews, we have done a systematic synthesis of all available primary and secondary prevention cohorts to quantify the risk of both composite and various cause specific death outcomes in a single comprehensive investigation. Thirdly, we have analysed and presented standardised pooled risk estimates comparing extreme thirds of baseline distribution of vitamin D, and by pre-specified vitamin D cut-offs. Fourthly, unlike previous reviews that included all randomised studies with mixed interventions,[18 21] our most up to date meta-analysis of randomised controlled trials included exclusively the studies that administered vitamin D alone. Finally, we did detailed analyses under a broader range of individual and study level circumstances to explore the potential sources of heterogeneity. Nevertheless, the findings from our trial component are consistent with the earlier meta-analysis (based on randomised controlled trials irrespective of concomitant supplementation with calcium) that also reported heterogeneity in efficacy between the two forms of supplement.[18]

## Implications of findings

Our findings may have several implications. They underscore a potentially deleterious role of low vitamin D in all cause and cause specific mortality in both primary and secondary prevention cohorts. Additionally, a beneficial effect was observed for supplementation with vitamin $D_3$ in the randomised controlled trials. This is of significant public health importance, as the gradual decline in circulating 25-hydroxyvitamin D concentrations reported globally is likely to continue owing to the increase in the proportion of older populations, obesity, and lack of adequate sun exposure combined with sunscreen use.[61] Our review further highlights existing scientific gaps in the trial evidence and, therefore, stimulates future research.[62] For instance, available intervention studies were generally insufficiently powered to reliably assess the optimum dosage, not able to examine potential toxic effects over prolonged use, and unable to reliably assess the efficacy in low risk general populations as most of the included community based studies involved solely older participants. Finally, compared with other conventional risk factors of ill health, the estimated population attributable risk of death due to suboptimal vitamin D in our study seems to be substantial. For example, in the United States, we estimated the population attributable risk due to vitamin D deficiency to be about 13%. The corresponding estimates in the United States were about 20% for smoking,[63] about 11% for physical inactivity,[64] and about 9% for alcohol consumption.[65]. This reinforces the potential importance of scalable, cost effective public health strategies (such as moderate sun exposure, supplementation, and food fortification) in improving the overall vitamin D status to reduce premature deaths worldwide.

## Strengths and limitations of study

The generalisability of our findings has been enhanced by the involvement of data from almost 900 000 participants in 26 nations. We used standardised estimates to allow consistent comparisons and examined a wide range of characteristics. However, the review was limited by the moderate amount of available data on several cause specific mortality outcomes. For example, even in aggregate, fewer than 1000 site specific cancer deaths were generally recorded in the observational cohort studies. Observational data also provide limited clarity on whether observed associations with mortality outcomes are direct (that is, due to suboptimal vitamin D) or indirect (due to shared determinants such as obesity, body composition, or social status). Furthermore, as all included observational studies lacked serial assessment of circulating 25-hydroxyvitamin D concentration in the same individuals, reliable assessment of the extent of any within person variability in circulating 25-hydroxyvitamin D concentration was not possible. Because most characteristics of epidemiological studies are measured with a degree of error and are subject to fluctuations within individuals over time, correction of such variability in future studies would help to avoid "regression dilution."[66 67] Although the observational studies are unable to assess the causal association, evidence from the intervention studies could provide concluding evidence in this respect. However, such trials are generally sparse, include chiefly older people (that is, a population with a high competing risk of death due to comorbidities[68]), and do not typically present data on cause specific deaths as the primary outcomes of interest. Furthermore, although most of the trials included in this review seem to have a low risk of bias, our findings should be interpreted with some caution, owing to the relatively small number of trials for each intervention subtype, especially for primary prevention. Therefore, our findings intensify the need for detailed future intervention studies that involve free living general populations, quantify efficacy in important subgroups such as non-white ethnicities, are adequately powered and sufficiently prolonged to help judge appropriate dosage and safety, aim to ascertain a broader range of fatal and non-fatal outcomes than has been customary in the randomised controlled trials thus far, and study both vitamin $D_2$ and $D_3$ to identify which form of vitamin D supplementation can be most efficient and safe.

## Conclusions

Evidence from observational studies indicates inverse associations of circulating 25-hydroxyvitamin D concentration with risks of death from cardiovascular disease, death from cancer, and non-vascular, non-cancer death. Supplementation with vitamin $D_3$ reduced overall mortality significantly among older adults; however, before any widespread supplementation, further studies will be required to determine the optimal dose and duration and to reliably establish whether vitamin $D_3$ affects the mortality risk differently than vitamin $D_2$.

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**What is already known on this topic**

Vitamin D may be associated with many extraskeletal disease conditions, including overall mortality outcomes

However, associations of vitamin D concentrations with risk of death from a broad range of causes, under different circumstances, and across primary and secondary prevention settings, remain less well understood

**What this study adds**

Updated meta-analyses of observational studies indicate inverse associations of circulating vitamin D concentrations with risks of deaths from cardiovascular disease, cancer, and other causes

Combined data from all relevant randomised intervention studies show that, when given alone, vitamin D supplementation may not reduce overall mortality significantly among older adults

When data were stratified by type of supplementation, vitamin $D_3$ given singly, reduced mortality significantly by 11%

By contrast, supplementation with vitamin $D_2$ alone had no overall effect on mortality

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# Table

**Table 1| Summary characteristics of included studies. Values are number of studies (number of participants) unless stated otherwise**

|  | Observational cohort studies | Intervention studies |
|---|---|---|
| **Eligible studies** | | |
| No of unique studies | 73 | 22 |
| Median (IQR) follow-up (years) | 6.0 (3.0-9.5) | 1.4 (0.5-3.0) |
| **Participants** | | |
| Total No of participants | 849 412 | 30 716 |
| Median (IQR) No of participants | 1073 (510-2429) | 343 (124-2578) |
| Total No of deaths | 66 511 | 5114 |
| Median (IQR) No of deaths | 224 (106-633) | 22.5 (7-471) |
| Median (IQR) % male | 51 (35-62) | — |
| Median (IQR) age (years) | 63 (59-71) | 77 (56-85) |
| **Baseline population** | | |
| Not selected on basis of prior chronic disease | 29 (788 282) | 9 (24 828) |
| With pre-existing chronic disease | 44 (61 130) | 13 (9195) |
| **Location** | | |
| Europe | 38 (330 573) | 13 (25 789) |
| North America | 26 (90 342) | 5 (1939) |
| Asia-Pacific | 8 (427 515) | 4 (2988) |
| South America | 1 (982) | — |
| **Sample type** | | |
| Serum | 57 (822 340) | — |
| Plasma | 16 (27 072) | — |
| **Assay method** | | |
| Radioimmunoassay | 34 (61 013) | — |
| Automated immunoassays | 19 (753 285) | — |
| Chromatographic methods | 20 (35 114) | — |
| **25-hydroxyvitamin D concentration** | | |
| Median (IQR) pooled concentration at baseline (ng/mL) | 20.7 (17.5-24.3) | 15.2 (10.4-21.3) |
| **Outcome—No of studies (No of events)\*** | | |
| All cause mortality | 68 (64 636) | 22 (5114) |
| Cardiovascular mortality | 29 (10 203) | 3 (574) |
| Cancer mortality | 17 (6620) | 2 (291) |
| Other non-vascular, non-cancer mortality | 10 (2565) | — |

IQR=interquartile range.

\*Several studies provided data on multiple outcomes of interest.

# Figures

| | No of studies | No of participants | No of deaths | Relative risk (95% CI)* for cause specific mortality | Relative risk (95% CI)* for cause specific mortality |
|---|---|---|---|---|---|
| **Cardiovascular death** | | | | | |
| Primary prevention cohorts | 19 | 80 662 | 6416 | | 1.35 (1.13 to 1.61) |
| Secondary prevention cohorts | 10 | 20 987 | 3787 | | 1.60 (1.32 to 1.94) |
| All cohorts | 29 | 101 649 | 10 203 | | 1.43 (1.25 to 1.64) |
| **Cancer death** | | | | | |
| Primary prevention cohorts | 12 | 104 353 | 5003 | | 1.14 (1.01 to 1.29) |
| Secondary prevention cohorts | 5 | 16 382 | 1617 | | 1.59 (1.17 to 2.16) |
| All cohorts | 17 | 120 735 | 6620 | | 1.25 (1.10 to 1.43) |
| **Non-cardiovascular, non-cancer death** | | | | | |
| Primary prevention cohorts | 7 | 38 526 | 1444 | | 1.30 (1.07 to 1.59) |
| Secondary prevention cohorts | 3 | 13 035 | 1121 | | 1.49 (0.94 to 2.35) |
| All cohorts | 10 | 51 561 | 2565 | | 1.34 (1.13 to 1.60) |
| **All cause mortality** | | | | | |
| Primary prevention cohorts | 27 | 780 990 | 48 488 | | 1.35 (1.22 to 1.49) |
| Secondary prevention cohorts | 41 | 59 918 | 16 148 | | 1.50 (1.36 to 1.65) |
| All cohorts | 68 | 840 908 | 64 636 | | 1.44 (1.34 to 1.55) |

0.5    1    2.5

Relative risk (95% CI) for bottom versus top thirds
of baseline 25-hydroxyvitamin D concentration

**Fig 1** Association of circulating 25-hydroxyvitamin D concentrations with cause specific mortality in observational cohort studies. *Pooled estimates are based on random effects meta-analysis. Using fixed effects models, for primary prevention cohorts, secondary prevention cohorts, and all cohorts, the estimates were 1.40 (1.32 to 1.47), 1.50 (1.35 to 1.66), and 1.42 (1.35 to 1.49) for cardiovascular deaths; 1.10 (1.02 to 1.17), 1.45 (1.28 to 1.65), and 1.16 (1.10 to 1.24) for cancer deaths; 1.28 (1.12 to 1.47), 1.38 (1.09 to 1.75), and 1.30 (1.16 to 1.47) for non-vascular, non-cancer deaths; and 1.45 (1.41 to 1.49), 1.49 (1.42 to 1.56), and 1.44 (1.40 to 1.47) for all cause deaths. Size of data marker is proportional to inverse of variance of relative risk; horizontal line represents 95% CI. Corresponding forest plots and $I^2$ (95% CI) estimates are provided in supplementary material

| | No of studies | No of participants | No of deaths | Relative risk (95% CI) | Relative risk (95% CI) |
|---|---|---|---|---|---|
| **Pre-specified laboratory cut-offs (ng/mL)*** | | | | | |
| 21-29 v ≥30 | 14 | 500 732 | 27 093 | | 1.07 (1.01 to 1.15) |
| 10-20 v ≥30 | 12 | 457 801 | 22 997 | | 1.20 (1.12 to 1.27) |
| ‹10 v ≥30 | 11 | 457 262 | 23 993 | | 1.50 (1.21 to 1.87) |
| **Dose-response assessment** | | | | | |
| Each 10 ng/mL decrease | 18 | 480 579 | 29 345 | | 1.16 (1.08 to 1.23) |

0.75  1  1.25 1.5  2

Relative risk (95% CI)
of all cause death
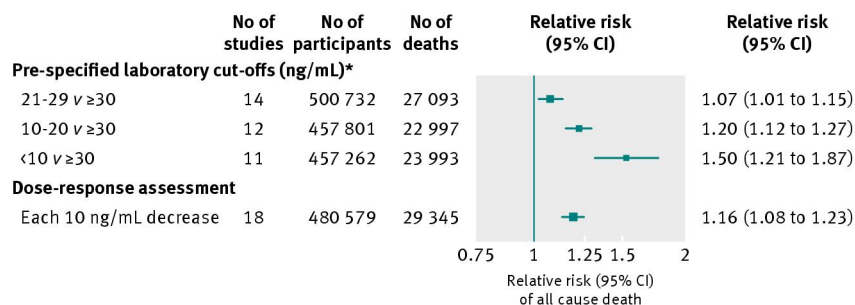
**Fig 2** Association of circulating 25-hydroxyvitamin D concentrations with all cause mortality, based on primary prevention cohorts. *Indirect comparisons based on available studies with relevant information in each category; summary estimates presented were calculated using random effects models. Using fixed effects models, the estimates were 1.09 (1.06 to 1.11) for clinical cut-off of 21-29 v ≥30, 1.20 (1.15 to 1.26) for 10-20 v ≥30, 1.23 (1.20 to 1.26) for <10 v ≥30, and 1.19 (1.18 to 1.21) per 10 ng/mL decrease

| Subgroups | No of studies | No of participants | No of deaths | Relative risk (95% CI) | Relative risk (95% CI) |
|---|---|---|---|---|---|
| **Latitude of study location** | | | | | |
| >40° N, <40° S | 10 | 47 695 | 3917 | | 1.41 (1.07 to 1.86) |
| Between 40° N and 40° S | 9 | 32 967 | 2499 | | 1.29 (1.09 to 1.53) |
| **Average age of participants** | | | | | |
| <70 years | 12 | 69 478 | 5217 | | 1.28 (1.09 to 1.51) |
| ≥70 years | 7 | 11 184 | 1199 | | 1.41 (1.01 to 1.96) |
| **Sex** | | | | | |
| Male | 2 | 2684 | 306 | | 1.55 (1.05 to 2.27) |
| Female | 2 | 3900 | 142 | | 1.15 (0.81 to 1.63) |
| Both | 15 | 74 078 | 5968 | | 1.36 (1.11 to 1.67) |
| **Baseline vitamin D supplement use†** | | | | | |
| ≥10% | 4 | 18 187 | 1340 | | 1.12 (0.94 to 1.32) ⎤ |
| None or <10% | 1 | 1194 | 196 | | 1.70 (0.96 to 3.00) ⎦ * |
| **Sample type** | | | | | |
| Plasma | 2 | 11 364 | 1718 | | 1.41 (1.24 to 1.61) |
| Serum | 17 | 69 298 | 4698 | | 1.34 (1.08 to 1.64) |
| **Assay method** | | | | | |
| Radioimmunoassay | 9 | 36 340 | 2481 | | 1.47 (1.11 to 1.95) |
| Automated assay | 5 | 29 044 | 3027 | | 1.21 (0.93 to 1.58) |
| Chromatographic plus CBPA | 5 | 15 278 | 908 | | 1.22 (0.99 to 1.51) |
| **Average follow-up** | | | | | |
| ≥5 years | 17 | 72 069 | 5806 | | 1.31 (1.09 to 1.59) |
| <5 years | 2 | 8593 | 610 | | 1.70 (0.90 to 3.22) |
| **No of events ascertained** | | | | | |
| ≥500 | 5 | 37 943 | 4276 | | 1.31 (1.09 to 1.58) |
| <500 | 14 | 42 719 | 2140 | | 1.36 (1.04 to 1.77) |
| **Level of adjustment** | | | | | |
| +++ | 9 | 46 923 | 3988 | | 1.26 (1.04 to 1.54) |
| ++/+ | 10 | 33 739 | 2428 | | 1.40 (1.07 to 1.83) |
| **Controlled for seasonality** | | | | | |
| Yes | 15 | 68 987 | 6038 | | 1.31 (1.08 to 1.61) |
| No | 4 | 11 675 | 378 | | 1.53 (0.95 to 2.49) |
| **Adjusted for socioeconomic status** | | | | | |
| Yes | 12 | 56 643 | 3908 | | 1.40 (1.09 to 1.80) |
| No | 7 | 24 019 | 2528 | | 1.26 (0.97 to 1.63) |
| **Risk of bias score‡** | | | | | |
| 9 | 6 | 34 661 | 3332 | | 1.29 (1.02 to 1.64) |
| <9 | 13 | 46 001 | 3084 | | 1.37 (1.06 to 1.76) |

0.75  1    1.75  2.5  3.5

Relative risk (95% CI) for bottom versus top thirds of baseline 25-hydroxyvitamin D concentration

**Fig 3** Association of circulating 25-hydroxyvitamin D concentration and risk of cardiovascular disease mortality in primary prevention cohorts, according to various characteristics. Based on available studies with relevant subgroup information. CPBA=competitive binding protein assay; +=relative risks adjusted for established cardiovascular risk factors such as age, sex, smoking status, lipids, hypertension, history of cardiometabolic disease; ++=adjusted for other potential risk factors such as physical activity, body mass index, social status; +++=adjusted for other additional variables such as bone minerals. *P<0.05 from meta-regression analyses. †Based on available studies with relevant subgroup information. ‡Based on Newcastle-Ottawa scale.

| Subgroups | No of studies | No of participants | No of deaths | Relative risk (95% CI) | Relative risk (95% CI) |
|---|---|---|---|---|---|
| **Latitude of study location** | | | | | |
| >40° N, <40° S | 4 | 27 079 | 1421 | | 1.16 (0.91 to 1.46) |
| Between 40° N and 40° S | 8 | 77 274 | 3582 | | 1.14 (0.97 to 1.35) |
| **Average age of participants** | | | | | |
| <70 years | 9 | 99 031 | 4499 | | 1.07 (1.00 to 1.15) |
| ≥70 years | 3 | 5322 | 504 | | 1.32 (0.63 to 2.73) |
| **Sex** | | | | | |
| Male | 3 | 50 484 | 2311 | | 1.16 (0.64 to 2.11) |
| Female | 1 | 2429 | 62 | | 1.33 (0.90 to 1.96) |
| Both | 8 | 51 440 | 2630 | | 1.10 (0.97 to 1.25) |
| **Baseline vitamin D supplement use†** | | | | | |
| ≥10% | 1 | 13 331 | 424 | | 0.92 (0.67 to 1.26) ⎤ |
| None or <10% | 1 | 1194 | 189 | | 2.35 (1.37 to 4.04) ⎦ * |
| **Sample type** | | | | | |
| Plasma | 2 | 48 994 | 2214 | | 1.57 (0.79 to 3.11) |
| Serum | 10 | 55 359 | 2789 | | 1.09 (0.96 to 1.25) |
| **Assay method** | | | | | |
| Radioimmunoassay | 6 | 74 830 | 3008 | | 1.19 (1.03 to 1.38) |
| Automated assay | 3 | 17 693 | 1408 | | 1.01 (0.91 to 1.11) |
| Chromatographic plus CBPA | 3 | 11 830 | 587 | | 1.18 (0.60 to 2.32) |
| **Average follow-up** | | | | | |
| ≥5 years | 10 | 95 868 | 4464 | | 1.12 (0.98 to 1.29) |
| <5 years | 2 | 8485 | 539 | | 1.29 (0.97 to 1.72) |
| **No of events ascertained** | | | | | |
| ≥500 | 1 | 47 800 | 2025 | | 1.16 (1.02 to 1.33) |
| <500 | 11 | 56 553 | 2978 | | 1.14 (0.98 to 1.33) |
| **Level of adjustment** | | | | | |
| +++ | 5 | 32 754 | 1641 | | 1.02 (0.80 to 1.29) |
| ++/+ | 7 | 71 599 | 3362 | | 1.21 (1.10 to 1.34) |
| **Controlled for seasonality** | | | | | |
| Yes | 8 | 45 492 | 2637 | | 1.13 (0.93 to 1.37) |
| No | 4 | 58 861 | 2366 | | 1.17 (1.05 to 1.31) |
| **Adjusted for socioeconomic status** | | | | | |
| Yes | 8 | 45 862 | 2201 | | 1.18 (0.95 to 1.46) |
| No | 4 | 58 491 | 2802 | | 1.12 (1.01 to 1.24) |
| **Risk of bias score‡** | | | | | |
| 9 | 2 | 20 492 | 922 | | 0.96 (0.80 to 1.15) |
| <9 | 10 | 83 861 | 4081 | | 1.19 (1.03 to 1.38) |

```
        0.5 0.75 1   1.75 2.5    5
```

Relative risk (95% CI) for bottom
versus top thirds of baseline
25-hydroxyvitamin D concentration

**Fig 4** Association of circulating 25-hydroxyvitamin D concentration and risk of cancer mortality in primary prevention cohorts, according to various characteristics. Based on available studies with relevant subgroup information. CPBA=competitive binding protein assay; +=relative risks adjusted for established cardiovascular risk factors such as age, sex, smoking status, lipids, hypertension, history of cardiometabolic disease; ++=adjusted for other potential risk factors such as physical activity, body mass index, social status; +++=adjusted for other additional variables such as bone minerals. *P<0.05 from meta-regression analyses. †Based on available studies with relevant subgroup information. ‡Based on Newcastle-Ottawa scale.

| Subgroups | No of studies | No of participants | No of deaths | Relative risk (95% CI) | Relative risk (95% CI) |
|---|---|---|---|---|---|
| **Latitude of study location** | | | | | |
| >40° N, <40° S | 16 | 295 232 | 27 646 | | 1.30 (1.18 to 1.42) |
| Between 40° N and 40° S | 11 | 485 758 | 20 842 | | 1.35 (1.16 to 1.57) |
| **Average age of participants** | | | | | |
| <70 years | 17 | 767 453 | 44 040 | | 1.35 (1.19 to 1.54) |
| ≥70 years | 10 | 13 537 | 4448 | | 1.33 (1.16 to 1.52) |
| **Sex** | | | | | |
| Male | 3 | 3466 | 1096 | | 1.36 (0.91 to 2.05) |
| Female | 4 | 6627 | 522 | | 1.21 (0.91 to 1.61) |
| Both | 20 | 770 897 | 46 870 | | 1.37 (1.22 to 1.53) |
| **Baseline vitamin D supplement use†** | | | | | |
| ≥10% | 8 | 23 169 | 4299 | | 1.25 (1.13 to 1.38)⎤ * |
| None or <10% | 3 | 424 414 | 12 993 | | 1.80 (1.72 to 1.88)⎦ |
| **Sample type** | | | | | |
| Plasma | 4 | 13 910 | 8118 | | 1.34 (1.18 to 1.52) |
| Serum | 23 | 767 080 | 40 370 | | 1.34 (1.19 to 1.51) |
| **Assay method** | | | | | |
| Radioimmunoassay | 11 | 33 314 | 4649 | | 1.28 (1.11 to 1.47) |
| Automated assay | 7 | 728 695 | 39 712 | | 1.39 (1.15 to 1.68) |
| Chromatographic plus CBPA | 9 | 18 981 | 4127 | | 1.33 (1.17 to 1.51) |
| **Average follow-up** | | | | | |
| ≥5 years | 22 | 71 673 | 17 618 | | 1.26 (1.16 to 1.36)⎤ * |
| <5 years | 5 | 709 317 | 30 870 | | 1.63 (1.44 to 1.84)⎦ |
| **No of events ascertained** | | | | | |
| ≥500 | 14 | 758 654 | 46 162 | | 1.34 (1.17 to 1.52) |
| <500 | 13 | 22 336 | 2326 | | 1.35 (1.17 to 1.56) |
| **Level of adjustment** | | | | | |
| +++ | 10 | 47 824 | 13 666 | | 1.22 (1.11 to 1.33) |
| ++/+ | 17 | 733 166 | 34 822 | | 1.42 (1.26 to 1.60) |
| **Controlled for seasonality** | | | | | |
| Yes | 20 | 738 356 | 45 365 | | 1.38 (1.22 to 1.56) |
| No | 7 | 42 634 | 3123 | | 1.27 (1.05 to 1.55) |
| **Adjusted for socioeconomic status** | | | | | |
| Yes | 17 | 481 332 | 22 014 | | 1.43 (1.23 to 1.67) |
| No | 10 | 299 658 | 26 474 | | 1.24 (1.10 to 1.39) |
| **Risk of bias score‡** | | | | | |
| 9 | 7 | 282 422 | 26 767 | | 1.26 (1.20 to 1.32) |
| <9 | 20 | 498 568 | 21 721 | | 1.39 (1.21 to 1.59) |

0.75   1   1.75   2.5

Relative risk (95% CI) for bottom
versus top thirds of baseline
25-hydroxyvitamin D concentration

**Fig 5** Association of circulating 25-hydroxyvitamin D concentration and risk of all cause mortality in primary prevention cohorts, according to various characteristics. Based on available studies with relevant subgroup information. CPBA=competitive binding protein assay; +=relative risks adjusted for established cardiovascular risk factors such as age, sex, smoking status, lipids, hypertension, history of cardiometabolic disease; ++=adjusted for other potential risk factors such as physical activity, body mass index, social status; +++=adjusted for other additional variables such as bone minerals. *P<0.05 from meta-regression analyses. †Based on available studies with relevant subgroup information. ‡Based on Newcastle-Ottawa scale.

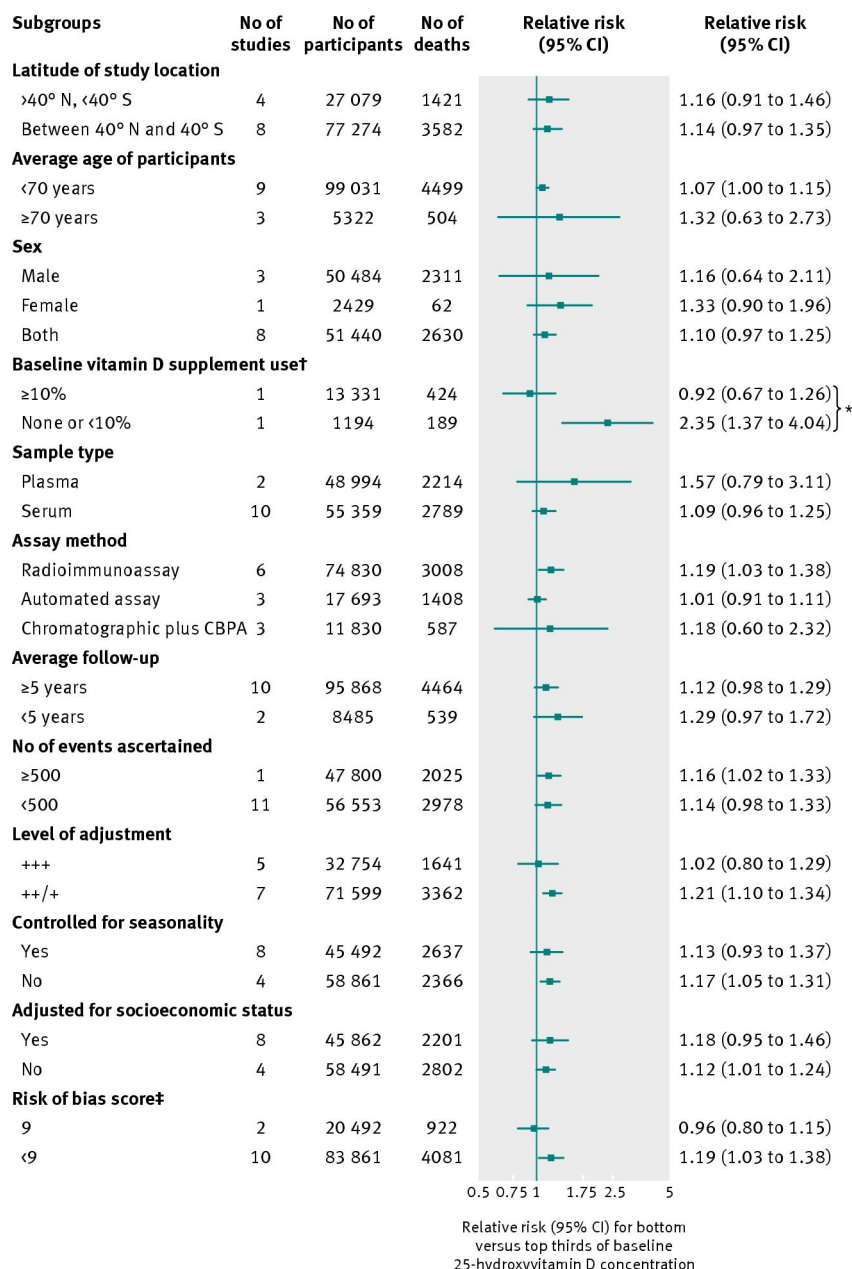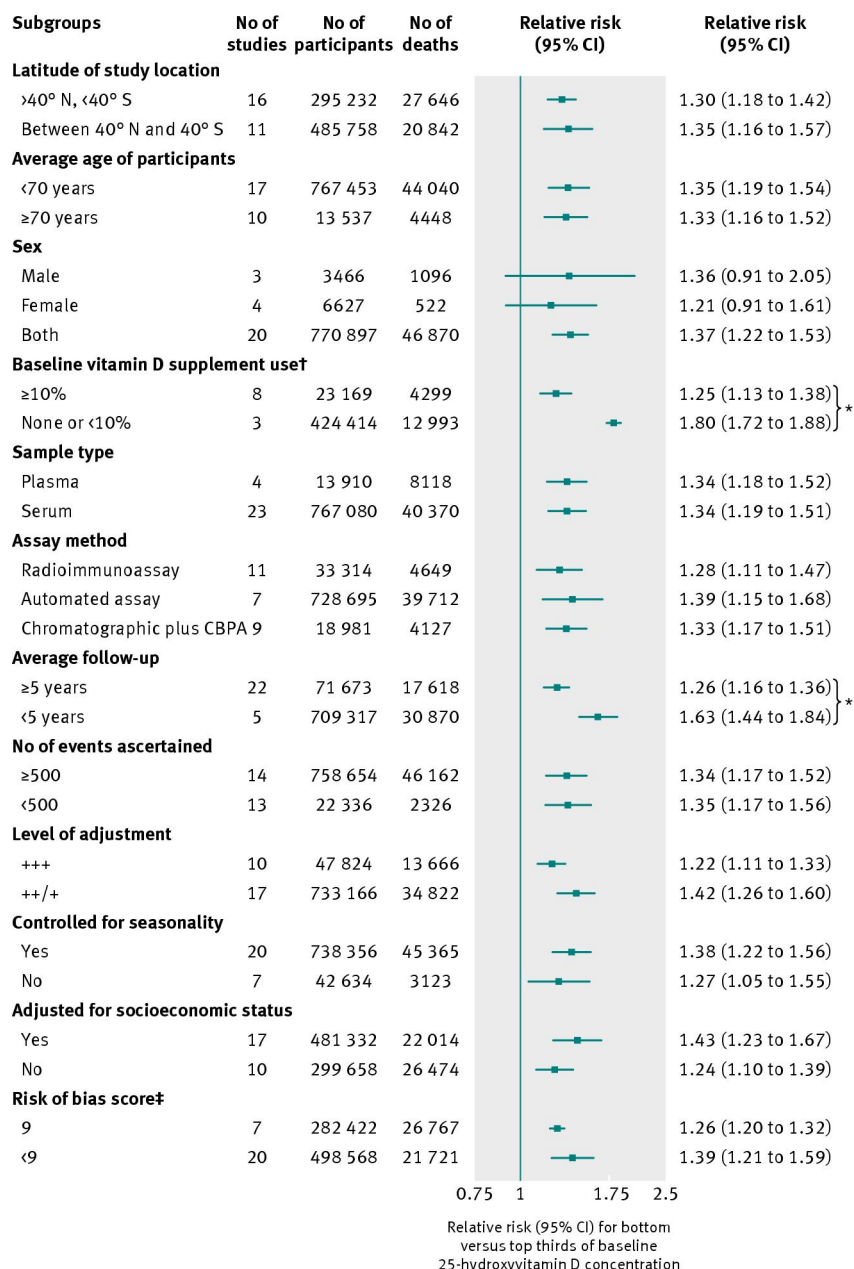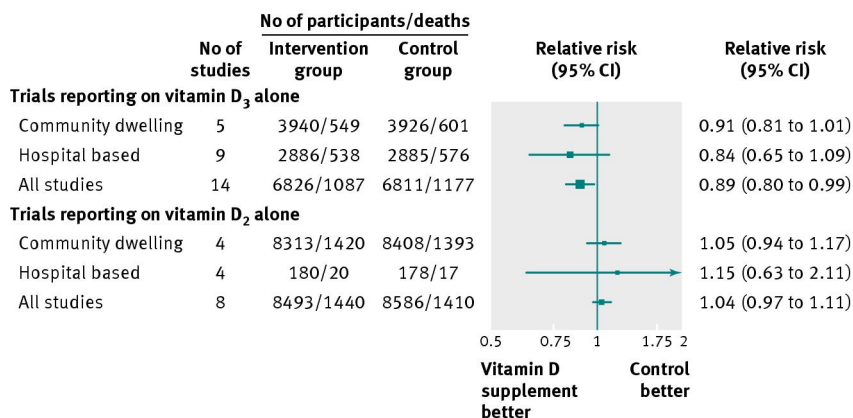| | No of studies | No of participants/deaths | | Relative risk (95% CI) | Relative risk (95% CI) |
|---|---|---|---|---|---|
| | | Intervention group | Control group | | |
| **Trials reporting on vitamin D₃ alone** | | | | | |
| Community dwelling | 5 | 3940/549 | 3926/601 | | 0.91 (0.81 to 1.01) |
| Hospital based | 9 | 2886/538 | 2885/576 | | 0.84 (0.65 to 1.09) |
| All studies | 14 | 6826/1087 | 6811/1177 | | 0.89 (0.80 to 0.99) |
| **Trials reporting on vitamin D₂ alone** | | | | | |
| Community dwelling | 4 | 8313/1420 | 8408/1393 | | 1.05 (0.94 to 1.17) |
| Hospital based | 4 | 180/20 | 178/17 | | 1.15 (0.63 to 2.11) |
| All studies | 8 | 8493/1440 | 8586/1410 | | 1.04 (0.97 to 1.11) |

**Fig 6** Effects of vitamin D supplementation on all cause mortality when given alone, derived from available randomised control trials. *Pooled estimates are based on random effects meta-analysis. Using fixed effects models, for community dwelling, hospital based, and overall population, the estimates were 0.91 (0.81 to 1.01), 0.88 (0.77 to 1.01), and 0.90 (0.82 to 0.98) for vitamin D₃ trials and 1.05 (0.94 to 1.17), 1.15 (0.63 to 2.11), and 1.03 (0.97 to 1.09) for vitamin D₂ trials. Overall fixed effect estimate for all trials was 0.98 (0.94 to 1.03). Size of data marker is proportional to inverse of variance of relative risk; horizontal line represents 95% CI. Corresponding forest plots and $I^2$ (95% CI) estimates are provided in supplementary material



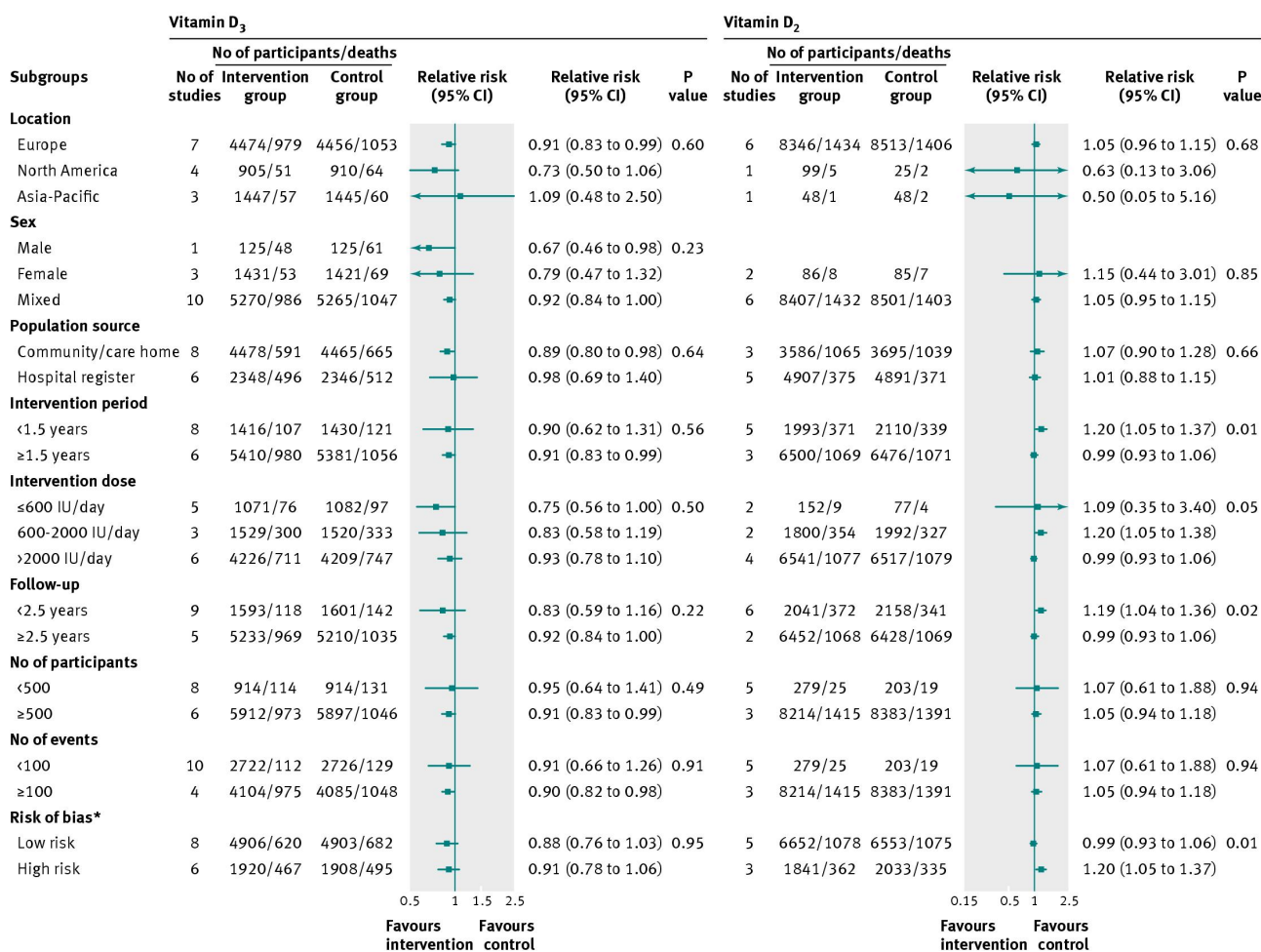| | Vitamin D₃ | | | | | | Vitamin D₂ | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No of participants/deaths | | | Relative risk (95% CI) | Relative risk (95% CI) | P value | No of participants/deaths | | | Relative risk (95% CI) | Relative risk (95% CI) | P value |
| Subgroups | No of studies | Intervention group | Control group | | | | No of studies | Intervention group | Control group | | | |
| **Location** | | | | | | | | | | | | |
| Europe | 7 | 4474/979 | 4456/1053 | | 0.91 (0.83 to 0.99) | 0.60 | 6 | 8346/1434 | 8513/1406 | | 1.05 (0.96 to 1.15) | 0.68 |
| North America | 4 | 905/51 | 910/64 | | 0.73 (0.50 to 1.06) | | 1 | 99/5 | 25/2 | | 0.63 (0.13 to 3.06) | |
| Asia-Pacific | 3 | 1447/57 | 1445/60 | | 1.09 (0.48 to 2.50) | | 1 | 48/1 | 48/2 | | 0.50 (0.05 to 5.16) | |
| **Sex** | | | | | | | | | | | | |
| Male | 1 | 125/48 | 125/61 | | 0.67 (0.46 to 0.98) | 0.23 | | | | | | |
| Female | 3 | 1431/53 | 1421/69 | | 0.79 (0.47 to 1.32) | | 2 | 86/8 | 85/7 | | 1.15 (0.44 to 3.01) | 0.85 |
| Mixed | 10 | 5270/986 | 5265/1047 | | 0.92 (0.84 to 1.00) | | 6 | 8407/1432 | 8501/1403 | | 1.05 (0.95 to 1.15) | |
| **Population source** | | | | | | | | | | | | |
| Community/care home | 8 | 4478/591 | 4465/665 | | 0.89 (0.80 to 0.98) | 0.64 | 3 | 3586/1065 | 3695/1039 | | 1.07 (0.90 to 1.28) | 0.66 |
| Hospital register | 6 | 2348/496 | 2346/512 | | 0.98 (0.69 to 1.40) | | 5 | 4907/375 | 4891/371 | | 1.01 (0.88 to 1.15) | |
| **Intervention period** | | | | | | | | | | | | |
| <1.5 years | 8 | 1416/107 | 1430/121 | | 0.90 (0.62 to 1.31) | 0.56 | 5 | 1993/371 | 2110/339 | | 1.20 (1.05 to 1.37) | 0.01 |
| ≥1.5 years | 6 | 5410/980 | 5381/1056 | | 0.91 (0.83 to 0.99) | | 3 | 6500/1069 | 6476/1071 | | 0.99 (0.93 to 1.06) | |
| **Intervention dose** | | | | | | | | | | | | |
| ≤600 IU/day | 5 | 1071/76 | 1082/97 | | 0.75 (0.56 to 1.00) | 0.50 | 2 | 152/9 | 77/4 | | 1.09 (0.35 to 3.40) | 0.05 |
| 600-2000 IU/day | 3 | 1529/300 | 1520/333 | | 0.83 (0.58 to 1.19) | | 2 | 1800/354 | 1992/327 | | 1.20 (1.05 to 1.38) | |
| >2000 IU/day | 6 | 4226/711 | 4209/747 | | 0.93 (0.78 to 1.10) | | 4 | 6541/1077 | 6517/1079 | | 0.99 (0.93 to 1.06) | |
| **Follow-up** | | | | | | | | | | | | |
| <2.5 years | 9 | 1593/118 | 1601/142 | | 0.83 (0.59 to 1.16) | 0.22 | 6 | 2041/372 | 2158/341 | | 1.19 (1.04 to 1.36) | 0.02 |
| ≥2.5 years | 5 | 5233/969 | 5210/1035 | | 0.92 (0.84 to 1.00) | | 2 | 6452/1068 | 6428/1069 | | 0.99 (0.93 to 1.06) | |
| **No of participants** | | | | | | | | | | | | |
| <500 | 8 | 914/114 | 914/131 | | 0.95 (0.64 to 1.41) | 0.49 | 5 | 279/25 | 203/19 | | 1.07 (0.61 to 1.88) | 0.94 |
| ≥500 | 6 | 5912/973 | 5897/1046 | | 0.91 (0.83 to 0.99) | | 3 | 8214/1415 | 8383/1391 | | 1.05 (0.94 to 1.18) | |
| **No of events** | | | | | | | | | | | | |
| <100 | 10 | 2722/112 | 2726/129 | | 0.91 (0.66 to 1.26) | 0.91 | 5 | 279/25 | 203/19 | | 1.07 (0.61 to 1.88) | 0.94 |
| ≥100 | 4 | 4104/975 | 4085/1048 | | 0.90 (0.82 to 0.98) | | 3 | 8214/1415 | 8383/1391 | | 1.05 (0.94 to 1.18) | |
| **Risk of bias*** | | | | | | | | | | | | |
| Low risk | 8 | 4906/620 | 4903/682 | | 0.88 (0.76 to 1.03) | 0.95 | 5 | 6652/1078 | 6553/1075 | | 0.99 (0.93 to 1.06) | 0.01 |
| High risk | 6 | 1920/467 | 1908/495 | | 0.91 (0.78 to 1.06) | | 3 | 1841/362 | 2033/335 | | 1.20 (1.05 to 1.37) | |

**Fig 7** Effects of vitamin D supplementation on all cause mortality, derived from available randomised controlled trials and according to various characteristics. Based on available studies with relevant subgroup information; P values are from meta-regression analyses. *Low risk and high risk categories are defined by studies that met ≥5 criteria versus those that met <5 criteria in Cochrane Collaboration's tool, respectively