RESEARCH

Vitamin D and mortality: meta-analysis of individual participant data from a large consortium of cohort studies from Europe and the United States

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Abstract

Objective To investigate the association between serum 25-hydroxyvitamin D concentrations (25(OH)D) and mortality in a large consortium of cohort studies paying particular attention to potential age, sex, season, and country differences.

Design Meta-analysis of individual participant data of eight prospective cohort studies from Europe and the US.

Setting General population.

Participants 26 018 men and women aged 50-79 years.

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Main outcome measures All-cause, cardiovascular, and cancer mortality.

Results 25(OH)D concentrations varied strongly by season (higher in summer), country (higher in US and northern Europe) and sex (higher in men), but no consistent trend with age was observed. During follow-up, 6695 study participants died, among whom 2624 died of cardiovascular diseases and 2227 died of cancer. For each cohort and analysis, 25(OH)D quintiles were defined with cohort and subgroup specific cut-off values. Comparing bottom versus top quintiles resulted in a pooled risk ratio of 1.57 (95% CI 1.36 to 1.81) for all-cause mortality. Risk ratios for cardiovascular mortality were similar in magnitude to that for all-cause mortality in subjects both with and without a history of cardiovascular disease at baseline. With respect to cancer mortality, an association was only observed among subjects with a history of cancer (risk ratio, 1.70 (1.00 to 2.88)). Analyses using all quintiles suggest curvilinear, inverse, dose-response curves for the aforementioned relationships. No strong age, sex, season, or country specific differences were detected. Heterogeneity was low in most meta-analyses.

Conclusions Despite levels of 25(OH)D strongly varying with country, sex, and season, the association between 25(OH)D level and all-cause and cause-specific mortality was remarkably consistent. Results from a long term randomised controlled trial addressing longevity are being awaited before vitamin D supplementation can be recommended in most individuals with low 25(OH)D levels.

Introduction

Although vitamin D is obtained from diet and dietary supplements, the main source of vitamin D is its production in skin under the influence of solar ultraviolet B radiation. The most commonly measured vitamin D metabolite is serum 25-hydroxyvitamin D (25(OH)D) because of its greater half life (~3 weeks) and up to 1000-fold higher serum levels compared with the physiologically active metabolite 1,25-dihydroxyvitamin D (half life of a few hours).[1] As ultraviolet B exposure varies with the latitude of regions and during the year, so mean 25(OH)D concentrations of populations also vary accordingly.[2][3] Furthermore, women are generally more prone to low 25(OH)D concentrations than men (possibly because of a positive correlation of 25(OH)D with testosterone levels),[4] and vitamin D deficiency is especially common among elderly people, who often have less sun exposure (because of reduced outdoor activity) and limited capacity of the skin to produce vitamin D metabolites.[5] However, it is unclear if and to what extent these influences on production and maintenance of sufficient vitamin D metabolite concentrations affect the prognostic association of low 25(OH)D concentrations with mortality.

The objective of this meta-analysis of a consortium of eight prospective cohort studies from different European countries and the United States was to investigate the prognostic association of 25(OH)D measurements with all-cause, cardiovascular, and cancer mortality, paying particular attention to potential differences between countries, sexes, age groups, and seasons of blood draw.

Methods

This manuscript was written in adherence to the Strengthening The Reporting of Observational Studies in Epidemiology (STROBE) and the Meta-analysis Of Observational Studies in Epidemiology (MOOSE) statements.[6][7]

Study design and study population

This investigation is a meta-analysis of individual participant data of seven population based cohorts with measured 25(OH)D of the Consortium on Health and Ageing: Network of Cohorts in Europe and the United States (CHANCES, www.chancesfp7.eu) together with the third US National Health And Nutrition Examination Survey (NHANES III). The cohorts of the CHANCES consortium were chosen because all variables needed for this project were harmonised according to pre-defined rules. The harmonisation rules were discussed among the CHANCES partners until a consensus was reached. The NHANES survey was added to include a North American cohort and because it has an open access policy that enabled data access with subsequent conversion according to the CHANCES data harmonisation rules. The cohorts cover 16 European countries and the United States. The cohorts' key characteristics are summarised in table 1⇓, and the study designs are described in the supplemental data and previous publications.[2][8-12] All CHANCES cohorts and the NHANES are conducted in accordance with the declaration of Helsinki, and written informed consent was obtained from all study participants.

Inclusion and exclusion criteria

To make cohorts more comparable, analyses were restricted to study participants aged 50-79 years. Current smokers were excluded from the Tromsø study because the 25(OH)D assay used resulted in smokers having 15-20% higher 25(OH)D concentrations than non-smokers which was not reproducible with other assays.[9] In addition, study participants with missing 25(OH)D measurement, missing values for covariates used in the main model, or participants lost to follow-up due to unknown reasons were excluded. The derivation of the final number of included study participants is shown for each cohort in table 1⇓.

Mortality ascertainment

All cohorts ascertained deaths by region, state, or country wide registries with a coverage of ≥98.5% and obtained information about the underlying cause of death for at least 97.6% of the deceased. The mean follow-up time ranged from 4.2 to 15.9 years (table 1⇓). All deaths coded with ICD-10 codes I00-I99 were considered cardiovascular deaths, and cancer deaths were defined by the ICD-10 codes C00-C97.

Measurement of 25(OH)D

Measurement of 25(OH)D was conducted in the full cohorts of ESTHER, Tromsø, and NHANES III, whereas in SENECA and MONICA/KORA random subsamples were measured, and in the three HAPIEE cohorts 25(OH)D was measured in a nested case-control design (including study participants who died from any cause as cases). Details of the sampling procedures, the design of the nested case-control study, and the manufacturers of the 25(OH)D assays are given in table 1⇓, and further details have been described previously.[2][8][9][11][13][14]

Covariate assessment

Sociodemographic, lifestyle, anthropometric, and disease history data were assessed by self administered questionnaires or in interviews. In addition to self reported information, some studies measured weight and height and validated the history of common chronic diseases by consulting medical records or registries (such as cancer registries) (supplementary table 1). If measured anthropometric data or validated diagnoses were available, these were used in the analysis, and self reported

information was used only to fill missing information. Prevalent cardiovascular disease was defined as a history of myocardial infarction or stroke.

Statistical analyses

This meta-analysis of individual participant data followed a two step approach by analysing the cohorts individually first and conducting meta-analyses of the obtained effect estimates thereafter.

Differences in 25(OH)D concentrations according to baseline characteristics were assessed with the Wilcoxon rank-sum test. The longitudinal analyses with mortality outcomes were conducted using 25(OH)D quintiles because there is still no consensus about cut off values for 25(OH)D risk categories[8 15] and the applied 25(OH)D assays in the cohorts were not commonly standardised. The cut off values for 25(OH)D quintiles were allowed to vary by cohort and by analysed subgroup to ensure that there was always 20% of the analysed study population in each quintile (see supplementary table 2 for applied cut off values).

Cox proportional hazards or logistic regression models (the latter for case-control studies nested in the HAPIEE cohorts) were used to estimate hazard ratios or odds ratios, respectively, with 95% confidence intervals for comparison of increasing 25(OH)D quintiles compared with the highest quintile. When the risk is low, logistic and Cox regression usually produce similar results and the odds ratio can be regarded as an approximation of the more accurate hazard ratio that also takes time to the event into account. We use the term "risk ratios" for both the effect estimates. Analyses for cardiovascular and cancer mortality were stratified by presence or absence of a history of the disease. For sensitivity analyses of all-cause mortality, data were restricted to subjects without a history of cardiovascular disease or cancer, respectively. For analyses on cause-specific mortality, competing risks analysis was employed to correct for the competing risk of death by other causes. Weights for subjects who died of competing causes of death were determined according to an extension of the Fine and Gray method.[16]

For all outcomes, three models were applied with adjustment for an increasing number of established risk factors for all-cause mortality and determinants of serum 25(OH)D concentrations. In model 1, adjustment was made for age, sex, and season of blood draw. Model 2 was the fully adjusted model with additional adjustment for education, body mass index, smoking, and physical activity. Model 3 additionally included diseases that could potentially be intermediates in the association of 25(OH)D and mortality (that is, a history of diabetes, hypertension, cardiovascular disease, and cancer). Model 2 results were considered the main results. Age was modelled linearly and the other variables categorically with dummy variables using the categories shown in table 2⇓. In addition, the following adjustments for cohort-specific characteristics were made in all three models: survey for MONICA/KORA, latitude of study centre for SENECA, and region (west, mid-west, north-east, south) as well as ethnicity (non-Hispanic white, non-Hispanic black, Mexican-American, other) for the NHANES III. Proportions of non-white study participants in the European studies were negligible.

For all meta-analyses, Mantel-Haenszel weighting and random effects models were used,[17] taking the sample size of the cohorts and the possibility of statistical heterogeneity among the studies into account. The latter was examined with Cochrane's Q test and the I^2 statistic. In sensitivity analyses, the nested case-control studies and, separately, death in the first three years of follow

up were excluded from the meta-analyses, but this did not change the overall findings and these results are therefore not shown. Dose-response relationships were assessed by estimation of a trend underlying the summary effect estimates of the 25(OH)D quintiles applying the best fitting equation of the form $y = m \ x^b$ (y, effect estimate; m, constant$_1$, x=number of quintile (that is, 1, 2, 3, 4, or 5), b=constant$_2$).

Subgroups to be analysed were chosen a priori with the aim to stratify for the important non-modifiable risk factors for vitamin D deficiency: sex, age, and season of blood draw.[8 18 19] Cohort-specific subgroup analysis results were pooled in meta-analyses, and in a sensitivity analysis the pooling of results was restricted to those cohorts that could contribute data to all subgroup analyses (ESTHER, Tromsø and NHANES III). In a further sensitivity analysis, analyses were stratified by smoking status (current; yes or no) to examine whether excluding current smokers from the Tromsø study could have affected the results of the overall meta-analysis.

All statistical tests were two sided using an α level of 0.05. The meta-analyses were conducted with the statistical software Comprehensive Meta-Analysis 2.0 (Biostat, Englewood NJ, USA), the dose-response trend was assessed with Microsoft Excel 2010 (Redmond, Washington, USA), and all other analyses were conducted with SAS, version 9.2 (Cary, North Carolina, USA). The competing risks analysis was carried out with a SAS macro provided by G Heinze, Medical University of Vienna, Austria.[20]

Results

The baseline characteristics of the analytical samples from participating cohorts and the nested case-control studies are shown in supplementary tables 3 and 4, respectively. All cohorts included men and women and all age groups (50-79 years), with exception of the SENECA study, which only recruited in the age range 70-79 years. The median 25(OH)D concentrations by cohort varied between 24 and 62 nmol/L. Lower values in the Tromsø, MONICA/KORA, SENECA, and HAPIEE cohort studies can in part be explained by there being few or no serum samples being collected in summer or autumn. Interestingly, the medians of 25(OH)D concentrations in the nested case-control studies were consistently lower among cases than controls (supplementary table 4).

Table 2⇓ shows 25(OH)D concentrations by baseline characteristics of the study participants (HAPIEE cohorts are again shown separately for cases and controls). There was no clear trend in 25(OH)D by age, but the median 25(OH)D concentrations were consistently lower among women than men. As expected, there was a clear pattern of 25(OH)D concentrations measured in winter or spring being substantially lower than those measured in summer or autumn. In addition, the median 25(OH)D concentrations were, with few exceptions among the cases and controls of the HAPIEE cohorts, increasing with categories of education, lowest in obese individuals, lowest in current smokers, and higher among subjects who undertook vigorous physical activity. There were no systematic differences in 25(OH)D concentrations between subjects with and without history of chronic diseases (diabetes, hypertension, cardiovascular disease, or cancer).

The median follow-up time in individual cohorts varied between 4.2 and 15.8 years, during which 6695 study participants died, including 2624 deaths from cardiovascular diseases and 2227 deaths from cancer (table 1⇓). Compared with models adjusted for age, sex, and season (model 1), the risk ratios by 25(OH)D quintile (using the top quintile as reference) were attenuated for

all outcomes by additional adjustment for conventional risk factors for premature mortality (model 2; supplementary tables 5-7). Further addition of common chronic diseases to the model (model 3) did not lead to further material attenuations of the risk ratios.

The forest plots of the meta-analyses of main results (model 2), comparing the bottom and the top 25(OH)D quintile are shown for all-cause mortality in fig 1⇓ and for cardiovascular and cancer mortality in fig 2⇓. A low degree of heterogeneity of effects in the individual cohort studies can be seen from the forest plots. The horizontal bars (the confidence intervals) overlap, and the boxes (the effect estimates) are mostly close together. The pooled effect estimate in the meta-analysis for all-cause mortality revealed a 1.6-fold higher mortality in the bottom quintile compared with the top quintile (risk ratio 1.57 (95% confidence interval 1.36 to 1.81), fig 1⇓). Furthermore, the association with cardiovascular mortality in subjects without a history of cardiovascular disease (risk ratio 1.41 (1.18 to 1.68)) and in subjects with a history of cardiovascular disease (risk ratio 1.65 (1.22 to 2.22)) and the association with cancer mortality in subjects with a history of cancer (1.70 (1.00 to 2.88)) were of similar strength and all statistically significant (fig 2⇓; detailed results shown in supplementary tables 6-9). The only exception was cancer mortality in subjects without a history of cancer, for whom there was association (risk ratio 1.03 (0.89 to 1.20)).

The dose-response trends across all 25(OH)D quintiles show curvilinear inverse associations for all-cause mortality and cardiovascular mortality, and for cancer mortality in subjects with a history of cancer (fig 3⇓; detailed results shown in supplementary tables 5-9). For all-cause mortality, the risk ratio for bottom versus top 25(OH)D quintile was almost unchanged if subjects with a history of cardiovascular disease or of cancer were excluded (supplementary table 10-11).

In analyses stratified for sex, age, and season, the association of the bottom quintile of 25(OH)D concentration with the outcomes did not differ substantially in the subgroups (pooled results are shown in fig 4⇓, detailed results are shown in supplementary tables 12-20). Restricting the analyses to cohorts that can contribute to all subgroups produced similar results (not shown), as did stratification by smoking status or exclusion of events in the first three years of follow-up (not shown).

Heterogeneity statistics for the meta-analyses usually indicated a low, statistically non-significant level of heterogeneity. When statistically significant, heterogeneity could be attenuated to non-significant levels by removal of one outlier study (supplementary tables 5-20). In the numerous meta-analyses, six out of the eight studies were identified as the outlier between one and three times, which shows that being the outlier was rather randomly distributed among the studies and did not indicate incomparability of the study's results.

Discussion

Principal findings

In this meta-analysis of eight prospective cohort studies from Europe and the United States, the lowest quintile of serum 25(OH)D concentration was associated with increased all-cause and cardiovascular mortality, with a curvilinear association between 25(OH)D concentration and these outcomes. An association with cancer mortality was only observed if subjects had a history of cancer. Results were consistent across study populations, sexes, age groups, and seasons of blood draw.

Comparison with other studies

This is the first meta-analysis of individual participant data on the relationship of 25(OH)D and mortality. Previous meta-analyses of published study results faced problems with hardly comparable vitamin D categories (including tertiles, quartiles, quintiles, or clinically defined categories). Nevertheless, the pooled effect estimates from this meta-analysis for all-cause mortality (risk ratio 1.57 (95% confidence interval 1.36 to 1.81)) and cardiovascular mortality (1.41 (1.18 to 1.68)) in subjects without cardiovascular disease at baseline) comparing the lowest and highest quintile of 25(OH)D concentration agreed with the meta-analyses of Zittermann et al[21] for all-cause mortality (risk ratio 1.41 (1.04 to 1.90)) and of Wang et al[22] for cardiovascular mortality (1.42 (1.19 to 1.71)). The most recent meta-analysis, by Chowdhury et al,[23] that recalculated published effect estimates to derive comparisons of the bottom versus the top tertile of vitamin D levels came to similar effect estimates for all-cause mortality (relative risk 1.35 (1.22 to 1.49)) and cardiovascular mortality (1.35 (1.13 to 1.61)). None of the studies included in the reviews was conducted in eastern European populations, which differ from western European populations with respect to lifestyle (such as alcohol consumption and nutrition) and socioeconomic characteristics.[24] Our meta-analysis shows that the associations of low 25(OH)D concentrations with all-cause and cardiovascular mortality outcomes are as strong in eastern Europe as in western Europe, northern Europe, or the US.

With respect to cancer mortality, the recent meta-analyses of Chowdhury et al[23] and Yin et al[25] showed weak, albeit statistically significant, elevated pooled risk ratios (1.14 (1.01 to 1.29) and 1.20 (1.02 to 1.43), respectively, after change of reference category to highest 25(OH)D category in the meta-analysis of Yin et al). Our meta-analysis revealed a clear difference in the association of 25(OH)D and cancer mortality between subjects with a history of cancer (risk ratio 1.70 (1.00 to 2.88)) and those without (1.03 (0.89 to 1.20)). This difference might explain why the pooled effect estimates of the previous meta-analyses were weak and why a large degree of heterogeneity was seen among the included studies. Moreover, it speaks for an important role of vitamin D in cancer prognosis (as recently shown by others for colorectal and breast cancer prognosis[26]). However, we cannot exclude reverse causality, that is, that the cancer might have led to low 25(OH)D levels. Furthermore, our study with the endpoint cancer mortality cannot make assumptions about a potential role of vitamin D in early phases of the carcinogenic process. A recent systematic umbrella review of meta-analyses of observational studies assessing the association of 25(OH)D levels with site-specific cancer incidences concluded that there is evidence for an association of 25(OH)D levels with colorectal cancer whereas there is inconclusive or no evidence for an association of 25(OH)D with other cancer sites.[27]

The median 25(OH)D concentrations varied strongly between northern, central and eastern Europe and the US in the same seasons of blood collection. However, it is uncertain how much of these differences are related to different ultraviolet B exposure, vitamin D consumption in the diet, and vitamin D fortification habits in the countries or are just artefacts of the different 25(OH)D assays employed. The distributions of various covariates were similar across the studies in this meta-analysis, but differences in the season of sample collection could have led to some of the observed differences. Importantly, the 25(OH)D assays used in the eight cohort studies were not standardised to a common standard, and large differences in concentrations may simply be due to different assay standards

and materials.[28 29] However, a previous analysis of the SENECA study that used a common 25(OH)D assay for analysis in 12 European countries was in agreement with our finding that population means of 25(OH)D concentrations are higher in northern than in southern Europe (differences of ≥20 nmol/L were reported).[2] The authors of the SENECA study mainly suspected country differences in vitamin D fortification of foods, such as margarine, as possible causes of the large regional differences.[30] This could also explain the higher 25(OH)D levels in the US, where fortification of milk is the rule whereas it is the exception in Europe.[31]

In addition, this analysis in eight population based studies confirmed the observation of previous studies that 25(OH)D concentrations vary strongly by age, sex, season, education, obesity, physical activity, and smoking.[8 18 19] Nevertheless, in our meta-analyses of mortality endpoints, the results were comparable despite cut-off values set at the quintiles varying by cohort (and therefore country), or by other factors such as age, sex, and season. Overall, this finding may support the view that low 25(OH)D concentrations might be a marker for a poor health status rather than a cause of premature mortality. The direction from a poor health state to vitamin D deficiency seems plausible because subjects with poor health typically spend less time outdoors because of reduced capacity for physical activity, limiting their production of provitamin D_3 in the skin under solar ultraviolet B radiation.[32]

Implications for vitamin D supplementation recommendations

The question of causality could be best answered by randomised controlled trials. Two recent systematic reviews of such trials confirmed that vitamin D supply has an effect on mortality.[23 33] However, the effects were weaker than suggested by observational studies, and vitamin D supplementation seemed to be effective only for the administration of vitamin D_3 in subjects with low 25(OH)D levels at baseline.[23 33] Mortality was only a secondary outcome in most of the trials, initially designed to study the effects of vitamin D and calcium supplementation on osteoporotic outcomes. New trials with a focus on non-skeletal outcomes are needed. Four large trials have started in 2012 and are described elsewhere.[34] Among these, the VIDAL study, which is currently undertaking a feasibility study, will be particularly informative with respect to mortality outcomes because it will be specifically designed to assess these endpoints in older adults (65-84 year old citizens of Great Britain). First results of this trial are expected between 2017 and 2020. Until then, it seems reasonable to follow the US Institute of Medicine recommendations that evidence is not yet sufficient to recommend vitamin D_3 supplementation for subjects with vitamin D insufficiency (defined as 30-50 nmol/L 25(OH)D) but that subjects with vitamin D deficiency (<30 nmol/L 25(OH)D) could profit from vitamin D_3 supplementation by maintaining bone health and reduction of fracture risk.[35]

However, there is no consensus on the ideal cut-off values for vitamin D deficiency, because they are currently based on the prevention of osteoporotic outcomes, and other outcomes might also be relevant.[8 15] In our view, all-cause mortality is an outcome with particularly high public health relevance and should therefore be given high priority. A recent analysis of the ESTHER cohort showed that the Institute of Medicine cut-offs for vitamin D deficiency and insufficiency are not only appropriate for osteoporotic outcomes but also for mortality prediction.[8] The former ESTHER analysis had been adjusted for the main determinants of 25(OH)D levels and therefore corrected for variations in 25(OH)D levels according to season

and sex. In this analysis, widely varying cut-off points specific for country, sex, and season produced similar results, which raises the question whether country, sex, and season might be considered for defining vitamin D deficiency. Future population based cohort studies with adequately standardised 25(OH)D measurements are essential to obtain sex- and season-specific cut-points for vitamin D deficiency. Caution should be used in generalising existing cut-offs to other regions with different latitudes.

Limitations and strengths

This meta-analysis of individual participant data has several strengths: the variety of cohorts from all over Europe and the US, the overall large size enabling subgroup analyses, almost complete registry-based follow-ups, and the common statistical analysis strategy. We did not use repeated 25(OH)D measurements during follow-up because this would not have had a strong influence on the results. Previous analyses of the Tromsø and ESTHER study showed that 25(OH)D concentrations are fairly stable even up to 14 years.[8 36] The main limitation of this meta-analysis of prospective cohort studies is its observational nature. Despite adjustment for known potential confounders, we cannot rule out the possibility that the observed associations are confounded by other unmeasured factors, such as impairments of the immune system.[32] In addition, residual confounding by variables that could have been measured with a higher degree of detail (such as physical activity) is possible but not a likely explanation for the observed strong associations between 25(OH)D concentrations and mortality outcomes.

Conclusion

In this meta-analysis of eight prospective cohort studies, the lowest 25(OH)D quintile was associated with increased all-cause mortality, cardiovascular mortality, and cancer mortality (in subjects with a history of cancer). The inverse association across quintiles was curvilinear, and the effects were remarkably consistent across countries, sexes, seasons of blood draw, and age groups despite 25(OH)D cut-off values varying according to these characteristics. To identify cut-off values for vitamin D deficiency, 25(OH)D variation by geographic region, sex, and season might need to be taken into account.

What is already known on this subject

Mean serum 25-hydroxyvitamin D (25(OH)D) concentrations vary by country, sex, age, and season of blood draw

Vitamin D deficiency has been linked to all-cause and cardiovascular mortality, whereas results on cancer mortality have been inconsistent

What this study adds

In this large consortium of eight cohort studies from Europe and the United States, the bottom 25(OH)D quintile was associated with increased all-cause and cardiovascular mortality and with cancer mortality in subjects with a history of cancer but not in subjects without a history of cancer. These relationships were compellingly consistent across countries, sexes, age groups, and seasons of blood draw, although cut-off values for the 25(OH)D quintiles varied among the analyses

In clinical practice, cut-off values for vitamin D deficiency might need to be made region-, sex-, and season-specific to identify those in the population with the relatively lowest 25(OH)D concentrations

Vitamin D may play an important role in cancer prognosis

1   Zerwekh JE. Blood biomarkers of vitamin D status. *Am J Clin Nutr* 2008;87:1087S-91S.

2   van der Wielen RP, Löwik MR, van den Berg H, de Groot LC, Haller J, Moreiras O, et al. Serum vitamin D concentrations among elderly people in Europe. *Lancet* 1995;346:207-10.

3   Kestenbaum B, Katz R, de Boer I, Hoofnagle A, Sarnak MJ, Shlipak MG, et al. Vitamin D, parathyroid hormone, and cardiovascular events among older adults. *J Am Coll Cardiol* 2011;58:1433-41.

4   Chang EM, Kim YS, Won HJ, Yoon TK, Lee WS. Association between sex steroids, ovarian reserve, and vitamin D levels in healthy non-obese women. *J Clin Endocrinol Metab* 2014:jc20133873.

5   Mosekilde L. Vitamin D and the elderly. *Clin Endocrinol (Oxf)* 2005;62:265-81.

6   von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP; STROBE Initiative. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *BMJ* 2007;335:806-8.

7   Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. *JAMA* 2000;283:2008-12.

8   Schöttker B, Haug U, Schomburg L, Köhrle J, Perna L, Müller H, et al. Strong associations of 25-hydroxyvitamin D concentrations with all-cause, cardiovascular, cancer, and respiratory disease mortality in a large cohort study. *Am J Clin Nutr* 2013;97:782-93.

9   Hutchinson MS, Grimnes G, Joakimsen RM, Figenschau Y, Jorde R. Low serum 25-hydroxyvitamin D levels are associated with increased all-cause mortality risk in a general population: the Tromsø study. *Eur J Endocrinol* 2010;162:935-42.

10   Thorand B, Kolb H, Baumert J, Koenig W, Chambless L, Meisinger C, et al. Elevated levels of interleukin-18 predict the development of type 2 diabetes: results from the MONICA/KORA Augsburg Study, 1984-2002. *Diabetes* 2005;54:2932-8.

11   Melamed ML, Michos ED, Post W, Astor B. 25-hydroxyvitamin D levels and the risk of mortality in the general population. *Arch Intern Med* 2008;168:1629-37.

12   Peasey A, Bobak M, Kubinova R, Malyutina S, Pajak A, Tamosiunas A, et al. Determinants of cardiovascular disease and other non-communicable diseases in Central and Eastern Europe: rationale and design of the HAPIEE study. *BMC Public Health* 2006;6:255.

13   Thorand B, Zierer A, Huth C, Linseisen J, Meisinger C, Roden M, et al. Effect of serum 25-hydroxyvitamin D on risk for type 2 diabetes may be partially mediated by subclinical inflammation: results from the MONICA/KORA Augsburg study. *Diabetes Care* 2011;34:2320-2.

14   Karakas M, Thorand B, Zierer A, Huth C, Meisinger C, Roden M, et al. Low levels of serum 25-hydroxyvitamin D are associated with increased risk of myocardial infarction, especially in women: results from the MONICA/KORA Augsburg case-cohort study. *J Clin Endocrinol Metab* 2013;98:272-80.

15   Brouwer-Brolsma EM, Bischoff-Ferrari HA, Bouillon R, Feskens EJ, Gallagher CJ, Hypponen E, et al. Vitamin D: do we get enough? A discussion between vitamin D experts in order to make a step towards the harmonisation of dietary reference intakes for vitamin D across Europe. *Osteoporos Int* 2013;24:1567-77.

16   Geskus RB. Cause-specific cumulative incidence estimation and the fine and gray model under both left truncation and right censoring. *Biometrics* 2011;67:39-49.

17   DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials* 1986;7:177-88.

18   De Boer IH, Levin G, Robinson-Cohen C, Biggs ML, Hoofnagle AN, Siscovick DS, et al. Serum 25-hydroxyvitamin D concentration and risk for major clinical disease events in a community-based population of older adults: a cohort study. *Ann Intern Med* 2012;156:627-34.

19   Durup D, Jørgensen HL, Christensen J, Schwarz P, Heegaard AM, Lind B. A reverse J-shaped association of all-cause mortality with serum 25-hydroxyvitamin D in general practice: the CopD study. *J Clin Endocrinol Metab* 2012;97:2644-52.

20   Kohl M, Heinze G. PSHREG: A SAS macro for proportional and nonproportional subdistribution hazards regression with competing risk data. 2012. http://cemsiis.meduniwien.ac.at/kb.

21   Zittermann A, Iodice S, Pilz S, Grant WB, Bagnardi V, Gandini S. Vitamin D deficiency and mortality risk in the general population: a meta-analysis of prospective cohort studies. *Am J Clin Nutr* 2012;95:91-100.

22   Wang L, Song Y, Manson JE, Pilz S, März W, Michaëlsson K, et al. Circulating 25-hydroxy-vitamin D and risk of cardiovascular disease: a meta-analysis of prospective studies. *Circ Cardiovasc Qual Outcomes* 2012;5:819-29.

23   Chowdhury R, Kunutsor S, Vitezova A, Oliver-Williams C, Chowdhury S, Kiefte-de-Jong JC, et al. Vitamin D and risk of cause specific death: systematic review and meta-analysis of observational cohort and randomised intervention studies. *BMJ* 2014;348:g1903.

24   Boylan S, Lallukka T, Lahelma E, Pikhart H, Malyutina S, Pajak A, et al. Socio-economic circumstances and food habits in Eastern, Central and Western European populations. *Public Health Nutr* 2011;14:678-87.

25   Yin L, Ordóñez-Mena JM, Chen T, Schöttker B, Arndt V, Brenner H. Circulating 25-hydroxyvitamin D serum concentration and total cancer incidence and mortality: a systematic review and meta-analysis. *Prev Med* 2013;57:753-64.

26   Maalmi H, Ordóñez-Mena JM, Schöttker B, Brenner H. Serum 25-hydroxyvitamin D levels and survival in colorectal and breast cancer patients: systematic review and meta-analysis of prospective cohort studies. *Eur J Cancer* 2014;50:1510-21.

27   Theodoratou E, Tzoulaki I, Zgaga L, Ioannidis JP. Vitamin D and multiple health outcomes: umbrella review of systematic reviews and meta-analyses of observational studies and randomised trials. *BMJ* 2014;348:g2035.

28 Schöttker B, Jansen EH, Haug U, Schomburg L, Köhrle J, Brenner H. Standardization of misleading immunoassay based 25-hydroxyvitamin D levels with liquid chromatography tandem-mass spectrometry in a large cohort study. *PLoS One* 2012;7:e48774.

29 Carter GD, Carter R, Jones J, Berry J. How accurate are assays for 25-hydroxyvitamin D? Data from the international vitamin D external quality assessment scheme. *Clin Chem* 2004;50:2195-7.

30 Chun RF. New perspectives on the vitamin D binding protein. *Cell Biochem Funct* 2012;30:445-56.

31 Holick MF. The vitamin D deficiency pandemic: a forgotton hormone important for health. *Public Health Rev* 2010;32:267-83.

32 Autier P, Boniol M, Pizot C, Mullie P. Vitamin D status and ill health: a systematic review. *Lancet Diabetes Endocrinol* 2014;2:76-89.

33 Bjelakovic G, Gluud LL, Nikolova D, Whitfield K, Wetterslev J, Simonetti RG, et al. Vitamin D supplementation for prevention of mortality in adults. *Cochrane Database Syst Rev* 2014;1:CD007470.

34 Kupferschmidt K. Uncertain verdict as vitamin D goes on trial. *Science* 2012;337:1476-8.

35 Committee to Review Dietary Reference Intakes for Vitamin D and Calcium. *Food and Nutrition Board, Institute of Medicine. Dietary reference intakes for calcium and vitamin D.* National Academies Press, 2011.

36 Jorde R, Sneve M, Hutchinson M, Emaus N, Figenschau Y, Grimnes G. Tracking of serum 25-hydroxyvitamin D levels during 14 years in a population-based study and during 12 months in an intervention study. *Am J Epidemiol* 2010;171:903-8.

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Tables

Table 1| Description of cohort studies included in meta-analyses of the associations between serum 25-hydroxyvitamin D concentrations and mortality outcomes

Study name	Place and years of recruitment	25(OH)D assay*	Sampling	Study population aged 50-79 years				Mortality follow-up					
				Total	With measured 25(OH)D	Included in analysis†	Age range	Registry	End of follow-up	Mean (SD) follow-up (years)	Total deaths	CVD deaths	Cancer deaths
ESTHER	Federal state Saarland, south west Germany, 2000-02	Men: IDS-iSYS; women: Diasorin. Both standardised to LC-MS/MS	Total cohort	9 949	9 563	9 083	50-74	State wide	2012	11.0 (2.2)	1 338	420	531
Tromsø	Town Tromsø, north Norway, 1994-95 (Tromsø 4)	Modular E170 Roche	Total cohort	10 262	6 571	4 406‡ non-smokers	50-79	Country wide	2010	14.2 (3.6)	1 141	498	413
MONICA/KORA	Town Augsburg and district, south Germany, 1984-85, 1989-90, 1994-95	IDS-iSYS	Random subsample§	6 316	962	939	50-74	Region wide	2009	15.9 (5.7)	366	165	66
SENECA	19 towns in 12 European countries, 1988-89	Competitive protein binding assay	Random subsample¶	2 586	823	661**	70-75	Region wide	1998	8.3 (2.9)	270	94	51
HAPIEE Czech Republic	6 towns in Czech Republic, 2002-05	DIAsource ELISA	Nested case-control††	7 099	2 280	2 029	50-73	Country wide	2011	7.9 (1.5)	453	165	208
HAPIEE Poland	Town Krakow, Poland, 2002-05	DIAsource ELISA	Nested case-control††	8 121	1 879	1 700	50-71	Region wide	2011	7.0 (1.2)	411	133	194
HAPIEE Lithuania	Town Kaunas, Lithuania, 2006-08	DIAsource ELISA	Nested case-control††	6 339	1 691	1 574	50-74	Region wide	2011	4.2 (1.2)	368	146	152
NHANES III	USA 1988-94	Diasorin RIA	Total cohort	6 822	5 740	5 626	50-79	Country wide	2006	12.3 (4.5)	2 348	1 003	612
Total	—	—	—	57 376	29 509	26 018	50-79	—	—	—	6 695	2 624	2 227

25(OH)D=25-hydroxyvitamin D; CVD=cardiovascular disease; ESTHER=Epidemiologische Studie zu Chancen der Verhütung, Früherkennung und optimierten Therapie chronischer Erkrankungen in der älteren Bevölkerung [German]; HAPIEE=Health, Alcohol and Psychosocial factors In Eastern Europe; LC-MS/MS=liquid chromatography coupled with tandem mass spectrometry; MONICA/KORA=Monitoring of trends and determinants in cardiovascular disease—Cooperative Health Research in the Region of Augsburg; NHANES=National Health and Nutrition Examination Survey; SENECA=Survey in Europe on Nutrition and the Elderly: a Concerted Action.

*Manufacturers of 25(OH)D assays: IDS-iSYS (Immunodiagnostic Systems, Frankfurt Main, Germany); Diasorin-Liaison (Diasorin, Stillwater, USA); DIAsource ELISA (DIAsource ImmunoAssays, Louvain-La-Neuve, Belgium); Modular E170 Roche (Roche Diagnostics, Rotkreuz, Swizerland) and competitive protein-binding assay (not commercially available).

†Numbers are lower than numbers with measured 25(OH)D due to missing covariates.

‡2 012 smokers were excluded from the Tromsø study because of concerns regarding the validity of the Roche 25(OH)D assay in smokers.

§Randomly selected stratified survey, sex and 10 year age groups.

¶25(OH)D concentrations were measured in random samples stratified by age and sex in those 16 of the 19 towns with blood collected during January and March 1989.

**Further two towns with 73 participants with measured 25(OH)D were excluded from this analysis because these towns did not participate in the mortality follow-up, leaving the following 14 towns from 12 European countries for inclusion in this analysis. North Europe (Latitude >50° north): Elverum, Norway; Roskilde, Denmark; Culemborg, Netherlands; Hamme, Belgium; central Europe (Latitude 45-59° north): Haguenau, France; Monor, Hungary; Burgdorf, Switzerland; Bellinzona, Switzerland;

Table 1 (continued)

				Study population aged 50-79 years			Mortality follow-up					
Study name	Place and years of recruitment	25(OH)D assay*	Sampling	Total	With measured 25(OH)D	Included in analysis†	Age range	Registry	End of follow-up	Mean (SD) follow-up (years)	Total deaths	CVD deaths	Cancer deaths

Yverdon, Switzerland; Romans, France; south Europe (Latitude <45° north): Betanzos, Spain; Fara Sabina, Magliano Sabina, Poggio Mirteto, Italy; Vila Franca de Xira, Portugal; Anogia, Archanes, Greece.

††25(OH)D was measured in cases (any death or non-fatal cardiovascular disease (CVD) event during follow-up) and controls frequency matched by sex and 5 year age groups. For this analysis on mortality, subjects with non-fatal CVD during follow-up were excluded from the cases in a first step. In order to add a representative sample of subjects with non-fatal CVD events to the controls, in a second step, the observed proportion of non-fatal CVD events in each age and sex specific stratum in the total cohort was calculated. In a third step, these proportions were multiplied by the sample sizes in each stratum in the controls with measured 25(OH)D. In a fourth step, the resulting numbers were randomly selected in each stratum from the group of subjects with non-fatal CVD during follow-up and added to the controls.

Table 2| 25-hydroxyvitamin D concentrations by baseline characteristics of the study populations

Characteristic	ESTHER	Tromsø	MONICA-KORA	SENECA†	NHANES‡	HAPIEE Czech Rep Controls	Cases	HAPIEE Poland Controls	Cases	HAPIEE Lithuania Controls	Cases
Age (years):											
50-59	47 (35-63)*	56 (46-67)*	40 (29-54)	NA	60 (44-80)*	26 (18-39)	21 (14-30)	38 (29-48)	34 (24-43)	46 (37-58)	40 (30-56)
60-69	46 (35-62)*	53 (42-64)*	42 (31-56)	NA	61 (45-80)*	31 (21-43)	24 (16-36)	39 (30-51)	36 (26-49)	46 (37-59)	41 (30-53)
70-79	43 (33-57)*	51 (41-63)*	36 (27-50)	33 (23-47)	64 (48-82)*	29 (20-45)	34 (21-56)	41 (34-49)	43 (28-76)	46 (37-59)	45 (33-56)
Sex:											
Women	43 (33-54)*	52 (42-64)*	37 (29-49)*	31 (21-44)*	57 (42-76)*	26 (18-38)*	20 (14-29)*	37 (28-47)*	32 (23-40)*	44 (35-56)*	38 (29-49)*
Men	52 (37-71)*	56 (46-66)*	44 (31-60)*	35 (26-49)*	67 (50-85)*	31 (21-44)*	26 (17-38)*	40 (31-51)*	37 (27-50)*	47 (37-60)*	44 (32-57)*
Season§:											
Winter	40 (31-54)*	51 (41-63)*	36 (27-52)*	32 (22-45)*	58 (43-77)*	28 (19-40)*	24 (16-35)*	38 (29-49)	35 (25-47)	41 (35-51)*	40 (30-55)
Spring	40 (32-52)*	57 (46-68)*	37 (27-49)*	37 (27-53)*	58 (43-76)*	28 (18-40)*	21 (14-30)*	39 (30-50)	35 (26-47)	44 (35-56)*	38 (29-50)
Summer	53 (41-70)*	64 (53-75)*	49 (36-61)*	NA	67 (49-84)*	NA	NA	NA	NA	48 (40-58)*	45 (31-51)
Autumn	50 (37-67)*	51 (42-63)*	48 (38-65)*	NA	65 (48-85)*	35 (24-45)*	28 (19-43)*	43 (33-54)	35 (23-49)	51 (40-64)*	45 (34-58)
Education¶:											
Low	45 (34-61)*	52 (41-63)*	35 (27-46)*	32 (23-47)*	60 (45-79)*	25 (16-38)*	20 (13-32)*	36 (28-46)*	32 (25-43)	46 (38-56)	45 (34-55)
Medium	47 (35-64)*	55 (45-67)*	42 (31-57)*	36 (26-50)*	62 (46-81)*	29 (20-41)*	24 (16-35)*	39 (30-50)*	37 (25-48)	45 (37-57)	39 (29-50)
High	49 (35-67)*	57 (46-68)*	46 (33-64)*	40 (29-56)*	65 (47-84)*	34 (21-45)*	29 (21-38)*	39 (30-53)*	35 (26-46)	47 (37-60)	44 (31-56)
BMI:**											
Normal or underweight	47 (35-63)*	56 (45-67)*	41 (30-57)	32 (22-51)*	65 (47-86)*	30 (18-44)*	23 (17-40)	39 (29-52)*	31 (22-42)	46 (36-58)	36 (26-56)
Overweight	47 (35-64)*	54 (44-65)*	41 (31-55)	35 (26-48)*	63 (47-82)*	31 (21-43)*	24 (15-39)	40 (32-51)*	37 (28-49)	48 (38-61)	44 (31-54)
Obesity	42 (33-57)*	49 (39-60)*	38 (27-53)	30 (22-43)*	57 (42-74)*	28 (18-39)*	25 (15-33)	37 (29-46)*	36 (26-46)	45 (36-56)	42 (33-51)
Smoking:											
Never	44 (34-59)*	52 (42-64)*	39 (29-53)*	32 (22-46)*	61 (46-79)*	30 (21-42)*	25 (14-39)*	40 (31-52)*	34 (26-44)*	46 (37-59)*	43 (31-52)*
Former	50 (37-68)*	55 (44-66)*	48 (34-61)*	38 (28-52)*	66 (49-85)*	31 (20-43)*	27 (17-38)*	39 (30-50)*	39 (30-50)*	48 (38-61)*	46 (35-57)*
Current	41 (31-58)*	Excluded	37 (26-51)*	31 (22-46)*	56 (41-78)*	26 (18-38)*	20 (15-29)*	37 (29-46)*	32 (22-45)*	43 (33-54)*	35 (26-49)*
Vigorous physical activity:											
No	44 (33-58)*	52 (42-64)*	37 (28-51)*	32 (23-46)*	58 (43-77)*	28 (18-40)*	22 (14-33)	36 (28-45)*	35 (26-43)	45 (35-58)*	39 (30-51)*
Yes	49 (36-66)*	57 (46-68)*	48 (35-64)*	44 (34-53)*	67 (50-86)*	30 (20-42)*	25 (16-38)	40 (31-51)*	35 (25-49)	47 (37-59)*	45 (32-57)*
History of diabetes:											
No	46 (35-62)*	54 (43-65)*	41 (30-55)	32 (23-46)	64 (47-83)*	30 (20-42)	25 (17-36)*	39 (30-50)	35 (25-47)	46 (37-59)*	44 (31-55)*
Yes	44 (32-59)*	51 (42-60)*	40 (30-52)	33 (25-42)	55 (41-72)*	29 (18-39)	19 (12-33)*	39 (29-48)	37 (27-47)	40 (33-50)*	38 (29-43)*

Table 2 (continued)

Characteristic	Median (interquartile range) concentration of 25(OH)D (nmol/L)*					HAPIEE Czech Rep		HAPIEE Poland		HAPIEE Lithuania	
	ESTHER	Tromsø	MONICA-KORA	SENECA†	NHANES‡	Controls	Cases	Controls	Cases	Controls	Cases
History of hypertension:											
No	47 (35-63)*	52 (41-64)*	42 (31-57)*	33 (23-47)	64 (47-83)*	30 (19-43)	24 (16-35)	39 (30-49)	35 (25-49)	46 (36-59)	40 (30-56)
Yes	44 (34-60)*	54 (44-66)*	38 (28-53)*	32 (22-44)	60 (44-80)*	29 (20-41)	24 (15-36)	39 (30-50)	35 (26-46)	46 (37-58)	42 (31-52)
History of CVD:											
No	46 (34-62)	54 (43-65)	41 (29-55)	32 (23-46)	62 (46-81)	30 (20-42)	24 (16-36)	39 (30-50)	36 (26-49)	45 (36-59)*	42 (31-54)
Yes	46 (34-64)	54 (44-67)	41 (33-54)	34 (22-46)	60 (44-81)	29 (20-40)	23 (14-30)	37 (28-49)	33 (25-42)	48 (40-61)*	41 (29-50)
History of cancer:											
No	46 (34-62)	54 (43-65)*	43 (32-57)	32 (23-46)	61 (45-81)*	30 (20-42)	24 (16-36)	39 (30-50)	35 (25-47)	46 (37-59)	42 (31-53)
Yes	44 (33-60)	52 (42-63)*	33 (23-57)	34 (14-40)	65 (47-83)*	32 (20-43)	21 (12-28)	39 (32-46)	34 (26-47)	45 (37-59)	43 (29-55)

25(OH)D=25-hydroxyvitamin D; BMI=body mass index; CVD=cardiovascular disease; NA=not applicable.

*Statistically significant (P<0.05) by Wilcoxon rank-sum test.

†Regional differences in SENECA: median (interquartile range) 25(OH)D concentration (nmol/L); north Europe 39 (28-49), central Europe 34 (26-50), south Europe 26 (18-34).

‡Regional differences in NHANES: median (interquartile range) 25(OH)D (nmol/L); northeast 61 (47-82), mid-west 66 (47-84), south 62 (45-82), west 58 (44-76). Differences by race and ethnicity: non-Hispanic white 70 (54-89), non-Hispanic black 48 (37-65), Mexican-American 56 (44-75), other (mainly Asian) 58 (46-78).

§Winter=December-February; Spring=March-May; Summer=June-August; Autumn=September-November.

¶Definition of categories of education by highest level of full-time education or years of full-time educations: low (primary school or less or ≤9 years), medium (more than primary school but less than college or university or 10-12 years), high (college or university or ≥13 years).

**Definition of categories of weight by World Health Organization BMI categories: normal or underweight (<25 kg/m²), overweight (25-<30); obesity (≥30).

Figures

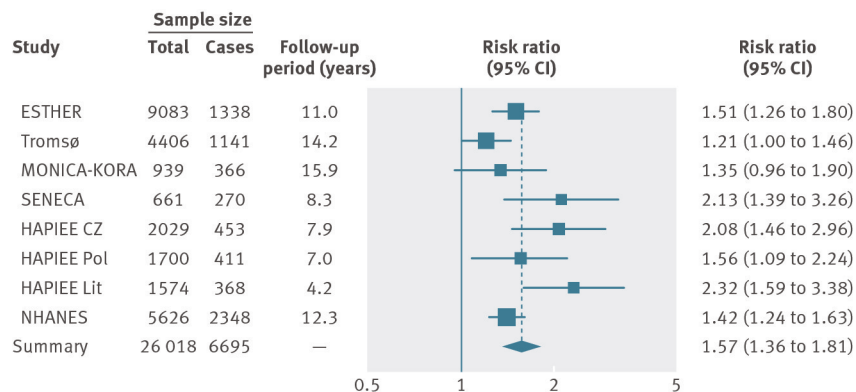|	Sample size				
Study	Total	Cases	Follow-up period (years)	Risk ratio (95% CI)	Risk ratio (95% CI)
ESTHER	9083	1338	11.0		1.51 (1.26 to 1.80)
Tromsø	4406	1141	14.2		1.21 (1.00 to 1.46)
MONICA-KORA	939	366	15.9		1.35 (0.96 to 1.90)
SENECA	661	270	8.3		2.13 (1.39 to 3.26)
HAPIEE CZ	2029	453	7.9		2.08 (1.46 to 2.96)
HAPIEE Pol	1700	411	7.0		1.56 (1.09 to 2.24)
HAPIEE Lit	1574	368	4.2		2.32 (1.59 to 3.38)
NHANES	5626	2348	12.3		1.42 (1.24 to 1.63)
Summary	26 018	6695	—		1.57 (1.36 to 1.81)

Fig 1 Risk ratios of all-cause mortality for bottom versus top quintiles of 25-hydroxyvitamin D concentration in eight cohorts (meta-analysis of individual participant data)
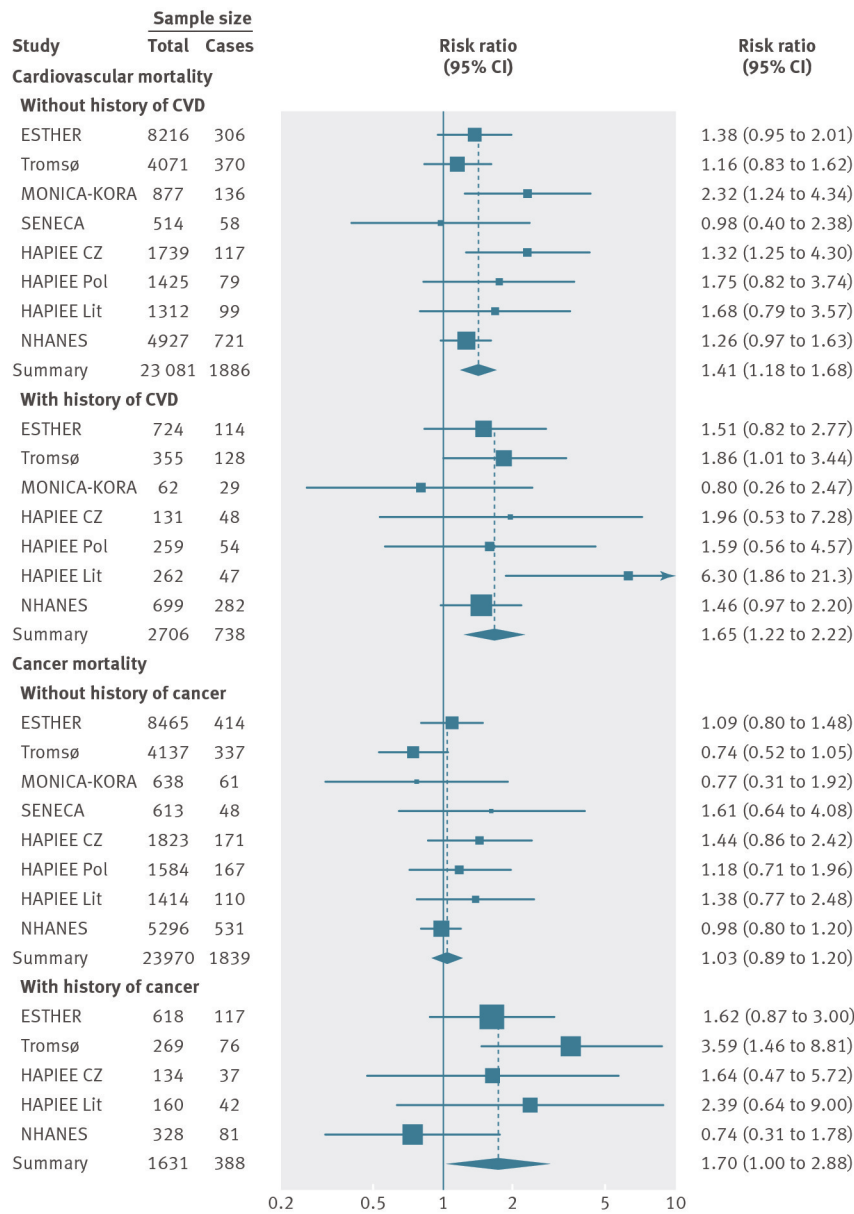
Fig 2 Risk ratios of cardiovascular mortality in subjects with and without a history of cardiovascular disease (top panel) and of cancer mortality in subjects with and without a history of cancer (bottom panel) for bottom versus top quintiles of 25-hydroxyvitamin D concentration in eight cohorts (meta-analysis of individual participant data)
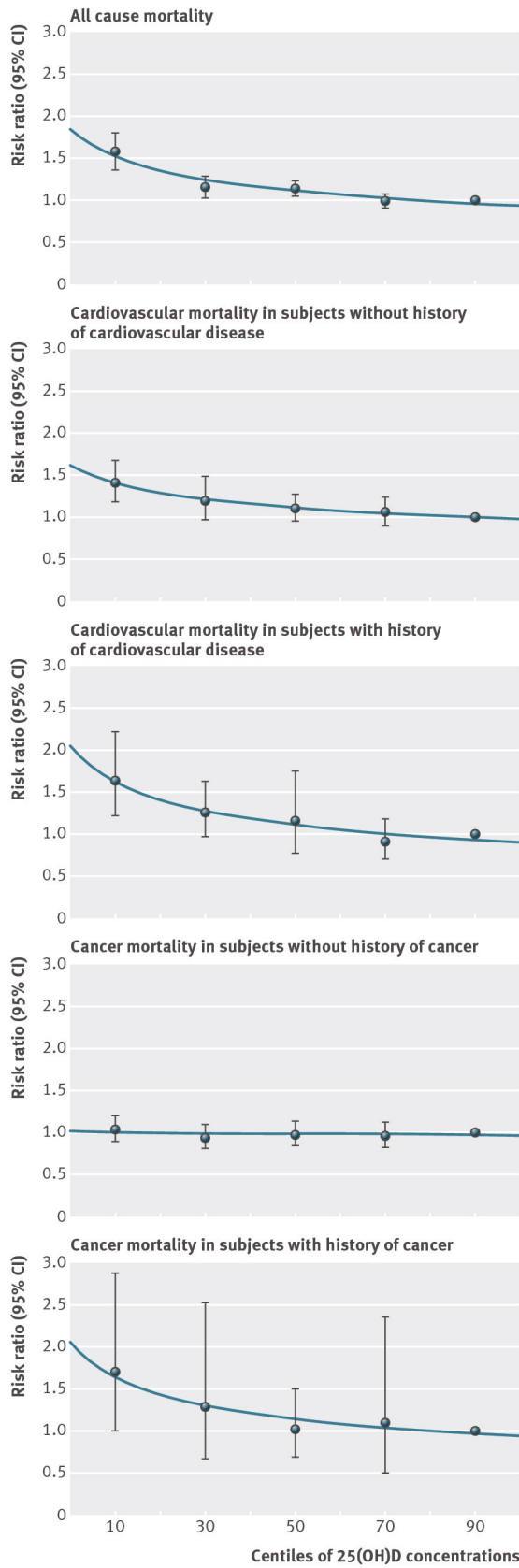
Fig 3 Dose-response trend of pooled effect estimates for the comparison of 25-hydroxyvitamin D quintiles (top quintile as reference) with respect to mortality outcomes
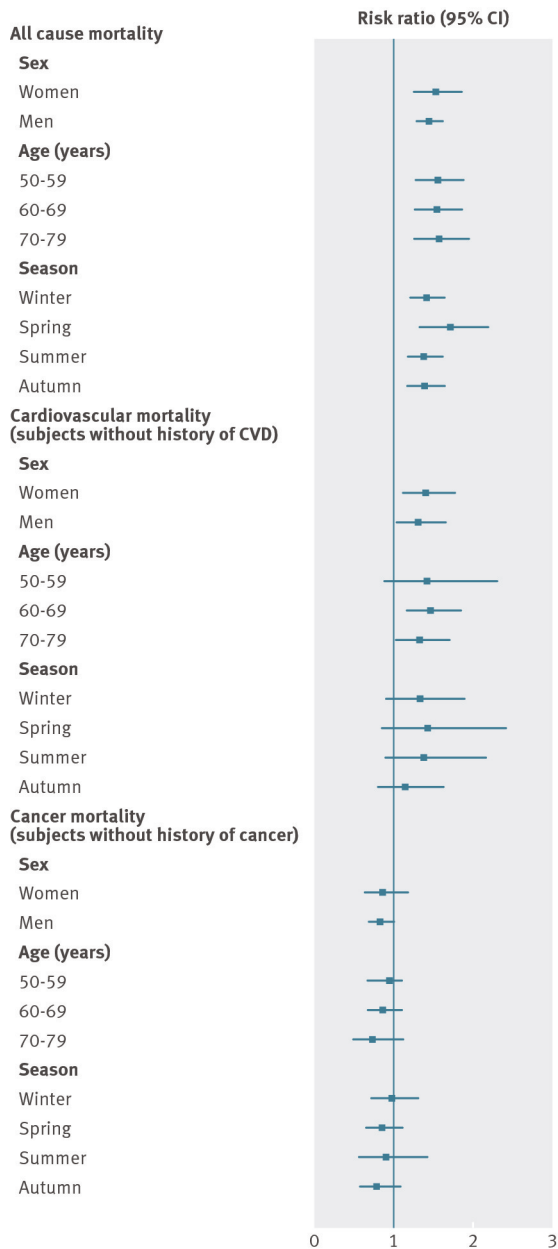
Fig 4 Sex-, age-, and season-specific pooled effect estimates for bottom versus top quintiles of 25-hydroxyvitamin D concentration with respect to all-cause mortality, cardiovascular mortality in subjects without a history of cardiovascular disease (CVD), and cancer mortality in subjects without a history of cancer