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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; correspondance 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

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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 χ^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

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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

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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.

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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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Ethical approval: Not needed.

Transparency declaration: The lead author (the manuscript's guarantor) affirms that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned have been explained.

Data sharing: No additional data available.

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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.

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Figures

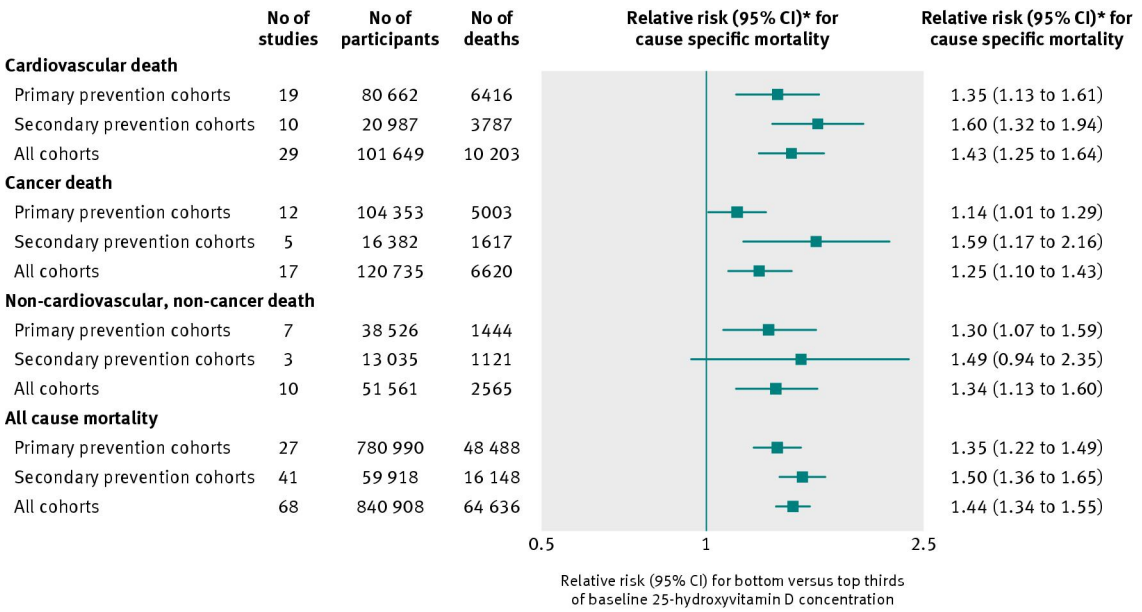

Fig 1 Association of circulating 25-hydroxyvitamin D concentrations with cause specific mortality in observational cohort studies. *Pooled estimates are based on random effects meta-analysis. Using fixed effects models, for primary prevention cohorts, secondary prevention cohorts, and all cohorts, the 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

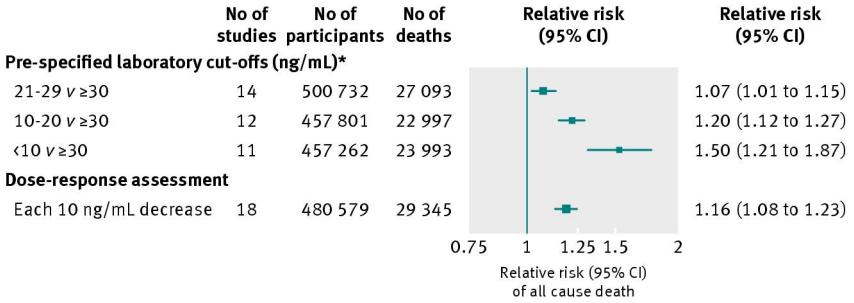

Fig 2 Association of circulating 25-hydroxyvitamin D concentrations with all cause mortality, based on primary prevention cohorts. *Indirect comparisons based on available studies with relevant information in each category; summary estimates presented were calculated using random effects models. Using 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

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Subgroups	No of studies	No of participants	No of deaths	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)

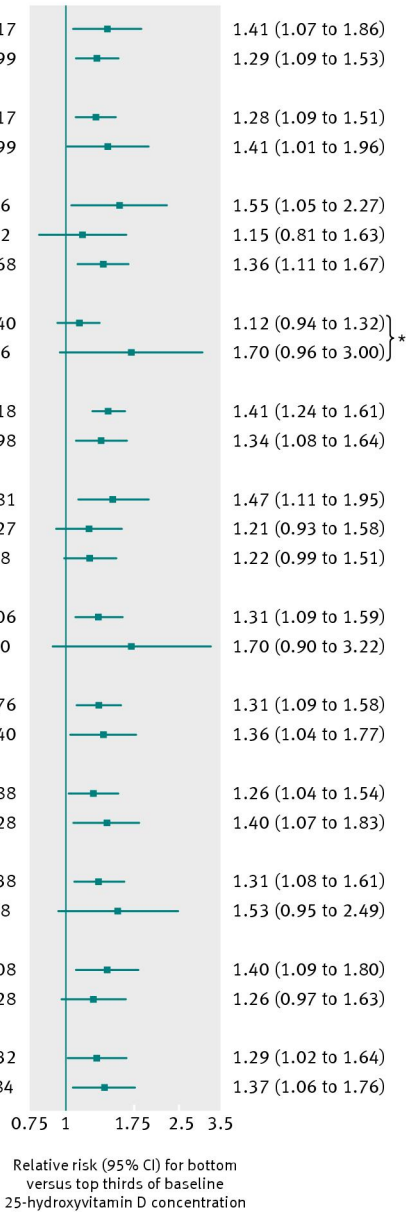

Fig 3 Association of circulating 25-hydroxyvitamin D concentration and risk of cardiovascular disease mortality in primary prevention cohorts, according to various characteristics. Based on available studies with relevant subgroup information. CPBA=competitive binding protein assay; +=relative risks 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.

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Subgroups	No of studies	No of participants	No of deaths	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)

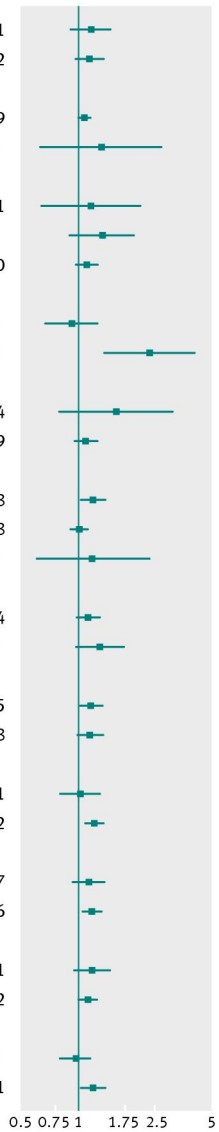

Fig 4 Association of circulating 25-hydroxyvitamin D concentration and risk of cancer mortality in primary prevention cohorts, according to various characteristics. Based on available studies with relevant subgroup information. CPBA=competitive binding protein assay; +=relative risks adjusted for established 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.

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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)

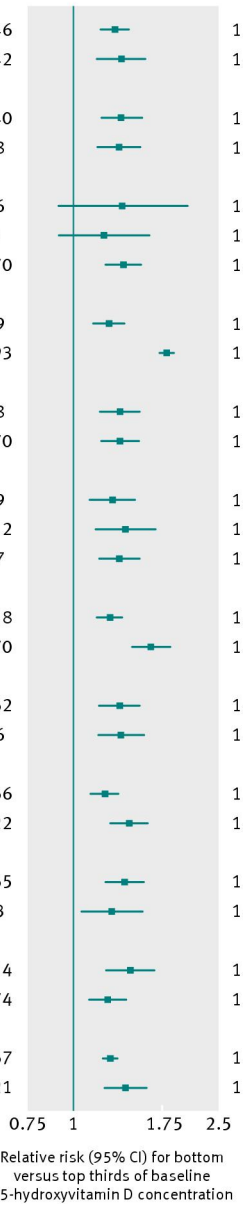

Fig 5 Association of circulating 25-hydroxyvitamin D concentration and risk of all cause mortality in primary prevention cohorts, according to various characteristics. Based on available studies with relevant subgroup information. CPBA=competitive binding protein assay; +=relative risks adjusted for 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.

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	No of studies	No of participants/deaths: Intervention group	No of participants/deaths: Control group	Relative risk (95% CI)
Trials reporting on vitamin D_3 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 D_2 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)

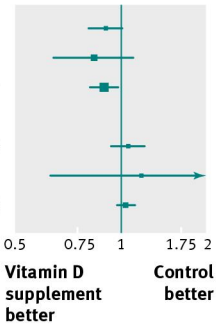

Fig 6 Effects of vitamin D supplementation on all cause mortality when given alone, derived from available randomised control trials. *Pooled estimates are based on random effects meta-analysis. Using fixed effects models, for community dwelling, hospital based, and overall population, the estimates 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 D_3 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 D_2 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

Subgroups	Vitamin D_3: No of studies	No of participants/deaths: Intervention group	No of participants/deaths: Control group	Relative risk (95% CI)	P value	Vitamin D_2: No of studies	No of participants/deaths: Intervention group	No of participants/deaths: Control group	Relative risk (95% CI)	P value
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

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