Vitamin D deficiency and mortality risk in the general population: a meta-analysis of prospective cohort studies[1–4]

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ABSTRACT

Background: Low vitamin D status may increase mortality risk.

Objective: We used nonparametric ("highest compared with lowest" categories) and parametric (>2 categories) statistical models to evaluate associations of 25-hydroxyvitamin D [25(OH)D] serum concentrations and mortality in observational studies among general populations.

Design: We searched PubMed, EMBASE, Web of Science, and reference lists for relevant articles. We included studies that contained data on relative risks (RRs) for mortality for different 25(OH)D concentrations, which included a corresponding measure of uncertainty, and this yielded 14 prospective cohort studies that involved 5562 deaths out of 62,548 individuals. We applied log-transformed RRs and CIs, adjusted for the maximal number of confounding variables. In the parametric model, which is based on 11 studies and 59,231 individuals, we used the lowest quantile as the reference category.

Results: For "highest compared with lowest" categories of 25(OH)D, the estimated summary RR of mortality was 0.71 (95% CI: 0.50, 0.91). In the parametric model, the estimated summary RRs (95% CI) of mortality were 0.86 (0.82, 0.91), 0.77 (0.70, 0.84), and 0.69 (0.60, 0.78) for individuals with an increase of 12.5, 25, and 50 nmol 25(OH)D serum values/L, respectively, from a median reference category of ~27.5 nmol/L. There was, however, no significant decrease in mortality when an increase of ~87.5 nmol/L above the reference category occurred.

Conclusion: Data suggest a nonlinear decrease in mortality risk as circulating 25(OH)D increases, with optimal concentrations ~75–87.5 nmol/L. *Am J Clin Nutr* 2012;95:91–100.

INTRODUCTION

Vitamin D deficiency is increasing globally and is considered an important public health problem (1–3). Vitamin D is derived mainly from UV-B–induced synthesis in the skin, but current lifestyle and environmental factors often limit sunlight exposure, which results in a high prevalence of vitamin D deficiency (1–3). 25(OH)D[5], which is produced by hydroxylation of vitamin D in the liver and then released into the circulation, is the generally accepted indicator of human vitamin D status (3). Based on several clinical outcomes, target concentrations of between 75 and 100 nmol serum 25(OH)D/L (divide by 2.496 to convert to nanograms per liter) have been proposed (4, 5). However, the Institute of Medicine has recently declared that circulating concentrations of only 50 nmol/L are sufficient for the general population (6), a statement that has been criticized by vitamin D researchers (7).

Almost all human tissues express the vitamin D receptor, and vitamin D metabolites regulate ~3% of the human genome (3, 8). Randomized, controlled trials have largely, but not consistently, shown that vitamin D supplementation reduces fractures and falls (9–11). Beyond the beneficial effects on bone and musculoskeletal health, evidence increasingly suggests that vitamin D metabolites may protect against colorectal cancer (12), cardiovascular (13) and autoimmune diseases (14), and infections (15). A meta-analysis of randomized, controlled trials that were designed mainly to evaluate musculoskeletal outcomes among frail, elderly people showed a significant reduction of total mortality in the vitamin D treatment groups (16). In the majority of studies included in that meta-analysis, baseline circulating concentrations were <50 nmol 25(OH)D/L. Even in apparently healthy subjects, circulating concentrations <50 nmol 25(OH)D/L are common around the world (1, 17, 18). It is thus of great interest for health authorities to know whether or not indicators of global health, such as mortality, are related to the population's vitamin D status.

There is some evidence for a nonlinear association of 25(OH)D with outcomes such as breast cancer (19), CVD incidence (20), and all-cause mortality (21). We performed a meta-analysis to evaluate associations of 25(OH)D serum concentrations and mortality in observational studies among general populations, with the use of nonparametric and parametric statistical models for assessment of dose–risk relations.

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[5] Abbreviations used: AIC, Akaike's information criterion; CVD, cardiovascular disease; MFHS, Mini-Finland Health Survey; 25(OH)D, 25-hydroxyvitamin D.

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MATERIALS AND METHODS

We planned, conducted, and reported this systematic review in accordance with Meta-analysis of Observational Studies in Epidemiology guidelines (22).

Search strategy

We performed a systematic literature search through September 2010 of PubMed (http://www.ncbi.nlm.nih.gov/pubmed), Embase (http://www.embase.com), and ISI Web of Science (apps.webofknowledge.com) without restrictions, with the use of the following search terms: [vitamin D or 25(OH)D or cholecalciferol or calcidiol or calcitriol] and (overall or total or all-cause mortality or cardiovascular mortality or death). We searched for the keywords in the headers and in the abstract, when available. We also manually searched references that the selected articles and published reviews cited. Two academic investigators carried out the literature search independently. Group discussion resolved any disagreement about article selection. Additional studies and missing information in published reports were searched via direct author contact and referral by experts in the field (eg, conferences and meetings).

Selection

Inclusion criteria

Inclusion criteria were observational studies published as original articles that reported RR estimates of, or crude data on, overall mortality by serum 25(OH)D concentrations. We selected studies that reported the minimal information on RRs necessary to perform meta-analyses: sufficient information to estimate the RR and 95% CIs for the different quantiles used to categorize serum 25(OH)D concentrations (ORs, RRs, or crude data, and corresponding SEs, variances, CIs, or *P* values of the significance of the estimates). For the parametric models, only studies with >2 categories compared with reference categories were included.

When results of the same study were published in more than one article, we used only the most recent or the one with the largest sample of individuals.

We extracted information on serum 25(OH)D concentrations across all published categories to construct dose–response models.

We applied no language or time restrictions.

Exclusion criteria

We excluded studies or estimates when *1*) study populations differed from the general population with respect to life expectancy (eg, cancer patients); or *2*) participants suffered from any disease or condition that might interfere with vitamin D metabolism, such as chronic kidney disease or diabetes.

Main outcomes

Outcome was all-cause mortality, defined as such in the underlying studies.

Data extraction

We performed data extraction with the use of a data set designed before we conducted the data searches. This data set was constructed as a series of records and included information about the study, participants' characteristics, and dose–response relationships. In brief, we recorded study characteristics such as date of publication, geographic origin, and setting. We also extracted data about the study participants. With regard to dose–response information, we recorded the value of 25(OH)D concentrations (expressed as nmol/L) assigned as the midpoints of the ranges of the reported categories, as suggested previously (23). We documented frequency counts and adjusted estimates of log RRs and corresponding 95% CIs for each exposure level. Finally, we recorded covariates that described the characteristics of the study.

Most studies presented the data in nanograms per milliliter. With the use of a correction factor of 2.496, we converted 25(OH)D concentrations presented in nanograms per milliliter to nanomoles per liter.

Statistical analysis

Estimates of risk and model fitting

A nonparametric meta-analysis was carried out in all studies that presented at least one RR for one category compared with the reference level to compare the highest and lowest categories.

We transformed every measure of association, adjusted for the maximal number of confounding variables, and the corresponding CIs into log RRs, and we calculated the corresponding variance with the use of the Greenland (24) formula. For studies that lacked estimates, we calculated crude estimates from tabular data. We used Woolf's formula to evaluate the SE of the log RRs (25). We assessed the homogeneity of the effects across studies with the use of the large-sample test based on the chi-square statistic. A further measure of heterogeneity, I^2, a transformation of the square root of the chi-square divided by its df, has been considered as a way to compare heterogeneities with regard to different numbers of pooled studies. Greater values of I^2 indicate greater heterogeneity (26).

For the parametric models, we chose the lowest quantile as the reference category (27, 28). In fact, the highest category is generally open-ended and too heterogeneous. We observed >50 nmol of difference/L among the upper categories of included studies, whereas the highest difference among the upper level of the reference category was 25 nmol/L only. Because 25(OH)D concentrations never reach null values, we adopted the method proposed by Liu et al (27) for meta-analysis of studies with nonzero exposure dose as reference. We investigated the relation between 25(OH)D concentrations and mortality, based on the contrast of each vitamin D concentration with the reference within a study. As a consequence, the value for the linear term is the difference of each exposure level from the reference category ($x_j - x_0$) (27). Because log RR for the reference category is zero (RR = 1 when $x_j = x_0$), our model is forced to pass through the origin, which results in a no-intercept regression model (23).

When the reference category was not the lowest, to obtain CIs of RR for each 25(OH)D category, we needed a measure of the risk estimate variability. We used the method proposed by Greenland et al (29) to estimate the covariance useful to calculate the CIs. When crude data were not available from the original

articles, we did not estimate covariances, which resulted in more conservative CIs.

To explore the relation between 25(OH)D serum concentrations and overall mortality, we applied an approach that implemented a random-effects meta-regression model in a nonlinear dose–risk relation framework (30). This method allows one to deal simultaneously with *1*) the correlation within the same study among reported dose-specific RRs due to a common reference group; *2*) the heterogeneity between studies; and *3*) the nonlinear trend components of the dose–risk relation, which allow one to fit data among a rich set of possible functions that includes some so-called "U-shaped" and "J-shaped" relations.

The summary estimates of the effect were based on a 2-step procedure. First, for each study we estimated the 2 trend coefficients (β_1 and β_2) of the following second-order fractional polynomial log-linear model: $\ln(\mathrm{RR}) = \beta_1 x^{p_1} + \beta_2 x^{p_2}$, and p_1 and p_2 were chosen from a predefined set, $P = (-2, -1, -0.5, 0, 0.5, 1, 2, 3)$. The powers are expressed in accordance with the Box–Tidwell transformation (31), in which x^{p_1} denotes x^{p_1} if $p_i \neq 0$ and $\ln(x)$ if $p_i = 0$. Consideration of the family of second-order fractional polynomials specifically is worthwhile, because the first-order models can model only monotonic curves and because fractional polynomials with an order >2 are rarely required in practice (28).

In the second step, the pooled dose–response relation is estimated in accordance with a bivariate random-effects model, which estimates the summary trend components, along with an estimate of the covariance matrix, with the use of restricted maximum-likelihood estimates. We estimated the summary RR with the use of standard mixed-model software packages, such as SAS PROC MIXED (SAS Institute) (32).

The best fit among the family of models thus generated is defined as that with the lowest AIC. We also fitted a linear model within each study to estimate the RR per unit increase of serum 25(OH)D, to evaluate the adequacy of the optimal bivariate random-effects model with respect to the conventional linear one, and compared the AICs in the 2 models. When sufficient information was published (the number of subjects at each serum concentration category), we fitted the linear model in accordance with Greenland and Longnecker (33). This method provides the natural logarithm of the RR and an estimator of its SE, and the fact that the estimates for separate categories depend on the same reference group is taken into account. When the number of subjects at each serum concentration category was not available from a publication, we calculated coefficients and ignored the correlation between the estimates of risk at the separate exposure levels.

Heterogeneity and sensitivity analyses

We conducted several sensitivity analyses to evaluate the stability of the pooled estimates and to examine changes in results after exclusion or inclusion of specific studies or single estimates considered in the inclusion criteria. We performed meta-regressions, subgroup analyses, and sensitivity analyses to assess between-study heterogeneity (34). Because the number of studies was limited and the model with 2 parameters is complex, the meta-regression analysis was carried out based on the classical model, which compares the highest and lowest categories.

To investigate variability among the estimates we looked at all the possible factors that could influence the estimates: assigned values used for reference categories of 25(OH)D, upper limits of 25(OH)D, features of the study populations, assay method, and length of follow-up. To investigate whether publication bias might affect the validity of the estimates, we constructed funnel plots of the regression of log RR on the sample size, weighted by the inverse of the pooled variance (35).

RESULTS

Study selection and data extraction

The detailed steps of our systematic literature search are shown in **Figure 1**. We identified 1585 abstracts in PubMed, 2404 abstracts in EMBASE, and 1699 abstracts in ISI Web of Science. Of these 5688 abstracts, we excluded 5655 on the basis of screening or titles, which left 33 articles to source in full text. We identified no additional references from a search of reference lists of the 33 full-text articles. After further inspection we excluded 16 articles from the 33 full-text articles. Three studies gave no information on 25(OH)D concentrations, and 13 studies provided no data to estimate the risk of overall mortality. From the 17 studies that remained eligible for inclusion in the meta-analysis, we excluded 2 articles (36, 37) because they overlapped NHANES III data from Melamed et al (21) and investigated only cause-specific mortality (37). Three articles (38–40) were included only in the nonparametric statistical model because the authors presented the risk estimate for only one category compared with baseline exposure, and that information is not sufficient to investigate nonlinear trends. One study (41) was excluded because the population comprised patients that

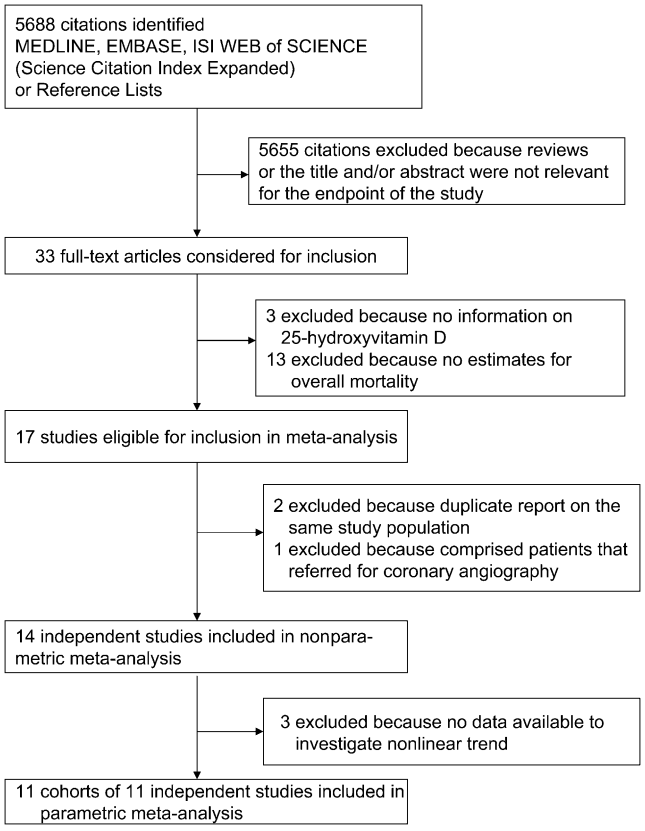

FIGURE 1. Flowchart of selection of studies for inclusion in the meta-analysis of 25-hydroxyvitamin D and overall mortality.

were referred for coronary angiography in a tertiary-care medical center. Thus, we ultimately could include 14 independent studies of 14 cohorts in the nonparametric statistical model and 11 cohorts in the parametric statistical models of our meta-analysis. Our search did not identify relevant prospective, nested, case-control studies or case-cohort studies. Moreover, we did not identify articles of interest for our meta-analysis in languages other than English.

Study characteristics

The characteristics of the 14 prospective cohort studies that we included in the final nonparametric analysis are shown in **Table 1**. All studies were published between 2006 and 2010. Overall, the studies include 5562 deaths out of 62,548 participants. All studies reported results for overall mortality, except for the MHFS study (44), which was based on cardiovascular mortality. We included this investigation in our meta-analysis because cardiovascular mortality was the main cause of death. However, we evaluated in a sensitivity analysis the effect of inclusion of this study. Four studies were conducted in the United States, one in Japan, and one in New Zealand. Eight studies were European. All studies were community based.

Two articles (43, 48) included only white participants, 3 (21, 45, 50) included a mixed-ethnicity group, and 9 (38–40, 42, 44, 46, 47, 49, 51) did not specify the ethnicity of their study population. The mean age of the participants ranged from 45 to 80 y. The mean follow-up ranged from 1.3 to 27 y. Three studies (38, 39, 46) included only women and 3 other studies (47, 50, 51) only men.

Overall mortality rates according to 25(OH)D category of the 11 studies included in the parametric models and the adjustments made for confounding are shown in **Table 2**.

Effects of 25(OH)D on total mortality risk

In the nonparametric meta-analysis, the summary estimate for highest compared with lowest categories of 25(OH)D showed a significant mortality risk reduction of 0.71 (95% CI: 0.50, 0.91; I^2 = 58%) (**Figure 2**). In the parametric analyses, the median of the assigned reference categories was 27.5 nmol/L (IQR: 20.0–32.5). The random-effects model with power terms $p_1 = 1$ and $p_2 = 2$ presented the lowest AIC value among the estimable second-order fractional polynomial models tested. The best-fitting model presented the following estimates: $\beta_1 = -0.085$ (95% CI: −0.120, −0.050; $P = 0.0003$) and $\beta_2 = 0.0018$ (95% CI: 0.0008, 0.0030; $P = 0.0045$). This model fitted significantly better than did the simple linear one (change in deviance = 107).

The study-specific dose–risk functions, with the best-fitting fractional polynomial model, along with the RR estimates and their 95% CIs, are plotted in **Figure 3**. The overall pooled dose–risk function and the 95% CIs are shown in **Figure 4**. The estimated summary RRs of mortality, obtained with the best-fitting fractional polynomial model, were 0.86 (95% CI: 0.82, 0.91), 0.77 (95% CI: 0.70, 0.84), and 0.69 (95% CI: 0.60, 0.78) for individuals with an increase of 12.5, 25, and 50 nmol 25(OH)D serum values/L, respectively, from a median reference category of 27.5 nmol/L. In terms of risk assessment, the meta-analysis shows that an increase in 25(OH)D serum concentrations is

TABLE 1
Study features of 14 cohort studies included in the meta-analysis of 25(OH)D and overall mortality[1]

Reference	Country	Cohort name	Accrual period	Follow-up[2]	Deaths	Overall patients	Estimated annual mortality rate	Mean age	Sex	Assay method	Season of blood sampling[3]
				y	*No.*	*No.*		*y*			
Visser et al, 2006 (42)	Netherlands	LASA	1995–1996	6	380	1260	0.050	75	MF	Protein binding assay, Nichols	—
Jia et al, 2007 (43)	UK		1999–2000	6	129	398	0.054	80	MF	RIA, not specified	Adj
Melamed et al, 2008 (21)	USA	NHANES III	1988–1994	9	777	13,331	0.007	45	MF	RIA, DiaSorin	Adj
Kuroda et al, 2009 (39)	Japan	—	1993–2007	7	107	1232	0.013	64	F	Protein binding assay, not specified	—
Pilz et al, 2009 (40)	Netherlands	Hoorn	1989–1992	6.2	51	614	0.013	70	MF	Competitive binding assay, DiaSorin	Adj
Kilkkinen et al, 2009 (44)	Finland	MFHS	1978–1980	27	640	6619	0.004	49	MF	RIA, DiaSorin	Adj
Semba et al, 2009 (45)	USA	WHAS I, II	—	6	100	714	0.023	74	F	RIA, DiaSorin	Adj
Semba et al, 2010 (46)	Italy	InCHIANTI	1992–1994	7	228	1006	0.032	74	MF	RIA, Nichols	Adj
Szulc et al, 2009 (47)	France	MINOS	1995–1996	10	182	782	0.023	65	M	RIA, DiaSorin	Def
Bolland et al, 2010 (38)	New Zealand	—	1998–2003	5	63	1471	0.009	74	F	RIA, DiaSorin	Adj
Hutchinson et al, 2010 (48)	Norway	Tromsø	1994–1995	12	798	4751	0.014	63	MF	ECLIA Roche	Def
Anderson et al, 2010 (49)	USA	—	2000–2009	1.3	1193	27,686	0.033	71	MF	ECLIA, not specified	Def
Cawthon et al, 2010 (50)	USA	MrOS	2000–2002	7	330	1490	0.032	74	M	LC-MS	Adj
Michaëlsson et al, 2010 (51)	Sweden	ULSAM	1991–1995	13	584	1194	0.038	55	M	LC-MS	Adj

[1] Adj, adjustments; Def, definition; ECLIA, electrochemiluminescence immunoassay; InCHIANTI, Invecchiare in Chianti, Aging in the Chianti Area; LASA, Longitudinal Aging Study Amsterdam; LC-MS, liquid chromatography–mass spectrometry; MFHS, Mini-Finland Health Survey; MrOS, Osteoporotic Fractures in Men; RIA, radioimmunoassay; ULSAM, Uppsala Longitudinal Study of Adult Men; WHAS, Women's Health and Aging Study; 25(OH)D, 25-hydroxyvitamin D.
[2] Mean or median.
[3] Season of blood sampling was used for adjustments in the model or for definition of the 25(OH)D quartiles.

TABLE 2
Estimates reported by the authors of the 11 studies included in the nonlinear meta-analysis of 25(OH)D and overall mortality[1]

Reference	No. cases	No. participants	Categories of 25(OH)D[2]	Assigned dose	Adjusted RR (95% CI)	Adjustments
			nmol/L	*nmol/L*		
Visser, 2006 (42)	—	127	<25.0	18.8	1.28 (0.85, 1.92)	Age, sex, BMI, education, marital status, chronic diseases,
	—	462	25.0–49.9	37.0	1.00 (0.72, 1.40)	smoking, alcohol consumption, frailty indicators,
	—	440	50.0–75.0	62.3	0.91 (0.65, 1.26)	physical activity, cognitive status, depression
	—	231	≥ 75.0	90.0	1 (reference)	
Jia, 2007 (43)[3]	41	75	40.0	13.8	1.74 (0.91, 3.34)	Age, sex, season, medication, health status, diabetes,
	34	86	57.5	57.5	1.40 (0.73, 2.70)	heart problems
	21	80	75.0	75.0	0.90 (0.45, 1.79)	
	17	78	95.0	95.0	0.80 (0.39, 1.62)	
	16	79	127.5	127.5	1 (reference)	
Melamed, 2008 (21)	—	3386	<44.5	28.5	1.26 (1.08, 1.46)	Age, sex, BMI, smoking, season, chronic diseases,
	—	3344	44.5–60.7	52.8	1.06 (0.89, 1.24)	C-reactive protein, vitamin D supplementation, lipid
	—	3242	60.8–80.3	70.8	0.93 (0.79, 1.10)	status, physical activity, socioeconomic status
	—	3359	>80.3	96.3	1 (reference)	
Kilkkinen, 2009 (44)[4]	254	1258	16.3	16.3	1 (reference)	Age, sex, BMI, smoking, season, marital status, education,
	194	1202	31.3	31.3	1.04 (0.86, 1.26)	alcohol consumption, physical activity
	164	1284	40.5	40.5	0.81 (0.66, 1.00)	
	171	1222	51.3	51.3	0.86 (0.70, 1.06)	
	150	1253	111.3	111.3	0.76 (0.61, 0.95)	
Semba, 2009 (45)	34	177	<38.3	25.5	2.45 (1.12, 5.36)	Age, sex, BMI, smoking, season, chronic diseases, lipid
(WHAS I,II)	24	179	38.3–50.8	44.5	2.05 (0.97, 4.32)	status, race, physical activity, supplement use
	28	186	51–67.5	59.3	2.25 (1.08, 4.69)	
	14	172	>67.5	81.0	1 (reference)	
Semba, 2010 (46)	—	252	<26.3	19.5	2.11 (1.22, 3.64)	Age, sex, BMI, smoking, season, chronic diseases, lipid
(InCHIANTI)	—	254	26.3–40.0	33.3	1.41 (0.83, 2.40)	status, aspirin use, education
	—	247	40.1–64.0	52.3	1.12 (1.09, 1.15)	
	—	253	>64.0	76.8	1 (reference)	
Szulc, 2009 (47)[5]	—	—	32.5	32.5	1.70 (0.95, 3.05)	Age, season, BMI, smoking, chronic diseases, physical
	—	—	56.3	56.3	1.02 (0.56, 1.86)	performance and activity, vitamin D supplementation
	—	—	75.8	75.8	1.44 (0.82, 2.56)	
	—	—	106.8	106.8	1 (reference)	
Hutchinson, 2010 (48)	247	1184	34.0	34.0	1.32 (1.07, 1.62)	Age, sex, season, BMI, chronic diseases, physical activity
	198	1187	46.8	46.8	1.06 (0.86, 1.31)	
	190	1192	56.3	56.3	1.09 (0.88, 1.34)	
	163	1188	72.5	72.5	1 (reference)	
Anderson, 2010 (49)	—	—	<37.5	25.0	1.77 (1.51, 2.08)	Age, sex, season, chronic diseases
	—	—	40.0–75.0	57.5	1.20 (1.05, 1.40)	
	—	—	>75.0	90.0	1 (reference)	
Cawthon, 2010 (50)	—	373	<50.0	31.3	0.95 (0.68, 1.34)	Age, season, health status, weight, clinic, race, alcohol
	—	370	50.0–62.9	56.5	1.05 (0.75, 1.47)	consumption, activity level, marital status, education,
	—	372	63.0–74.9	69.0	0.89 (0.64, 1.24)	mobility, kidney function, serum calcium, and phosphate
	—	376	>75.0	90.0	1 (reference)	
Michaëlsson, 2010 (51)	76	119	<45.0	28.8	1.43 (1.11, 1.84)	Propensity score adjusted (age, sex, BMI, smoking, season,
	444	956	45.0–92.5	68.8	1 (reference)	chronic diseases, vitamin D supplementation, vitamin D
	64	119	>92.5	111.0	1.27 (0.97, 1.66)	intake, various biochemical parameters)

[1] InCHIANTI, Invecchiare in Chianti, Aging in the Chianti Area; WHAS, Women's Health and Aging Study; 25(OH)D, 25-hydroxyvitamin D.
[2] 25(OH)D concentrations presented in nmol/L were converted to ng/mL with the use of a correction factor of 0.401. To convert the values of 25(OH)D to nmol/L, multiply by 2.496.
[3] Median values were obtained from the authors.
[4] Cardiovascular disease mortality.
[5] Mean values were obtained from the author.

associated with decreased mortality, which tends to reach 31% of mortality reduction for 50 nmol/L from a baseline reference value. However, CIs suggest that at >87.5 nmol of difference/L from the reference category the decrease in mortality may not be significant any more. We estimated the model up to 112.5 nmol/L (which corresponds to a difference from the reference of ~87.5 nmol/L) because only 2 studies reach higher concentrations (Table 1).

Heterogeneity and publication bias

Evidence of heterogeneity for the RR when highest compared with lowest categories were compared was apparent ($P = 0.008$; $I^2 = 58\%$), but we observed no indication for publication bias with the method of Macaskill et al (35) ($P = 0.11$). In addition, no indication for publication bias was shown when we evaluated estimates for the parametric models ($P = 0.80$).

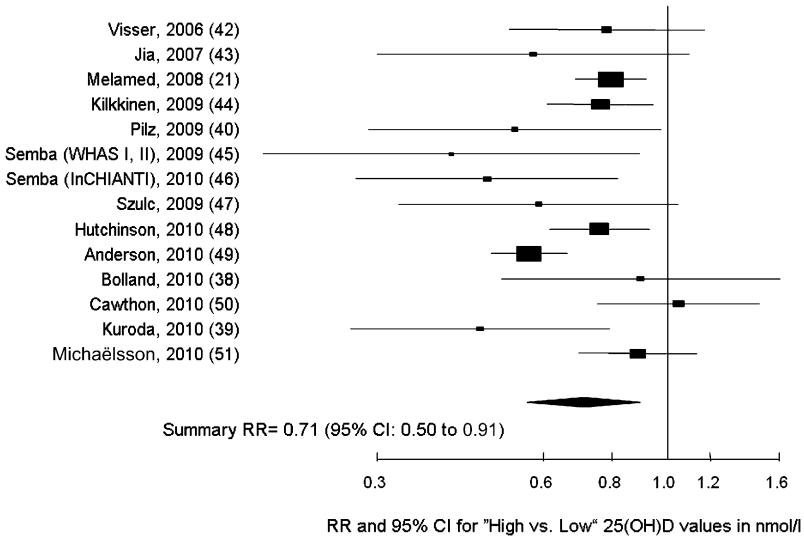

FIGURE 2. Forest plot and summary RR of the association between high and low concentrations of 25(OH)D and overall mortality; results are based on a total number of 62,548 individuals. To convert the values of 25(OH)D to ng/L, divide by 2.496. Note that vertical marks represent RRs, and horizontal bars represent 95% CIs; a statistically significant result was assumed when the 95% CI did not include 1 (vertical line). 25(OH)D, 25-hydroxyvitamin D.

To investigate heterogeneity in the nonlinear parametric model, we evaluated through meta-regression the effect of mean age, mean length of follow-up, mortality rates, and countries (European countries compared with the United States). None of the factors explained heterogeneity: age (P = 0.49), length of follow-up (P = 0.33), mortality rates (P = 0.68), or countries (P = 0.81). Furthermore, our choice of the 25(OH)D reference category did not affect the risk estimates (P = 0.56).

The only factor that explained some variability in mortality risk for higher compared with lower 25OHD values was the assay method. When we categorized the test procedures into the 3 subgroups "DiaSorin radioimmunoassay" (21, 44, 46, 47), "liquid chromatography–mass spectrometry" (50, 51), and "other/not specified methods" (42, 43, 45, 48, 49), the liquid chromatography–mass spectrometry presented significantly (P = 0.001) greater RR estimates (RR: 0.94; 95% CI: 0.77, 1.15) than did the DiaSorin radioimmunoassay (RR: 0.77; 95% CI: 0.68, 0.87) and the other/not specified methods (RR: 0.63; 95% CI: 0.56, 0.70).

We also carried out a subgroup analysis for the parametric models and stratified by the highest categories. In both groups of studies with upper categories $<$ or $>$75 nmol/L, the nonlinear model fit better than did the linear model.

Sensitivity analyses

Smokers from one study (48) were excluded with use of the Roche electrochemiluminescence immunoassay because this assay produces significantly higher 25(OH)D serum concentrations in smokers compared with nonsmokers, whereas there is no significant difference between smokers and nonsmokers when other laboratory methods for 25(OH)D measurements are used (ie, liquid chromatography–mass spectrometry/mass spectrometry methods, DiaSorin radioimmunoassay, HPLC, and Immunodiagnostic Systems radioimmunoassay) (52). We therefore performed a sensitivity analysis and included the RR for smokers from the aforementioned study. We also performed a sensitivity analysis with the exclusion of estimates from the MFHS study (44), which presented estimates only for CVD mortality. Results did not change when the aforementioned studies were included or excluded: the fractional polynomial still remains a good model to explain nonlinear trends (AIC = −180 and −90, respectively), and both results fit significantly better than did the linear ones. No significant change was seen when different upper limits of open-ended upper categories of 25(OH)D serum values were looked at: β_1 = −0.034 (95% CI: −0.047, −0.020) and β_2 = 0.0007 (95% CI: 0.0003, 0.0012).

DISCUSSION

This meta-analysis indicates a nonlinear decline in overall mortality in community dwellers as 25(OH)D concentrations increase. Compared with concentrations of 27.5 nmol 25(OH)D/L, concentrations of 50 nmol 25(OH)D/L above the reference category, which correspond to absolute values of 77.5 nmol 25(OH)D/L, are associated with a 31% reduction in mortality risk. Data also indicate that at 87.5 nmol of difference/L from the reference category, the decrease in mortality may not be significant any more.

Our meta-analysis has several strengths but also some limitations. First, the present analysis included only prospective cohort studies, and most follow-ups lasted years to decades, which largely avoids the problem of reverse causation bias. Second, our meta-analysis included only multivariable adjusted RRs. Third, we showed that the nonlinear trend predicts mortality better than does the linear trend. Note that the progressive increase in mortality risk with deficient 25(OH)D concentrations in our analysis agrees with the progressive rise in serum parathyroid hormone concentrations in vitamin D–deficient individuals (53). Fourth, low 25(OH)D concentrations, as reflected by the 2 categories with the highest mortality risk, are frequently and globally observed in the adult population (1, 17, 18, 21). A limitation is that our results may be hampered by heterogeneity, which can be explained by the multiple differences between studies with regard to the study design, analytic procedures of

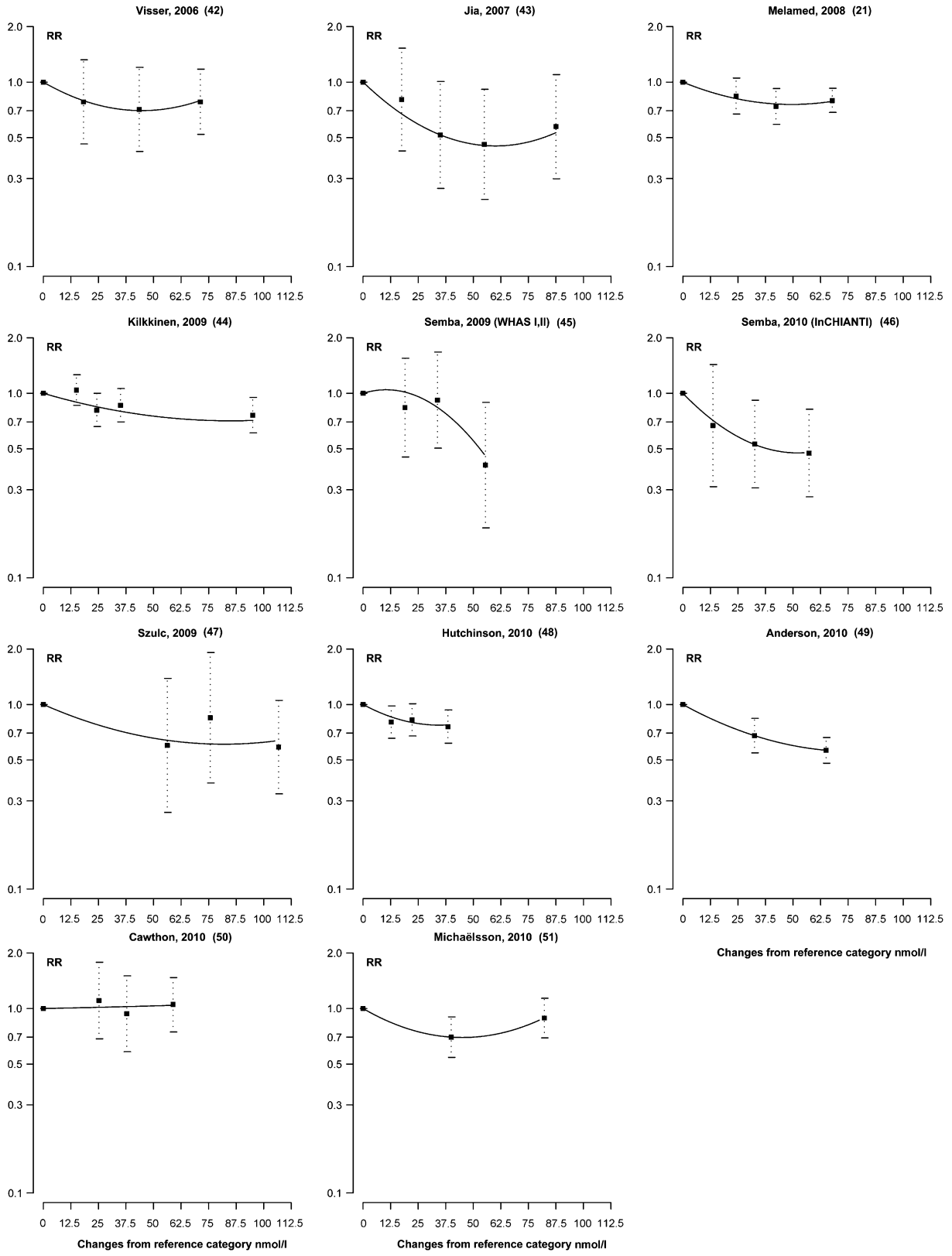

FIGURE 3. Study-specific RR estimates of overall mortality with increases in the concentration of 25(OH)D with respect to the study-specific reference value. Black squares indicate the RR estimates transformed to set the lowest concentration of 25(OH)D as the reference category, and dotted lines are 95% CIs. Lines indicate the predicted risk with the best nonlinear random-effects model. To convert the values of 25(OH)D to ng/L, divide by 2.496. InCHIANTI, Invecchiare in Chianti, Aging in the Chianti Area; WHAS, Women's Health and Aging Study; 25(OH)D, 25-hydroxyvitamin D.

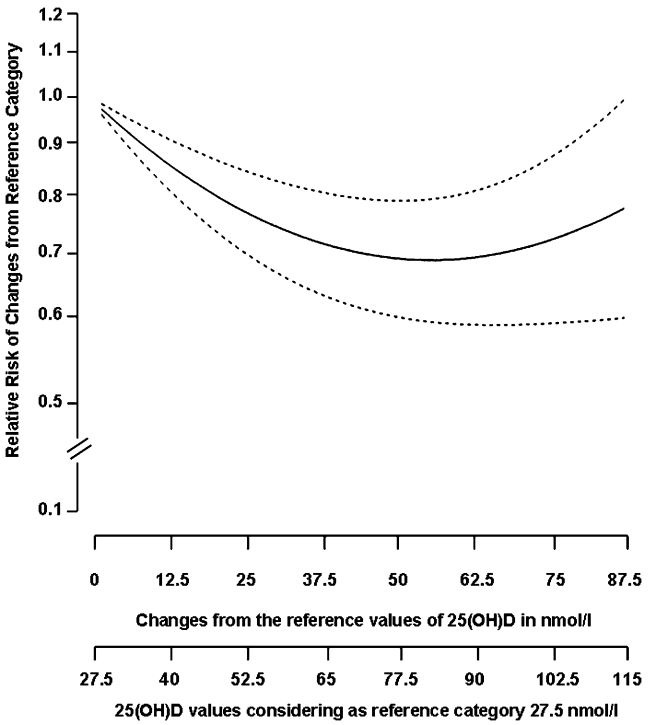

FIGURE 4. Summary nonlinear dose-response relation (dotted lines: 95% CI) from the best-fitting random-effects model, between an increase in the concentration of 25(OH)D (ng/mL) with respect to reference values and overall mortality RR. Results are based on a total number of 59,231 individuals. Summary RR = exp $(-0.0850x + 0.00175x^2)$. The lower x axis indicates the absolute 25(OH)D values. Note that 27.5 nmol/L is the median value of the reference categories of our included studies. To convert the values of 25(OH)D to ng/L, divide by 2.496. 25(OH)D, 25-hydroxyvitamin D.

25(OH)D measurement, and confounding and adjustment for confounders. Nevertheless, it is clear that the inverse association between circulating 25(OH)D and mortality appeared consistent. Despite some variation in mortality risk, which depended on the analytic method used for 25(OH)D measurement, the DiaSorin radioimmunoassay shows good correlation with the liquid chromatography–mass spectrometry method (40, 52), and the latter is considered the gold standard. Thus, the analytic procedure may only be an indicator for other study differences. Our estimates were not influenced by age, length of follow-up, or country of origin (European countries or United States). Moreover, the stability of our estimates was not affected by exclusion of estimates from the MFHS study (44), which presented estimates only for CVD mortality, or inclusion of estimates for smokers from the Tromsø study (48). Although ideally multiple measurements of 25(OH)D should be performed, the correlation coefficient between 2 measurements of 25(OH)D taken 3 y apart was moderately high (0.70) (54), which suggests that a single 25(OH)D measurement is a useful tool in epidemiologic studies.

Our results are in general agreement with an earlier meta-analysis of randomized trials in frail, elderly people (14). In that analysis, daily vitamin D supplements of 10–20 μg were related to a reduction in overall mortality of 8% during a mean follow-up of 5.7 y. In trials for which baseline 25(OH)D concentrations were available (6 of the 9 trials, which covered 15,979 of the 55,774 participants), values ranged from 22.0 to 47.0 nmol/L at baseline and increased to 62.0–105.0 nmol/L in vitamin D–supplemented individuals.

Whereas some researchers (18, 55) and the Institute of Medicine (6) already consider concentrations >50 nmol 25(OH)D/L to be adequate, others have pointed out that the most advantageous serum concentrations begin at 75 nmol 25(OH)D/L and that the best are between 90 and 100 nmol 25(OH)D/L (4, 5). Our data on overall mortality add further evidence for a desirable concentration of 75–87.5 nmol 25(OH)D/L. Serum concentrations of <75 nmol 25(OH)D/L are prevalent around the world, and even concentrations <25 nmol 25(OH)D/L are common in regions such as South Asia and the Middle East (1). In the US population, mean concentrations are 62.5 nmol 25(OH)D/L (4), clearly still below the range of 75–112.5 nmol 25(OH)D/L, the range for which mortality risk was lowest in our analysis. African Americans have mean concentrations of only 40 nmol 25(OH)D/L (56).

Although the underlying mechanism of how vitamin D deficiency reduces life expectancy is not completely clear, experimental evidence shows that vitamin D receptor–knockout mice have various metabolic and cardiovascular disturbances and a short lifespan (8, 57–60). In developed countries, cancer and CVD account for 60–70% of age-adjusted mortality rates, whereas an additional 10–20% encompasses type 2 diabetes mellitus, respiratory disease, and respiratory infections (61). Various articles (3, 62–64) reviewed the evidence of vitamin D deficiency for several of the aforementioned chronic diseases in humans. A population-based cohort study in female twins (65) highlighted a more general mechanism. This study reported longer leukocyte telomere length with higher circulating 25(OH)D concentrations. Leukocyte telomere length is a predictor of aging-related disease and is positively related to longevity. The difference in leukocyte telomere length between the highest and lowest tertiles of circulating 25(OH)D (mean values: 41.0 and 124.3 nmol/L, respectively) was 107 base pairs, which is equivalent to 5 y of telomeric aging.

In the present meta-analysis, it was not possible to assess the association between 25(OH)D concentrations and mortality risk above a concentration of 112.5 nmol 25(OH)D/L. Our data do not exclude the possibility that mortality risk may increase again at concentrations >112.5 nmol 25(OH)D/L. Some of the cohort studies we included in our meta-analysis indicate that there is a significant increase in mortality risk at concentrations >97.5 (51) and 125 nmol 25(OH)D/L (21). A biphasic vitamin D effect with an increased risk at low and high 25(OH)D concentrations has also been suggested for prostate cancer and cardiovascular incidence (20, 66). A possible mechanism for the adverse effects on prostate cancer incidence may be an increase in testosterone concentrations at high 25(OH)D concentrations (67). Excess vitamin D may also increase the risk of vascular calcification (68). Whether high 25(OH)D concentrations in observational studies reflect high exposure to UV-B radiation and/or high dietary vitamin D intake is unclear at present. Concentrations of 25(OH)D are also, to some extent, genetically determined (69). Future randomized controlled trials are needed to investigate whether vitamin D supplementation at higher doses could have potential benefit in the reduction of mortality risk in those with 25(OH)D deficiency.

In summary, this meta-analysis of prospective cohort studies offers further support that concentrations of 75–87.5 nmol 25(OH)D/L are desirable. Because many adults do not achieve this 25(OH)D value, large prospective randomized trials are

urgently needed to investigate whether vitamin D supplementation is able to reduce mortality risk in the general population.

The authors' responsibilities were as follows—SG (guarantor): had full access to all the data in the study and takes responsibility for its integrity and the accuracy of the data analysis; SG, SI, SP, and WBG: conceived and designed the study; SG, SI, SP, WBG, and AZ: acquired the data; SG, SI, VB, SP, and AZ: analyzed and interpreted the data; AZ, SP, SI, SG, and WBG: drafted the manuscript; SG, SI, VB, SP, and AZ: critically reviewed the manuscript for important intellectual content; SG, SI, and VB: undertook statistical analysis. AZ and SP received honoraria from DiaSorin, Germany. WBG received funding from the Ultraviolet Foundation, McLean, VA; the Vitamin D Society, Canada; the European Sunlight Association, Brussels, Belgium; and the Vitamin D Council, California. None of the other authors had a personal or financial conflict of interest.

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