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Vitamin D: Epidemiology of cardiovascular risks and events

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Vitamin D may influence blood pressure through the renin-angiotensin system, parathyroid hormone levels, myocardial function, inflammation, and vascular calcification. In the past several years, a number of high-quality prospective studies have examined 25(OH)vitamin D (25(OH)D) levels in relation to risk of cardiovascular disease (CVD). Studies consistently show that levels of 25(OH)D below 20–25 ng/mL are associated with an increased risk of CVD incidence or mortality. Risk appears especially elevated at 25(OH)D levels below 10 or 15 ng/mL. It is unclear if levels higher than 25 ng/mL provide further benefits for CVD disease. Currently, results from randomized clinical trials are sparse and do not allow a definitive conclusion. Given other potential benefits of vitamin D, and low potential for toxicity, deficient levels below 25–30 ng/mL should be avoided and treated when identified. Further observational and randomized clinical trial data are important to better characterize the optimal range for 25(OH)D.

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Introduction

Prior to the late 1970's, concerns had been raised that high vitamin D may have been a factor in increasing cardiovascular disease (CVD) risk. For example, based on findings from a small case–control, Linden concluded in the British Medical Journal that long-term high consumption of vitamin D may be

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a precipitating cause of myocardial infarction.¹ Such concerns led to some case–control studies to examine the association between vitamin D status as determined by circulating 25(OH) vitamin D (25(OH)D) in relation to risk of CVD. In the earliest study, conducted in Denmark in 1978,² circulating 25(OH)D levels were measured in 128 patients admitted with ischemic heart disease (75 with angina pectoris and 53 with acute myocardial infarction (MI)) and 409 controls. Contrary to expectation, the 25(OH)D levels were significantly lower in angina patients (23.5 ng/mL) or in those with MI (24.0 ng/mL) than in the controls (28.8 ng/mL). Shortly thereafter, a nested–case control study of MI (the Tromsø Heart Study in northern Norway) based on only 30 cases and 60 matched controls, reported a slightly non-significant lower 25(OH)D level in cases (23.6 ng/mL) compared to controls (25.4 ng/mL).³ More than a decade passed before the next publication, a case–control study of MI conducted in New Zealand in 1990.⁴ In that study MI cases ($n = 179$) had a lower mean 25(OH)D level than controls ($P = 0.017$), and the case–control difference was more pronounced in the winter–spring (-1.8 ng/mL; $P = 0.029$) than in the summer–autumn (-1.0 ng/mL; $P = 0.21$). The relative risk (RR) of MI decreased across increasing quartiles of 25(OH)D: compared with levels <10 ng/mL: 10–13 ng/mL: RR = 0.56; 13.1–16.8 ng/mL: RR = 0.33; >16.8 ng/mL: RR = 0.30 (95% confidence interval (CI), 0.15–0.61). The results were essentially unchanged after multivariate adjustment of major CVD risk factors.

These studies helped to dispel notions that vitamin D status at least up to a level of approximately 30 ng/mL was deleterious, and even suggested potential benefit of being at the higher end of the range of 25(OH)D. Yet, the relationship between vitamin D status and CVD received relatively little study in humans until a number of publications beginning in 2008. In the mean time, evidence has accumulated for a number of potential mechanisms whereby vitamin D status may influence CVD risk. Among the potentially relevant mechanisms uncovered, vitamin D may influence CVD risk by influencing blood pressure, the renin–angiotensin system, parathyroid hormone (PTH) levels, myocardial function, inflammation, ventricular hypertrophy and vascular calcification.⁵ Various tissues, including cardiomyocytes and endothelial cells, appear to express the vitamin D receptor and 1- α -hydroxylase and 24-hydroxylase, the enzymes required for the conversion of 25(OH)D to the active 1,25(OH)₂D form, and its subsequent breakdown. A study from 2006⁶ found that more than 170 genes in the coronary artery smooth muscle cells respond to 1,25(OH)₂D. An inadequate level of circulating 25(OH)D may impair normal function of genes related to vitamin D, potentially leading to increased risk of CVD. In general, the results from *in vitro* and animal studies provided support that inadequate vitamin D could increase risk of CVD disease in humans.

While the early case–control studies suggested that lower vitamin D status increased risk of MI, these studies were small and did not control for many potentially confounding factors. Also, since vitamin D status was assessed after the disease outcome, it is plausible that the disease could have affected 25(OH)D levels (reverse causation), or that the selection of controls were biased in some manner. It was thus critical to follow the leads from these early case–control studies with prospective studies, in which vitamin D status prior to the disease outcome is assessed. We thus review all prospective studies that assessed the association between baseline categories of 25(OH)D levels and risk of developing or dying from cardiovascular related causes. We focus on the dose–response relation between 25(OH)D and the various outcomes. In addition, we consider the limited randomized control trial (RCT) data that have assessed the potential role of vitamin D supplementation on CVD.

Methods

We searched for all prospective studies, published until December 2010, that assessed the association between baseline categories of 25(OH)D levels and risk of developing or dying from cardiovascular related causes. The measure of association was relative risk (RR) or hazard ratio (HR). In order to be included, the studies had to report at least one of the following end-points: cardiovascular mortality, cerebrovascular mortality, coronary mortality, heart failure, sudden cardiac death or myocardial infarction. Our search resulted in 13 studies. The mean age at inclusion spanned across studies from 44 to 75 years. The average follow-up time varied between studies from 5 years to 27 years. The study populations are originating from several countries, such as the United States, Finland, Norway, Sweden, Germany, the Netherlands and Italy, roughly spanning 30 to 70 degrees Northern latitude. The study design was usually based on a nested case–control design, in which archived blood samples in

a sufficiently large population are retrieved after study outcomes, such as MI occurrence, and 25(OH)D are compared between cases and randomly selected controls from the same archive. The dose-response relation of each identified study is summarized in the figures, showing the most fully adjusted model for the analyses based on categories of 25(OH)D level. In these figures, the highest 25(OH)D category within each study was used as the referent, unless otherwise specified. We have used a cubic spline interpolation of the point estimates in order to represent this relation. We describe below the main features of the studies that are included in this review, including what factors were controlled for in the multivariate analysis.

Included prospective studies

Study A

Data from the Third National Health and Nutrition Examination Survey (NHANES III)⁷ was used to examine the association between 25(OH)D levels and all-cause and CVD mortality in approximately 13,000 participants representative of the US adult population 20 years or older, with a physical examination and laboratory testing at baseline, 1988–1994.⁸ The original study oversampled Non-Hispanic blacks, Mexican Americans and elderly individuals. The mean age of the analytic cohort was 44.8 years, and participants were followed on average for 8.7 years. During follow-up, there were 1,806 deaths of which 777 were from CVD causes. Fig. 1 shows RR estimates from the model which accounted for age, sex, race, season of blood withdrawal, hypertension, history of CVD, diabetes mellitus, smoking, HDL and total cholesterol, use of cholesterol-lowering medications, glomerular filtration rate categories, serum albumin, log albumin to creatinine ratio, log C-reactive protein level, body mass index (BMI), physical activity level, use of vitamin D supplementation, and socioeconomic status. The estimates were similar in the model that did not adjust for diabetes mellitus and hypertension. For CVD mortality, RR in the lowest category was 1.20, 95% CI (0.87, 1.64), compared to the highest quartile (>32.1 ng/mL). Of note, an increased risk was primarily in the lowest group and the lowest risk was observed in the second highest quartile (RR = 0.83; relative to highest). Relative to those in the second highest quartile, those in the lowest had a 45% higher risk. The associations were stronger for women than for men, and stronger for those without a history of CVD.

Study B

The Mini-Finland Health Survey is a prospective population health survey carried out 1978–1980 on Finnish adults older than 30 years. The current study⁹ included participants free of CVD at baseline and with measured 25(OH)D levels, resulting in 6219 individuals, with mean age at inclusion 49.4 years. The median follow-up time was 27.1 years. During this time, 640 coronary disease death and 293 cerebrovascular disease deaths were reported. The study reported hazards ratio (HR) of total CVD mortality, coronary heart disease and cerebrovascular death across serum 25(OH)D quintiles, with the lowest quintile (mid-range value 8.8 ng/mL) as the referent category. Fig. 2 shows estimates from the models that adjusted for age, sex, marital status, educational level, BMI, alcohol, smoking, leisure-time physical activity and season of blood withdrawal. Adjustment for HDL, cholesterol diabetes and blood pressure (BP) did not impact on the estimates. The values on the X-axis are mid-ranges between sex-specific

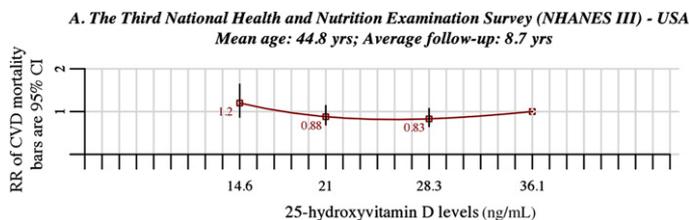


Fig. 1. Relative risks of cardiovascular mortality across quartiles of 25-hydroxyvitamin D levels, represented here by their mid-ranges.



Fig. 2. Hazard ratios of total cardiovascular mortality, cerebrovascular mortality and coronary mortality across quintiles of 25-hydroxyvitamin D levels, represented here by the mid-ranges between sex-specific quintile medians.

quintile medians of 25(OH)D levels. The strongest association was found for cerebrovascular mortality in the highest two quintiles, where there was a significant decrease of 31% and 52%, respectively, as compared to the lowest quintile (HR: 0.69; 95%CI (0.48, 1.00) and 0.48 (95% CI 0.31, 0.75), $P = .002$ for trend). For total CVD mortality a significant 24% decrease was seen in the highest quintile ($P = .005$ for trend).

Study C

The Framingham Offspring Study¹⁰ enrolled Caucasian participants who attended a medical examination during 1996–2001. A total of 1739 participants had 25(OH)D levels measured and were free of CVD or kidney disease at the time of enrollment into the current study. The mean age was 59 years and the mean follow-up time was 5 years. HR's of first cardiovascular events (120 individuals during follow-up), including MI, coronary insufficiency (prolonged chest pain with documented ECG changes), angina, stroke, transient ischemic attack, peripheral claudication, or heart failure, are given across categories of 25(OH)D, with the highest category (≥ 15 ng/mL) as the referent. In the model that adjusted for age, sex, systolic BP, antihypertensive medication, creatinine, diabetes mellitus, total to HDL cholesterol ratio, smoking and BMI, an 80% increase in the risk of first CVD events for the lowest 25(OH)D category (< 10 ng/mL) and a 53% increase for the 10–15 ng/mL category ($P = .01$ for trend) were observed; these estimates were even more dramatic for participants with hypertension, where hypertension was defined as systolic BP ≥ 140 mmHg, diastolic BP ≥ 90 mmHg or use of antihypertensive medication: 151% and 93% increase, respectively ($P = .002$ for trend). A figure from the paper on the dose-response based on restricted cubic splines suggested visually that risk of CVD decreased with increasing 25(OH)D levels up to a range of approximately 20 ng/mL, with a leveling of the benefit with higher levels (Fig. 3).

Study D

The Fourth Tromsø Study¹¹ is a prospective population-based study, initiated during 1994–1995, on adults aged 25 years or older living in the city of Tromsø, Norway, with oversampling of individuals

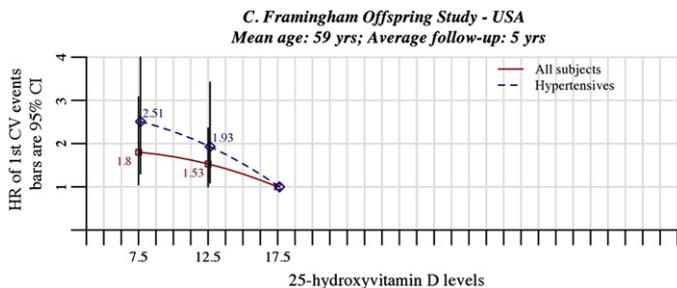


Fig. 3. Hazard ratios of first cardiovascular events, in all subjects and in hypertensive subjects, across three categories of 25-hydroxyvitamin D levels, represented here by their mid-ranges.

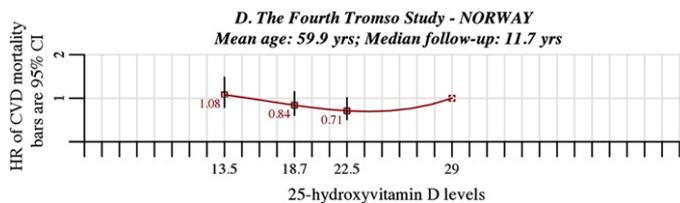


Fig. 4. Hazard ratios of cardiovascular mortality across quartiles of 25-hydroxyvitamin D levels represented by the mean-range points.

aged 50–74 years and a small random sample of the other age categories less than 84 years. The sampling scheme and the availability of 25(OH)D measurements resulted in 7161 participants, with a mean age of 59.9 years. They were followed for an average of 11.7 years. The study assessed HR's for all-cause and CVD mortality for the cohort of 4700 non-smoker participants. Among these 798 died, of whom 325 were from CVD causes. As the 25(OH)D levels seemed to be over-estimated in smokers as compared to non-smokers, the analysis was restricted to non-smokers in order to prevent confounding by a probable dose-response effect of smoking on 25(OH)D. Fig. 4 presents estimates across quartiles of 25(OH)D, with the highest quartile (mean-range 29 ng/mL) as reference, from the model that adjusts for age, gender, BMI, physical activity score, and diabetes, as well as hypertension, creatinine, history of CVD and of cancer. A statistically significant 32% increase in all-cause mortality was found for the lowest quartile (mean-range 13.5 ng/mL) (HR: 1.32 95% CI (1.07–1.62)), while no increase was found in the other categories or for CVD mortality. The lowest risk was found in the second highest quartile (the RR was 52% higher in the lowest quartile compared to the second highest quartile).

Study E

The Ludwigshafen Risk and Cardiovascular Health (LURIC) study¹² recruited patients at the Cardiac Center Ludwigshafen, Germany that were referred for coronary angiography during 1997–2000. The current study followed prospectively the vast majority of the cohort with available 25(OH)D measurements, were of Caucasian origin, did not have a malignancy within 5 years before enrollment or an acute disease except for cardiac diseases or acute coronary syndrome (3258 individuals) for 7.7 years. The mean age was 62 years. During follow-up, 737 deaths, 463 due to cardiovascular causes, were reported. The HR for all-cause and cardiovascular mortality are shown across quartiles of 25(OH)D, with the highest quartile (median 28.4 ng/mL) as reference. The HR values plotted in Fig. 5 correspond to the model that adjusted for age, sex, BMI, physical activity, smoking status, diabetes mellitus, systolic and diastolic BP, LDL- and HDL- cholesterol, albumin level, cystatin C level, triglyceride level, N-terminal pro-BNP level, and the use of statins, aspirin, β -blockers, bronchodilators, and angiotensin-converting enzyme inhibitors. Participants in the first quartile (median 7.6 ng/mL) and in the 2nd quartile (median 13.3 ng/mL) had an increase in the risk of CVD mortality of 122% and 82%, respectively

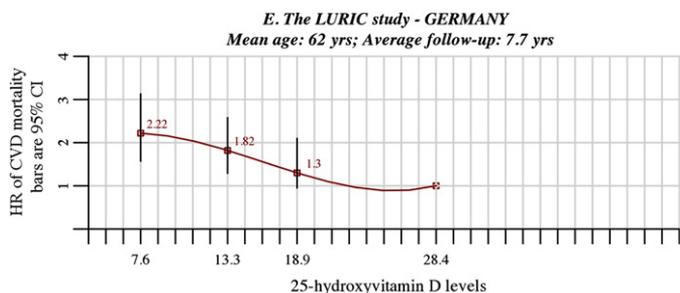


Fig. 5. Hazard ratios of cardiovascular mortality across quartiles of 25-hydroxyvitamin D levels, represented by their median points.

(HR: 2.22, 95% CI (1.57, 3.13) and 1.82, 95% CI (1.29, 2.58)). The increase for all-cause mortality was of similar magnitude (HR: 2.08, 95% CI (1.60, 2.70) and 1.53 (1.17, 2.01)).

Study F

The LURIC Study also followed the cohort prospectively for heart failure (HF) deaths and sudden cardiac death (SCD) across predefined 25(OH)D categories¹³ (see above). The highest category (30 ng/mL) was used as the reference. 116 HF deaths and 188 SCD deaths were reported. The HR estimates in Fig. 6 are shown from the model that adjusted for age and sex, month of blood withdrawal, BMI, smoking status, diabetes mellitus, arterial hypertension, coronary artery disease (CAD), glomerular filtration rate (GFR), LDL- and HDL-cholesterol, triglycerides, C-reactive protein (CRP), angiotensin converting enzymes (ACE) inhibitor, diuretics, and β -blockers. Subjects in the lowest category (<10 ng/mL) had a significant 5-fold increase in the risk of SCD and almost 3 times the risk of HF compared to the highest category (HR: 5.05, 95% CI (2.13–11.97) and 2.84, 95% CI (1.20–6.74), respectively). The risk of SCD remained highly elevated also in the middle categories (HR: 2.58, 95% CI (1.11–5.97) for subjects in the 10–20 ng/mL range and 2.52, 95% CI (1.06–6.00) for those in the 20–30 ng/mL range).

Study G

The Health Professionals Follow-up Study¹⁴ is a nested case-control study, conducted on 18,255 men, aged 40 to 75 years (mean age 64 years), who were free of CVD at the time of blood collection. During the 10 years of follow-up, 454 men developed MI, defined as incident non-fatal MI or fatal CHD. These cases were matched on age, month and year of blood withdrawal and smoking status to 900 men who were alive at the time of sampling and had no history of CVD (controls). Fig. 7 shows the RR of MI across categories of 25(OH)D levels, with the category ≥ 30 ng/mL as referent, from the model that adjusted for the matching variables as well as family history of MI before 60 years of age, diabetes mellitus, hypertension, alcohol intake, physical activity, BMI, region, ethnicity, multivitamin and marine omega-3 intake. Men with 25(OH)D levels less than 15 ng/mL had a 2 fold increase in the risk of MI (RR: 2.01, 95% CI (1.22, 3.30), $P = .02$ for trend). Furthermore, the multivariate RR for deficient vs sufficient 25(OH)D values did not change appreciably when further adjusted for C-reactive protein or estimated glomerular filtration rate based on measured plasma creatinine level, age, sex, and race.

Study H

The Hoorn Study¹⁵ is a population-based cohort of glucose metabolism and CVD risk factors among inhabitants of the municipality of Hoorn, the Netherlands. Subjects with type II diabetes at a follow-up visit during 1996–1998 and a random sample of the remaining cohort with impaired or normal glucose

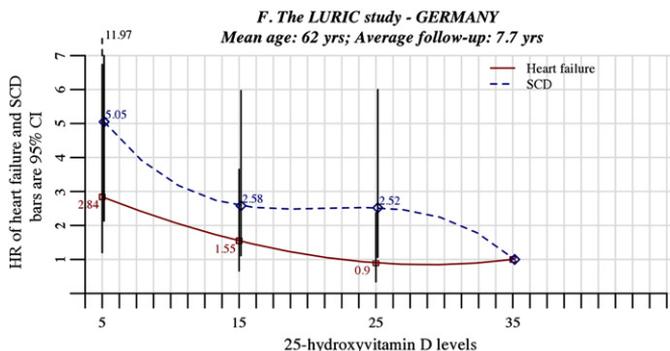


Fig. 6. Hazard ratios of heart failure and sudden cardiac death (SCD) across four predefined categories of 25-hydroxyvitamin D levels, represented here by their mid-ranges.



Fig. 7. Relative risks of myocardial infarction (MI) across four categories of 25-hydroxyvitamin D levels, represented here by their mid-ranges.

metabolism were invited to participate in a follow-up study during 2000–2001. The current study includes those 60% who accepted to participate ($n = 614$). The mean age is 69.8. During a mean follow-up time of 6.2 years, there were 51 deaths of which 20 were due to cardiovascular diseases. HR's estimates for all-cause and CVD mortality for subjects in the first 25(OH)D quartile (mid-range 12.3 ng/mL) compared to the upper 3 quartiles combined (mid-range 24.9 ng/mL) presented in Fig. 8 are from models that adjusted for age, sex, diabetes mellitus, smoking status, hypertension, HDL cholesterol, glomerular filtration rate, waist-to-hip ratio and physical activity level. The HR for CVD mortality was 5.02, 95% CI (1.88–13.42), $P = .001$ and for all-cause mortality, it was 1.93, 95% CI (1.06–3.51), $P = .032$.

Study I

The Uppsala Longitudinal Study of Adult Men (ULSAM)¹⁶ was initiated as a health survey with focus on cardiovascular health on men that were born and living by the age of 50 years in the city of Uppsala, Sweden. The current study is based on the cohort 71 years of age during 1991–1995 and had 25(OH)D measurements, resulting in 1194 participants that were followed on average for 12.7 years. The authors explored the non-linearity of the association between mortality from all-cause or cardiovascular disease and 25(OH)D levels in analyses where vitamin D status was treated as a continuous variable with linear and quadratic terms. During follow-up, 584 participants died, 196 from cardiovascular causes. The analyses indicated a U-shaped association for all-cause mortality ($P = .0009$ for 25(OH)D as a quadratic term), but not for cardiovascular mortality ($P = .52$). Thus, men at both low and high ends of 25(OH)D spectrum had a higher risk of dying from all-causes, whereas only those at the low end had an increase risk of CVD mortality. Fig. 9 shows HR's across 5 categories of 25(OH)D, with the 18–37 ng/mL (10th–90th percentile) as referent, from the model that adjusted for age (implicitly, since all participants were of

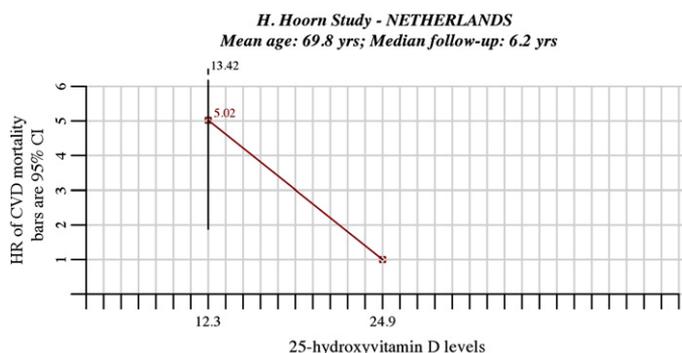


Fig. 8. Hazard ratios of cardiovascular mortality in the lowest 25(OH)D quartile compared to the upper three quartiles combined. The X-axis shows the mid-ranges in these two categories, as presented in the original study.

similar age), weight, height, calcium intake, physical activity, smoking status, socioeconomic status, season of blood withdraw as well as potential mediators of vitamin D's effect on mortality: PTH, serum calcium, phosphate, plasma cystatin C, systolic and diastolic BP, total cholesterol, diabetes mellitus and self-perceived health. Men with CVD before baseline were omitted from the analysis of CVD mortality and, similarly, those with CVD or cancer were excluded when estimating all-cause mortality risk. Across all 25(OH)D categories relative to those in the referent (18–37 ng/mL), there was a slightly elevated risk of dying from all causes than from CVD. Overall mortality was increased significantly by 60–70% among men in the 5th to 10th percentile of 25(OH)D (16–18 ng/mL) and those in the highest category (>39 ng/mL). CVD mortality was increased by 64% for men in the 16–18 ng/mL category (HR: 1.64, 95% CI (1.04, 2.57)) and did not reach statistical significance for the other categories.

Study J

The study that examined the association between vitamin D status and overall or CVD mortality among elderly from the Third National Health and Nutrition Examination Survey (*NHANES III*)¹⁷ included 3408 adults older than 65 years at enrollment that were followed for 7.3 years. *NHANES III* has already been described (see above). The mean age in this cohort was 73 years. During follow-up, 1493 participants died, 767 due to CVD causes. Fig. 10 shows HR's across 5 categories of vitamin D status, with the highest (>40 ng/mL) as referent, after adjustment for age, sex, race or ethnicity, poverty to income ratio, region, BMI, physical activity, smoking status, cigarette pack years, and chronic diseases such as asthma, chronic obstructive pulmonary disease, renal dysfunction, hypertension, diabetes mellitus, hyperlipidemia, history of myocardial infarction, history of stroke, and history of non-skin cancer. CVD and overall mortality were elevated across all non-referent categories, but the increase was statistically significant only in the lowest 2 categories: <10 ng/mL and 10–20 ng/mL, with HR for CV mortality = 2.36, 95% CI (1.17–4.75) and 1.54, 95% CI (1.01–2.34), respectively; the HR for all-cause mortality was slightly lower than cardiovascular mortality, HR = 1.83, 95% CI (1.14–2.94) and 1.47, 95% CI (1.09–1.97).

Study K

The Osteoporotic Fractures in Men (MrOS) Study¹⁸ is a prospective center-based study (6 US clinical centers) carried out on healthy community-dwelling men, aged at least 65 years, that were scheduled for a baseline visit between 2000–2002. The study focused mainly on osteoporosis. Eligible participants had to be able to walk without assistance and not have bilateral hip replacements. 1490 participants, with a mean age of 73.7 years were followed on average for 7.3 years. During this time, 330 died, 110 due to cardiovascular causes. At least 65% of the eligible subjects had one or more of the following medical conditions at enrollment: stroke, heart attack, non-skin cancer, chronic obstructive pulmonary disease, hypertension, congestive heart failure, thyroid disease, diabetes, or Parkinson's disease. HR's for all-cause and cardiovascular mortality are shown in Fig. 11 across 25(OH)D quartiles, with ≥ 30 ng/mL as referent, from models adjusting for age, clinic, season of blood draw, serum calcium and phosphate, glomerular filtration rate, percentage body fat, weight, race, health status, presence of at least one medical condition, alcohol use, education, activity level, marital status, and presence of

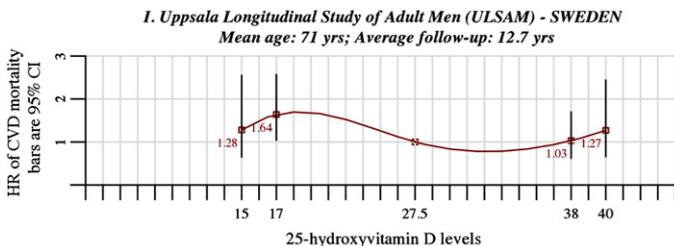


Fig. 9. Hazard ratios of cardiovascular mortality across five categories of 25-hydroxyvitamin D levels, with the middle category as referent. The X-axis shows the mid-ranges in these two categories.

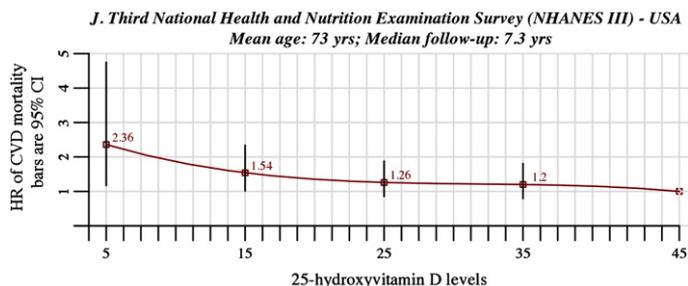


Fig. 10. Hazard ratios of cardiovascular mortality across five categories of 25-hydroxyvitamin D levels, represented here by their mid-ranges.

a functional or mobility limitation. Overall mortality was the same across all levels of 25(OH)D ($P = 0.96$ for trend), while there was a non-significant 12%–52% increase in the risk of CV mortality for the lowest categories ($P = 0.15$ for trend).

Study L

Data from the Aging in the Chianti Area Study (*InCHIANTI*) were used to examine the association of 25(OH)D status on overall and cardiovascular mortality in adults aged at least 65 years at enrollment from Tuscany, Italy.¹⁹ 1006 adults with mean age 74 years were followed for 6.5 years; 228 died, of whom 107 due to CVD causes. Fig. 12 shows HR's of CV mortality across 25(OH)D quartiles, with the highest (>25.6 ng/mL) as reference, from analyses that accounted for age, sex, education, season of blood withdrawal, BMI, smoking, aspirin use, physical activity, total and HDL cholesterol, cognitive impairment and renal insufficiency. In analyses of all-cause mortality, it was additionally adjusted for hypertension, diabetes mellitus, heart failure and stroke. Being in any of the non-referent categories conferred a doubling in the risk of CVD mortality (HR = 2.64, 95% CI (1.68, 2.19), 1.68, (0.76, 3.72) and 2.19, (1.05, 4.60) across increasing quartiles).

Study M

The Rancho Bernardo Study²⁰ Participants from a Caucasian cohort living in Southern California in 1972, who were alive for a medical examination during 1997–1999, had measurements taken during this time on the 25(OH)D, 1,25(OH)₂D and intact PTH levels and had measurements on the glomerular filtration rate (eGFR) were included in the current prospective study for examining the potential impact of vitamin D blood markers on CVD mortality. Thus 1073 participants with a median age of 76 years were followed on average for 6.4 years. 111 CVD deaths were recorded during this time. This

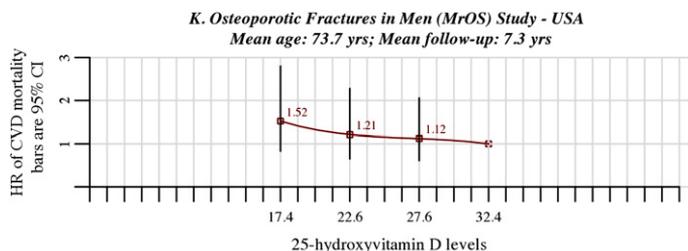


Fig. 11. Hazard ratios of cardiovascular mortality across quartiles of 25-hydroxyvitamin D levels, represented here by their mid-ranges.

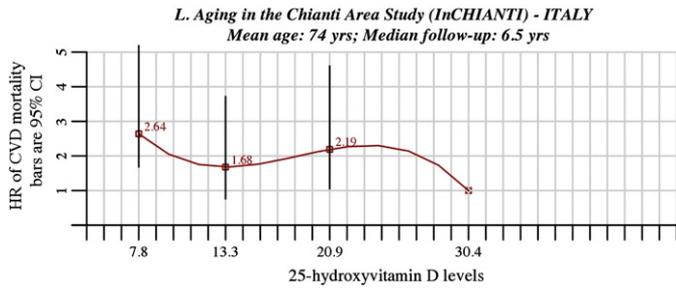


Fig. 12. Hazard ratios of cardiovascular mortality across quartiles of 25-hydroxyvitamin D levels, represented here by their mid-ranges.

cohort had remarkable high 25(OH)D levels, as 86% of the subjects had ≥ 30 ng/mL (mean 42 ng/mL). Because the RR's were not presented in categories, but only in terms of standard deviation (SD) increment, a figure was not included for this study. In models that accounted for age, sex, BMI, systolic BP, LDL cholesterol, glucose, physical activity, albumin to creatinine ratio, presence of CVD, season of blood withdrawal, use of diuretics, calcium channel blockers, β -blockers and angiotensin-converting enzyme, no additional risk of CVD death was found per SD increase in 25(OH)D (HR = 1.07, 95% CI (0.86, 1.33)). The authors note that the results were similar in analyses that compared subjects with 25(OH)D ≥ 30 ng/mL vs. < 30 ng/mL. As an interaction was found between 25(OH)D and eGFR ($P = .03$), the analyses were repeated separately in the group with eGFR < 60 and ≥ 60 . Although the results almost reach statistical significance, it is noteworthy that a 33% decrease in CVD death per 25(OH)D SD increase was found in the eGFR < 60 group (HR = 0.67, 95% CI (0.43, 1.05)) and a 25% increase in the ≥ 60 group (HR = 1.25, 95% CI (0.99, 1.57)).

Synthesis of prospective studies of 25(OH)D level and cardiovascular disease

Dose–response

We did not formally test for dose–response in a meta-analysis because the studies used different categorizations of 25(OH)D and different methods and laboratories were used. Nonetheless, we draw some conclusions based on the 13 studies identified. In all 12 studies that provided results by categories of 25(OH)D, the highest risk of CVD incidence or mortality occurred at the lowest level of 25(OH)D, a level generally lower than 10–15 ng/mL. Typically, the (negative) slope of the risk curve was steepest at the lowest level, and usually the slope was negative to a range of approximately 20–25 ng/mL. From the range of approximately 20–25 to 30–35 ng/mL, the results were more variable and the slopes flatter; with some studies suggesting potential benefits with increasing 25(OH)D over this range (B, cerebrovascular mortality; F, sudden death only; G; L), but some did not detect any apparent trend (C; F, heart failure; B; L, coronary mortality), had slight but non-significant inverse trends (E; J; K), slight non-significant positive trends (A; D; I), or inadequate data (C; H) in the ranges from 25–35 ng/mL. There are sparse data above the range of 35 ng/mL. One relatively small study with remarkable high 25(OH)D levels (M) (86% had ≥ 30 ng/mL; mean 42 ng/mL) showed no significant trend. Thus, we conclude based on fairly consistent results over these 13 studies, a higher risk below 25(OH)D levels of approximately 25 ng/mL, with increasing magnitude of risk as levels go lower, and no clear trend above a level of approximately 25 ng/mL.

Assessment of causality

Because these studies showing potential CVD benefits of vitamin D are observational, and exposure to vitamin D is not randomized, these associations could possibly be non-causal. We thus consider

potential non-causal explanations of the associations. Given the consistently higher risk at the lower end of 25(OH)D across many studies, this finding is highly unlikely due to chance. Most biases can be ruled out because the studies were prospective, follow-up was generally high and complete, and many studies had relatively long follow-up which probably precluded reverse causation.

The most likely alternative explanation to causality to consider is confounding. In general, the studies were of reasonably high quality, and assessed and adjusted for many behavioral and biochemical CVD risk factors in the multivariate models. Circulating 25(OH)D levels are determined largely by sun exposure, and studies have shown that physical activity level is positively correlated with 25(OH)D level, likely as an indicator of outdoor exposure to the sun. In addition, higher adiposity is relatively consistently shown to be related to lower 25(OH)D level. Almost all of the studies controlled for physical activity and body mass index, and the reported associations remained after their adjustment. Nonetheless, while body mass index is measured well, physical activity is difficult to measure and imperfect measurement might still have resulted in residual confounding. A number of studies also adjusted for glomerular filtration rate (or creatinine), as circulating 25(OH)D levels could be lower in individuals with chronic kidney disease, which is a risk factor for CVD.²¹ Although residual or uncontrolled confounding cannot be entirely ruled out, the studies as a group were of relatively high quality and excluded confounding to the extent possible by current standards.

Randomized controlled trial data

Randomized controlled trials (RCTs) would provide the strongest test of the hypothesis that vitamin D lowers CVD risk, but unfortunately data from RCTs are very limited to date. In a recent systematic review by Wang et al.,²² the authors concluded that evidence from limited data suggests that vitamin D supplements at moderate to high doses may reduce CVD risk. Two reasonably sized RCTs have reported on vitamin D and CVD. In a study of 2686 men and women in the United Kingdom, men and women were randomized to 830 IU vitamin D per day (administered as 100,000 IU oral vitamin D3 every 4 months) or placebo over a period of 5 years.²³ The in-study 25(OH)D levels were measured as 29.7 ng/mL in the vitamin D group and 21.4 ng/mL in the placebo group. A non-significant decrease was observed in the intervention group for CVD incidence (RR = 0.90, 95% CI 0.77–1.06) and CVD mortality (RR = 0.84, 95% CI 0.65–1.10). Notably, the mean level in the placebo group would suggest that a substantial number of individuals had adequately high 25(OH)D and were not likely to benefit from additional vitamin D if higher risk is mostly in levels below 20–25 ng/mL.

The largest RCT of vitamin D (and calcium) supplementation and CVD risk was from the Women's Health Initiative, in which 36,282 postmenopausal women received either calcium (1000 mg daily) and vitamin D3 (400 IU daily) or placebo.²⁴ No reduction was observed in MI or CHD death (hazard ratio, 1.04; 95% CI, 0.92–1.18). However, this study had three major limitations. First, the dose of 400 IU/day (coupled with sub-optimal compliance) may have been too low to cause a substantial contrast between the treated and non-treated group. Second, there was a relatively high use of non-protocol supplements in the study (including vitamin D), which may have reduced overall deficiency in the study population. Finally, vitamin D was given concurrently with 1000 mg/d of calcium, and some studies suggest high-dose calcium could increase risk of CVD.²⁵ A meta-analysis of RCTs of vitamin D and total mortality suggested some benefit of vitamin D supplementation on reducing total mortality (RR = 0.93 (95% confidence interval, 0.87–0.99)).²⁶

Future directions and current recommendations

A definitive proof of the utility of vitamin D supplementation on CVD incidence and mortality would come from "definitive" RCTs. Such trials would require large numbers, adequate duration and dose, sufficient numbers of subjects in the low range of 25(OH)D, such as below 20 ng/mL, who are most likely to benefit, and successful conduct of the trial (high compliance, minimal use of non-protocol vitamin D supplements). Some ongoing RCTs, e.g. VITAL (www.vitalstudy.org) may provide important information when completed. The duration required to observe an effect is unknown, and depends on the mechanism. For CVD, arguably several to five years may be adequate, as most CVD interventions

when successful have shown relatively immediate effects; for other endpoints, such as cancer, a longer duration for supplementation might be required for an unequivocal result to emerge.

Smaller scale RCTs on intermediate endpoints, such as coronary calcification, inflammatory markers, or blood pressure, might provide useful data, but would not inform on the overall impact of vitamin D on CVD as multiple mechanisms could be involved. The potential intermediate factor most definitively associated with vitamin D deficiency is an increase in PTH levels.²⁷ Patients with primary hyperparathyroidism are at increased risk of mortality, particularly from fatal CVD,^{28,29} and suffer from abnormalities in the renin-angiotensin system, cardiac arrhythmias, increased coronary calcification, and structural and functional abnormalities in the vascular wall. Cardiac mortality is markedly elevated in chronic renal failure patients, perhaps due in part to the profound functional vitamin D deficiency from loss of renal conversion of 25(OH)D to 1,25(OH)₂D. Chronic renal failure patients suffer from severe secondary hyperparathyroidism. In individuals without renal disease but with severe vitamin D deficiency, similar but less extreme processes involving PTH and the cardiovascular system might occur. In a recent analysis of The Uppsala Longitudinal Study of Adult Men, higher plasma PTH levels, even within the normal range, were associated with increased CVD mortality (RR for 1-SD increase in PTH, 1.38; 95% CI, 1.18 to 1.60; $P < 0.001$).³⁰ Further study of this topic is critical.

Despite the lack of “gold standard” evidence for an effect of vitamin D on CVD risk, recommendations on adequate intakes and levels of 25(OH)D cannot be avoided. Unlike a pure pharmaceutical agent, vitamin D is a natural compound and vitamin D levels are related to diet and lifestyle (sun exposure). Levels of 25(OH)D in most populations currently are likely to be much lower than levels humans evolved under because of industrialization and recommendations to limit sun exposure to prevent skin ageing and cancer. Further, albeit an important endpoint, CVD is only one consideration to base vitamin D recommendations. The vast majority of studies for various endpoints including some cancers, total mortality, musculoskeletal health, and some autoimmune and infectious diseases find higher risk of these outcomes at levels below 30 ng/mL.³¹

In our analysis, risk for CVD incidence and mortality was typically higher for those with 25(OH)D levels below approximately 25 ng/mL. To achieve levels in the range of 30–40 ng/mL generally requires oral doses in the range of 1800 to 4000 IU/day.³¹ Intake in this range has been shown to be non-toxic and safe for the majority of people. In most parts of the world, 25(OH)D levels below 25 ng/mL are common.³² The fact that we observed higher CVD risk associated with lower 25(OH)D in 13 diverse study populations, originating from countries that roughly span 30 to 70 degrees Northern latitude, indicates that substantial proportions of individuals in most populations do not have adequate vitamin D intakes and levels that would minimize their risk for CVD.

Summary

Because of laboratory and epidemiologic evidence that vitamin D deficiency may increase risk of CVD, we searched for all prospective studies, published until December 2010, that assessed the association between baseline categories of 25(OH)D levels and risk of dying from cardiovascular related causes. We identified 13 studies, which were mostly of high quality, and adjusted for many known and suspected CVD risk factors, including body mass index and physical activity. In most studies, the highest risk of CVD incidence or mortality occurred at the lowest level of 25(OH)D, a level typically lower than 10–15 ng/mL. Typically, the (negative) slope of the risk curve was steepest at the lowest level, and usually the slope was negative to a range of approximately 20–25 ng/mL. From the range of approximately 20–25 to 30–35 ng/mL, the results were more variable and equivocal. Thus, we conclude with fairly consistent results over 13 studies, a higher risk below 25(OH)D levels of approximately 25 ng/mL, with increasing magnitude of risk with decreasing levels, and no clear trend above a level of approximately 25 ng/mL. Currently, randomized trial data are not adequate to inform on the role of vitamin D supplementation on CVD risk. Prevention of vitamin D deficiency may have benefits on lowering risk of CVD. Risk appears to be minimized at 25(OH)D levels between 25–30 ng/mL, and levels of 30 ng/mL are generally regarded as safe. Thus, achieving a level of 30 ng/mL appears a reasonable target, though further studies can help refine and solidify recommendations.

Practice points

- 25(OH)vitamin D levels below 25 ng/mL have been consistently associated with increased risk of cardiovascular (CVD) endpoints.
- Currently, randomized trial data are not adequate to inform on the role of vitamin D supplementation on CVD risk.
- Achieving a level of 30 ng/mL appears a reasonable target, given that this level is associated with lower risk of CVD and possibly other outcomes.

Research agenda

- Further observational and randomized clinical trial data are important to better characterize the optimal range for 25(OH)D, and whether vitamin D supplementation lowers risk of CVD and other diseases.
- The mechanisms whereby vitamin D might influence CVD remain to be elucidated.

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