Received: 7 July 2017 | Revised: 18 September 2017 | Accepted: 21 September 2017

DOI: 10.1111/cen.13485

ORIGINAL ARTICLE

WILEY

Serum 25-hydroxyvitamin D as a predictor of mortality and cardiovascular events: A 20-year study of a community-based cohort

Kun Zhu[1,2] | Matthew Knuiman[3] | Mark Divitini[3] | Joseph Hung[2] | Ee Mun Lim[1,4] | Brian R. Cooke[5] | John P. Walsh[1,2]

[1]Department of Endocrinology and Diabetes, Sir Charles Gairdner Hospital, Nedlands, WA, Australia

[2]School of Medicine and Pharmacology, University of Western Australia, Crawley, WA, Australia

[3]School of Population and Global Health, University of Western Australia, Crawley, WA, Australia

[4]Department of Clinical Biochemistry, PathWest Laboratory Medicine, Queen Elizabeth II Medical Centre, Nedlands, WA, Australia

[5]Department of Clinical Biochemistry, PathWest Laboratory Medicine, Fiona Stanley Hospital, Murdoch, WA, Australia

Correspondence
Kun Zhu, Department of Endocrinology and Diabetes, Sir Charles Gairdner Hospital, Nedlands, WA, Australia.
Email: kun.zhu@uwa.edu.au

Funding information
The 1994/95 Busselton Health Survey was supported by a grant from the Health Promotion Foundation of Western Australia. Abbott Australasia Pty Ltd donated assay kits for the Abbott ARCHITECT® 25-OH Vitamin D Assay, and funded the relevant staff to perform the specimen preparation and biochemical analysis. None of the funding agencies had any role in the conduct of the study; collection, management, analysis, or interpretation of the data; or preparation, review, or approval of the manuscript.

Summary

Objective: Prospective studies, mostly from Europe and North America, suggest that serum 25-hydroxyvitamin D (25(OH)D) is inversely associated with mortality and cardiovascular disease (CVD) risk. Data from other regions are limited, and threshold levels for adverse cardiovascular outcomes are uncertain. We examined serum 25(OH)D as a predictor of total mortality and cardiovascular outcomes in an Australian cohort.

Design: A 20-year, community-based cohort study.

Patients: Participants in the 1994/1995 Busselton Health Survey (n = 3946, baseline age 25-84 years).

Measurements: Baseline serum 25(OH)D and mortality and cardiovascular outcomes to 2014 obtained by record linkage.

Results: The mean serum 25(OH)D concentration was 60.6 ± 18.0 nmol/L. During 20-year follow-up (excluding the first 2 years), 889 participants died (including 363 from CVD) and 944 experienced a CVD event (including 242 with heart failure). In the full cohort, controlling for Framingham risk score variables, higher baseline 25(OH)D was associated with significantly reduced all-cause mortality (adjusted HR 0.83 per SD increment of 25(OH)D, 95% CI 0.77-0.90), CVD death (HR 0.85, 95% CI 0.74-0.96) and heart failure (HR 0.81, 95% CI 0.69-0.94), but not CVD events (HR 0.99, 0.92-1.07). In restricted cubic spline regression models, serum 25(OH)D below 65 and 55 nmol/L was associated with higher total mortality and higher CVD mortality/heart failure, respectively. In participants without CVD at baseline (n = 3220), results were similar, but hazard ratios were attenuated and associations with CVD mortality no longer significant.

Conclusions: In an Australian community-based cohort, baseline vitamin D levels below 55-65 nmol/L are predictive of all-cause mortality, CVD death and heart failure.

KEYWORDS

Australia, cardiovascular disease, follow-up study, health survey, heart failure, mortality, vitamin D

1 | INTRODUCTION

The pivotal role of vitamin D in calcium homoeostasis and bone health is well known. Vitamin D may also have anti-inflammatory and immune-modulating effects, and vitamin D receptors are expressed in many tissues related to cardiovascular health.[1] Several previous cohort studies have shown that low vitamin D status, usually defined as serum 25-hydroxyvitamin D (25(OH)D) below 30 or 50 nmol/L, is associated with increased all-cause mortality.[2-5] Cohort studies of 25(OH)D as a predictor of cardiovascular disease (CVD) mortality and events have been less consistent, with some[2,3] but not others[6,7] finding an association; however, two recent meta-analyses did report a significant association between vitamin D and CVD outcomes.[5,8] For the most part, randomized controlled trials (RCTs) of vitamin D supplementation have reported no effect on cardiovascular risk factors such as blood pressure, arterial stiffness, carotid intima medial thickness or endothelial function.[9] To date, RCTs of vitamin D (alone or with calcium supplementation) have shown no protective effects on CVD events or death.[9-11] However, a number of RCTs had significant limitations which hamper interpretation, including being designed to detect musculoskeletal outcomes such as falls and fracture rather than CVD outcomes, inclusion of participants who were vitamin D replete or did not measure 25(OH)D at baseline, and the heterogeneity in the supplementation dose and interval.[10,12]

It is also possible that vitamin D level is more relevant to particular types of CVD, such as heart failure. In the RECORD trial, where participants received 800 IU vitamin D or placebo daily for 3 years then followed up for a further 3 years, vitamin D supplementation was related to lower risk for cardiac failure, but not MI and stroke.[11] Cardiomyocytes express vitamin D receptors, and there is experimental evidence that vitamin D deficiency results in maladaptive cardiac remodelling and dysfunction.[1,13] However, there have been few longitudinal community-based cohort studies on the association between vitamin D status and heart failure. In addition, most previous studies on vitamin D level and CVD have been carried out in Europe and North America, and there are no previous large prospective studies in the Australian population. The "dose–response" relationship between serum 25(OH)D level and CVD risk is also not well-characterized, and threshold levels for risk are uncertain. Previous studies have suggested a no-linear association and in some cases a reverse J-shaped relationship between vitamin D status and all-cause[14] and cardiovascular mortality,[15] with increased risk at both low and high serum 25(OH)D values, but this remains controversial.

In the Busselton Health 1994/1995 survey cohort of adults aged 25-84 at baseline,[16] complete hospital morbidity and death records for 20 years after the baseline survey are available from the Western Australian Data Linkage System. The aim of this study was to investigate serum 25(OH)D as a predictor of all-cause and CVD mortality, incident CVD and heart failure in this well-characterized general community Australian adult cohort, and to evaluate the "dose–response" relationship between vitamin D status and outcomes.

2 | PARTICIPANTS AND METHODS

2.1 | Study participants

The population of Busselton, a coastal area in the southwest of Western Australia, has been regularly surveyed since 1966.[17] Greater than 90% of this population consists of individuals with Anglo-Celtic ancestry. In 1994/1995, a follow-up health survey of 4843 survivors from previous surveys was conducted as previously described.[16] The survey received ethics approval from the University of Western Australia Human Research Ethics Committee (approval number 05/06/004/674) and written informed consent was obtained from each participant. The Human Research Ethics Committee of the Department of Health of Western Australia (Project number 2011/60) gave permission to access the hospital morbidity and death records of the survey participants using record linkage.[18]

2.2 | Baseline measurements

Participants completed a comprehensive health and lifestyle questionnaire, and underwent various measurements and tests.[16] Information on smoking, alcohol intake, minutes of moderate and vigorous leisure time physical activity (LTPA) per usual week, diabetes and use of medications and supplements was obtained by questionnaire. Alcohol consumption was categorized as light, moderate and heavy if intake was <140, 140-420 and >420 g/week, respectively. LTPA was calculated as (min/week of moderate activities) + 2 × (min/week of vigorous activity) and categorized as (0-149 or 150 + min/week) where 150 min/week is the recommended level of physical activity sufficient for health benefits.[19] Weight and height were measured following standardized protocols and body mass index (BMI) calculated as weight (kg)/height $(m)^2$. Blood pressure (BP, systolic and diastolic) was measured using a mercury sphygmomanometer after 5 minutes of rest in a sitting position.

2.3 | Biochemical analysis

Blood samples were obtained from the participants after an overnight fast at time of survey, serum separated and stored at −70°C. Serum 25(OH)D was measured in 2016 using the Abbott ARCHITECT® 25-OH Vitamin D Assay (Abbott Laboratories, Abbott Park, IL, USA). The reported total between run CVs for the serum samples ranged from 2.6% at 178.3 nmol/L to 4.0% at 57.5 nmol/L. In 117 samples (randomly selected within 3 strata of 25(OH)D) that were also assayed using isotope-dilution liquid chromatography/tandem mass spectrometry,[20] there was a strong correlation between the two techniques (r^2 = .94).

Blood measures that were obtained at the time of the survey and were available for this analysis include serum total and HDL cholesterol, triglycerides, plasma glucose, C-reactive protein (CRP), creatinine and estimated glomerular filtration rate (eGFR).[16,21]

2.4 | Mortality and cardiovascular outcomes

Cardiovascular hospitalizations and deaths for the period 1 January 1980 to 30 June 2014 were identified using the linked data from the Hospital Morbidity Data Collection and Mortality Register provided via the Western Australian Data Linkage System.[18] International Classification of Diseases, 9th revision (ICD-9) codes were used up to 30 June 1999, and ICD-10 codes were used for subsequent events. History of CVD at baseline was based on any CVD hospital admission (ICD-9 390-459) during the 15 years before the survey. Four outcome events were analysed: (i) time to death from any cause; (ii) time to death from cardiovascular diseases (ICD9 390-459; ICD10 I00-99, G45); (iii) time to first fatal or nonfatal cardiovascular event defined as a hospital admission with a principal diagnosis of coronary heart disease (ICD9 410-414; ICD10 I20-25), stroke (ICD9 430-437; ICD10 I60-68, G45), heart failure (ICD9 428; ICD10 I50), peripheral arterial disease (ICD9 440-448; ICD10 I70-79) or death from cardiovascular disease; and (iv) time to first heart failure event.

2.5 | Statistical analyses

Descriptive statistics are presented as mean ± SD or number (%) unless otherwise stated. Variables with skewed distributions were log transformed for use in regression models. The associations between vitamin D level and cardiovascular outcomes were examined using Cox proportional hazards regression modelling. Vitamin D level (in nmol/L) was examined as a continuous variable (both a linear trend and restricted cubic splines) and in categories corresponding to lower (<50 nmol/L), medium (≥50 to <75 nmol/L), and higher (≥75 nmol/L) to test the graded risk. Lower vitamin D status was defined as below the sufficient level recommended by the US Institute of Medicine Committee (50 nmol/L),[22] and the higher level was defined as above the sufficient level recommended by the Endocrine Society (75 nmol/L).[23] The estimated hazard ratios (HRs) with 95% confidence interval (CI) are reported for each of the four outcomes after three levels of adjustment for potential confounders. Model 1 adjusted for age, sex, season of blood sampling, use of vitamin D supplements and CVD history, Model 2 further adjusted for Framingham risk score variables (cholesterol, HDL cholesterol, smoking, diabetes, systolic BP, hypertension treatment) and Model 3 further adjusted for BMI, alcohol, LTPA, glucose, triglycerides, CRP and eGFR. The percentage of participant using lipid-lowering medication was very low in the cohort, and hence, this was not included as an additional confounder. Analyses were conducted for the full cohort and the subcohort of participants with no history of CVD at baseline (CVD-free cohort). Tests of the interaction between vitamin D level and sex, age group (<50, 50-64, 65 + years) or BMI group (<25, 25-29.9, ≥30 kg/m^2) showed that, for all outcomes, the effect of vitamin D level on risk was not significantly different between men and women and across age or BMI groups (all $P > .05$), except for all cause death in the CVD-free cohort with age (P for interaction = .03, weaker relationship in <50 year age group). A 25(OH)D concentration of 80 nmol/L, as in the paper by Schottker et al,[2] was chosen as the reference in fitting the restricted cubic spline curves, because it is close to the middle of 25(OH)D distribution which increases the precision of estimated HRs, and from an illustration viewpoint a value in the "higher" category is preferred as higher 25(OH)D was associated with lower risk of outcomes. A 95% CI that does not include the value 1 for the HR is significant at the 5% level (ie, $P < .05$). Statistical analyses were performed using SAS® 9.4 (SAS Institute Inc., Cary, NC, USA).

3 | RESULTS

3.1 | Study population

After excluding participants who were aged outside the range 25-84 years (377), those with missing baseline serum 25(OH)D measures (245) or data on key confounders (234), and those who died within the first 2 years of follow-up (41) to avoid the potential of reverse causation, there remained a total of 3946 for analyses. Compared with those included in the study, the 479 individuals with missing 25(OH)D or key confounders data were younger on average (49.4 vs 52.7 years), more likely to be men (51% vs 43%), have slightly higher eGFR (71.6 vs 69.5 mL/min/1.73 m^2) but otherwise similar in all other characteristics listed in Table 1.

The baseline characteristics of the cohort are shown in Table 1. The average age of participants was 52 years, and 43% were male. At baseline, 18% had a history of CVD. Blood sampling occurred in spring and winter for the majority of participants. The mean (±SD) serum 25(OH)D concentration in participants was 60.6 ± 18.0 nmol/L, and the median was 58.8 nmol/L. Serum 25(OH)D was less than 50 nmol/L in 28% of participants, between 50 and 75 nmol/L in 54%, and 75 nmol/L or higher in 18%.

Excluding the first 2 years of follow-up, in the full cohort (n = 3946), there were a total of 889 (22.5%) deaths with 363 due to CVD, 264 due to cancer, and 71 due to respiratory-related diseases. There was no significant difference in the mean 25(OH)D level between the 363 individuals who died from CVD and the 526 who died of non-CVD causes (54.6 vs 56.6 nmol/L, $P > .05$). A total of 944 (23.9%) experienced a CVD event including 242 (6.1%) who had a heart failure event.

3.2 | All-cause mortality

In the full cohort, there was a significant inverse relationship between baseline 25(OH)D and risk of all-cause death, with a HR of 0.83 (95% CI 0.77-0.90) per SD increment (18 nmol/L) of baseline 25(OH)D in Model 1 (Table 2). When analysed by categories of vitamin D status, participants with lower 25(OH)D had significantly higher all-cause mortality compared with those in the medium category (Model 1, HR = 1.30, 95% CI 1.12-1.51) (Table 3). Results were similar after further adjustment for Framingham risk score variables (Model 2), in the fully adjusted Model 3, and in the CVD-free cohort (Tables 2 and 3).

3.3 | CVD mortality and events

In the full cohort, there was a significant inverse relationship between baseline 25(OH)D and risk of CVD mortality, with a HR of

TABLE 1 Characteristics of cohort and number of outcome events in the whole cohort and by CVD history

	CVD history = no (n = 3220)	CVD history = yes (n = 726)	All (n = 3946)
Age (years)	50.2 ± 14.9	62.3 ± 14.0	52.5 ± 15.4
Sex male (%)	42.8	43.1	42.8
BMI (kg/m^2)	26.1 ± 4.2	26.7 ± 4.2	26.2 ± 4.2
Serum 25(OH)D (nmol/L)	61.0 ± 18.0	59.0 ± 18.0	60.6 ± 18.0
Serum 25(OH)D category (%)			
Lower (<50 nmol/L)	27.3	31.4	28.1
Medium (50-75 nmol/L)	54.1	50.8	53.5
Higher (≥75 nmol/L)	18.6	17.8	18.4
Season of blood sampling			
Autumn	13.8	12.3	13.5
Winter	18.2	12.7	17.2
Spring	66.5	73.1	67.7
Summer	1.5	1.9	1.6
Taking vitamin D supplements (%)	4.9	6.3	5.2
Smoking status (%)			
Never	52.4	49.2	51.8
Former	33.9	42.8	35.6
Current	13.6	8.0	12.6
Alcohol consumption (%)			
None	6.6	6.5	6.5
Ex	8.4	14.3	9.5
Light	60.4	54.8	59.4
Moderate/Heavy	21.0	17.8	20.4
Unknown	3.7	6.6	4.2
Leisure time physical activity (%)			
<150 mins/week	48.9	54.4	49.9
≥150 + mins/week	46.5	36.2	44.6
Unknown	4.6	9.4	5.5
Diabetes (%)	5.0	11.2	6.1
Hypertension treatment (%)	12.4	45.7	18.5
ACE-inhibitors (%)	4.2	16.8	6.5
Beta-blockers (%)	4.3	17.6	6.7
Calcium channel blockers (%)	2.5	14.0	4.6
Diuretics (%)	3.3	12.1	4.9
Other hypertension medication (%)	1.9	6.3	2.7
Lipid-lowering medication (%)	1.6	8.0	2.8
Total cholesterol (mmol/L)	5.59 ± 1.08	5.81 ± 1.01	5.63 ± 1.07
HDL cholesterol (mmol/L)	1.40 ± 0.39	1.37 ± 0.41	1.40 ± 0.39
Systolic blood pressure (mm Hg)	123 ± 17	132 ± 20	125 ± 18
Glucose (mmol/L)	4.96 ± 1.22	5.19 ± 1.69	5.00 ± 1.32
Log glucose	1.58 ± 0.17	1.62 ± 0.21	1.59 ± 0.17
Triglycerides (mmol/L)	1.27 ± 0.84	1.54 ± 1.18	1.32 ± 0.91
Log triglycerides	0.09 ± 0.53	0.25 ± 0.58	0.12 ± 0.55
CRP (mg/L)	3.02 ± 7.96	3.88 ± 10.05	3.18 ± 8.39

(Continues)

TABLE 1 (Continued)

	CVD history = no (n = 3220)	CVD history = yes (n = 726)	All (n = 3946)
Log CRP	0.36 ± 1.22	0.62 ± 1.23	0.41 ± 1.22
eGFR (mL/min/1.73 m^2)	71.1 ± 12.5	63.4 ± 14.1	69.7 ± 13.1
Event outcomes, n (%)			
Death	550 (17.1)	339 (46.7)	889 (22.5)
CVD death	192 (6.0)	171 (23.6)	363 (9.2)
CVD event	580 (18.0)	364 (50.1)	944 (23.9)
Heart failure event	122 (3.8)	120 (16.5)	242 (6.1)

Data are shown as mean ± SD, per cent or number (%) of event outcomes.
CVD, cardiovascular disease; BMI, body mass index; 25(OH)D, 25-hydroxyvitamin D; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate.

TABLE 2 Adjusted hazard ratio for serum 25-hydroxyvitamin D as continuous variable in relation to mortality and cardiovascular risk over the 20-year follow-up

	Model 1 HR (95% CI)	Model 2 HR (95% CI)	Model 3 HR (95% CI)
Full cohort			
All-cause death	**0.83 (0.77, 0.90)**	**0.83 (0.77, 0.90)**	**0.84 (0.77, 0.91)**
CVD death	**0.84 (0.74, 0.96)**	**0.85 (0.74, 0.96)**	**0.82 (0.72, 0.94)**
CVD events	0.98 (0.91, 1.06)	0.99 (0.92, 1.07)	1.00 (0.92, 1.08)
Heart failure	**0.82 (0.70, 0.95)**	**0.81 (0.69, 0.94)**	**0.84 (0.72, 0.98)**
CVD-free cohort			
All-cause death	**0.82 (0.74, 0.92)**	**0.83 (0.74, 0.92)**	**0.84 (0.75, 0.94)**
CVD death	0.85 (0.70, 1.03)	0.86 (0.71, 1.05)	0.87 (0.72, 1.06)
CVD events	0.98 (0.89, 1.08)	1.00 (0.91, 1.10)	1.01 (0.92, 1.12)
Heart failure	**0.76 (0.60, 0.96)**	**0.76 (0.60, 0.96)**	0.80 (0.62, 1.02)

CVD, cardiovascular disease; HR, hazard ratio, for a change of 18 nmol/L (one SD) in serum 25-hydroxyvitamin D.
Cox proportional hazards regression models adjusted for: Model 1: age, sex, season of blood sampling, taking vitamin D supplements and CVD history (whole cohort only); Model 2: Model 1 + cholesterol, HDL cholesterol, smoking, diabetes, systolic blood pressure and hypertension treatment; Model 3: Model 2 + BMI, alcohol consumption, LTPA 150 + mins/week, log(glucose), log(triglyceride), log(CRP) and eGFR. Bold values indicate statistical significance at the $P < .05$ level.

0.84 (95% CI 0.74-0.96) per SD increment in Model 1, and similar results in Models 2 and 3 (Table 2). The risk of CVD death was significantly higher in participants in the lower vitamin D category than in the medium category (Model 1, HR = 1.42, 95% CI 1.13-1.79), with similar results in all 3 models (Table 3). When the analysis was restricted to the CVD-free cohort, HR estimates for CVD mortality were attenuated and no longer significant (Tables 2 and 3).

There was no significant association between baseline 25(OH)D (as either a continuous or categorical variable) and the risk of all CVD events in the full cohort or the CVD-free cohort (Tables 2 and 3, Figure 1C).

3.4 | Heart failure

In the full cohort, there was a significant inverse relationship between baseline 25(OH)D and risk of heart failure, with a HR of 0.82 (95% CI 0.70-0.95) per SD increment in Model 1 (Table 2). The risk of heart failure was significantly higher in participants in the lower vitamin D category compared with the medium category (Model 1, HR = 1.35, 95% CI 1.02-1.79), with similar results in Model 2, although no longer significant in Model 3 (Table 3).

In the CVD-free cohort, results were similar, with a HR for heart failure of 0.76 (95% CI 0.60-0.96) per SD increment of baseline 25(OH)D in Model 1, and similar results in Model 2, although no longer significant in Model 3 (Table 2). The risk of heart failure was significantly higher in participants in the lower vitamin D category than in the medium category (Table 3).

The risk in the higher vitamin D category was not significantly different ($P > .05$) to the risk in the medium category for all the outcomes (Table 3), and to the risk in the lower category for all outcomes studied, except for all-cause mortality in Model 1 (HR = 1.26, 95% CI 1.00-1.57) and Model 2 (HR = 1.28, 95% CI 1.03-2.61).

To provide context for the magnitude of the effect of 25(OH)D, the effect of a one SD change in 25(OH)D and other risk factors was compared and in most cases the effect of 25(OH)D was similar to or greater than that of other risk factors. For example, a one SD change in 25(OH)D on CVD death had HR of 0.85 (ie, 15% decrease) which is similar to the effect of a one SD change in systolic BP (HR = 1.13, ie, 13% increase).

In a further sensitivity analysis, leaving out deaths within first 4 years made negligible difference to results, except that in the full cohort, the association with CVD death did not reach the significance level (HR = 0.88, 95% CI 0.77-1.01 per SD increment of 25(OH)D).

TABLE 3 Adjusted hazard ratio between vitamin D status categories in relation to mortality and cardiovascular risk over the 20-year follow-up

	Lower < 50 nmol/L n = 1109 HR (95% CI)	Medium 50-75 nmol/L n = 2111 Reference	Higher ≥75 nmol/L n = 726 HR (95% CI)
Full cohort			
All-cause death			
Model 1	**1.30 (1.12, 1.51)**	1.00	1.04 (0.84, 1.28)
Model 2	**1.29 (1.12, 1.50)**	1.00	1.01 (0.82, 1.24)
Model 3	**1.29 (1.11,1.50)**	1.00	1.05 (0.85, 1.29)
CVD death			
Model 1	**1.42 (1.13, 1.79)**	1.00	1.25 (0.90, 1.74)
Model 2	**1.40 (1.12, 1.76)**	1.00	1.20 (0.86, 1.68)
Model 3	**1.46 (1.16, 1.85)**	1.00	1.20 (0.86, 1.69)
CVD events			
Model 1	1.10 (0.95, 1.28)	1.00	1.17 (0.97, 1.41)
Model 2	1.07 (0.92, 1.24)	1.00	1.16 (0.96, 1.40)
Model 3	1.07 (0.93, 1.24)	1.00	1.19 (0.98, 1.44)
Heart failure			
Model 1	**1.35 (1.02, 1.79)**	1.00	0.90 (0.59, 1.39)
Model 2	**1.33 (1.00, 1.75)**	1.00	0.85 (0.56, 1.31)
Model 3	1.30 (0.98, 1.72)	1.00	0.95 (0.61, 1.47)
CVD-free cohort			
All-cause death			
Model 1	**1.27 (1.05, 1.53)**	1.00	0.97 (0.73, 1.29)
Model 2	**1.24 (1.03, 1.50)**	1.00	0.95 (0.72, 1.26)
Model 3	**1.24 (1.02, 1.49)**	1.00	0.99 (0.74, 1.31)
CVD death			
Model 1	1.30 (0.96, 1.78)	1.00	1.07 (0.64, 1.78)
Model 2	1.20 (0.87, 1.64)	1.00	1.02 (0.61, 1.70)
Model 3	1.18 (0.86, 1.62)	1.00	1.02 (0.61, 1.73)
CVD events			
Model 1	1.09 (0.90, 1.32)	1.00	1.11 (0.87, 1.43)
Model 2	1.03 (0.86, 1.25)	1.00	1.10 (0.86, 1.41)
Model 3	1.03 (0.85, 1.24)	1.00	1.12 (0.87, 1.44)
Heart failure			
Model 1	**1.53 (1.03, 2.25)**	1.00	1.10 (0.59, 2.06)
Model 2	**1.49 (1.01, 2.20)**	1.00	1.06 (0.56, 1.97)
Model 3	**1.58 (1.06, 2.37)**	1.00	1.35 (0.71, 2.54)

CVD, cardiovascular disease; HR, hazard ratio.
Cox proportional hazards regression models adjusted for: Model 1: age, sex, season of blood sampling, taking vitamin D supplements and CVD history; Model 2: Model 1 + cholesterol, HDL cholesterol, smoking, diabetes, systolic blood pressure and hypertension treatment; Model 3: Model 2 + BMI, alcohol consumption, LTPA 150 + mins/week, log(glucose), log(triglyceride), log(CRP), eGFR. Bold values indicate statistical significance at the $P < .05$ level.

3.5 | Levels of vitamin D associated with increased risk

Figure 1 shows the estimated Model 1 adjusted HRs for each level of 25(OH)D (vs a level of 80 nmol/L) as estimated from the restricted cubic spline regression model. In the full cohort, compared with participants with a 25(OH)D level of 80 nmol/L, those with 25(OH)D < 65 nmol/L had a significantly higher risk of all-cause death (ie, 95% CI is entirely above 1.00) (Figure 1A), and those with 25(OH)D < 55 nmol/L had a significantly higher risk of CVD death (Figure 1B)

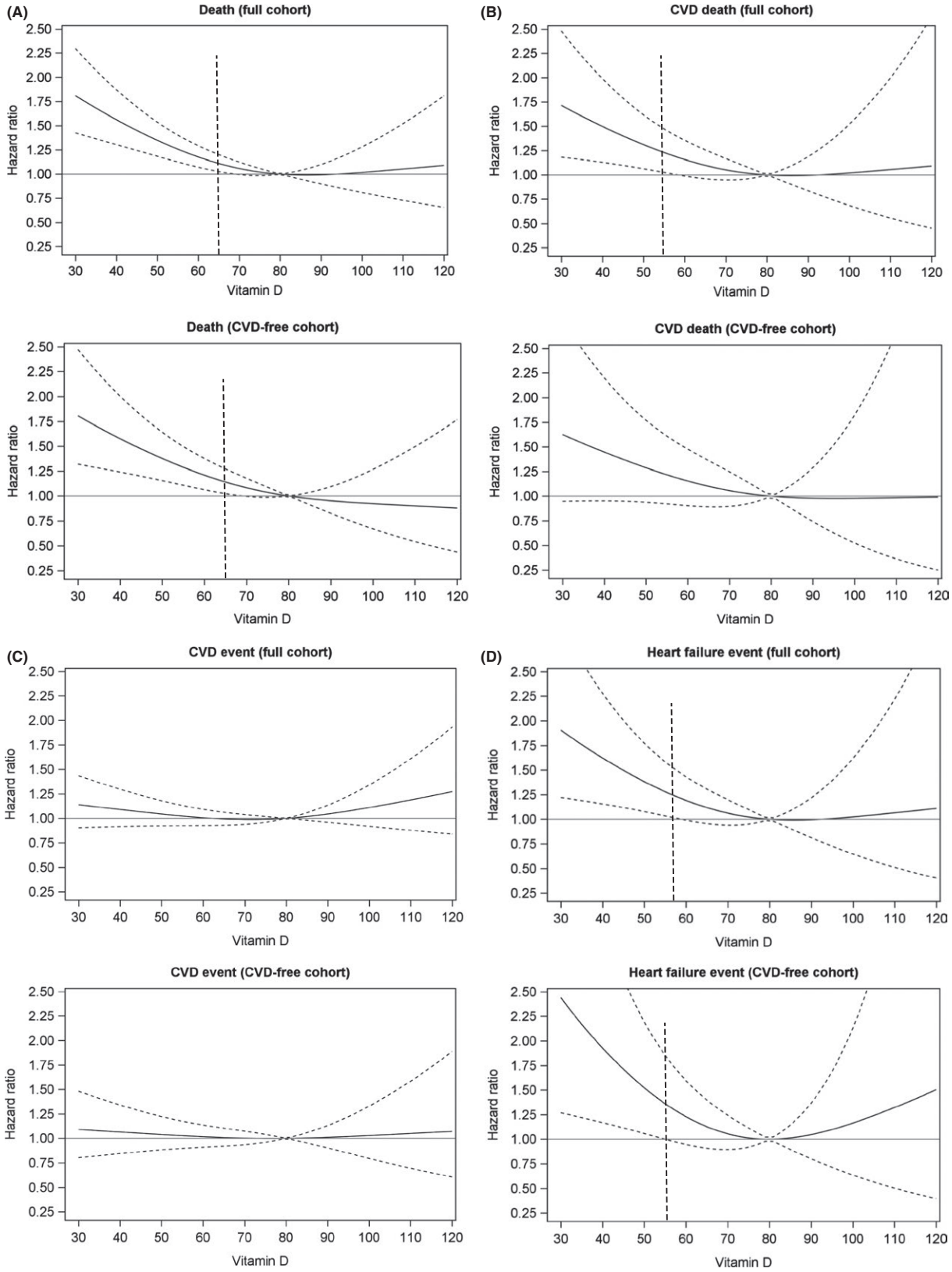

FIGURE 1 Adjusted hazard ratio (HR) from Model 1, for serum 25-hydroxyvitamin D (25(OH)D) concentration in relation to risk of all-cause death (A), cardiovascular disease (CVD) death (B), CVD event (C) and heart failure event (D) based on fitted restricted cubic spline regression with knots at 45, 75 and 105 nmol/L and using 80 nmol/L as the reference level, adjusted for age, sex, season of blood sampling, taking vitamin D supplements and CVD history (for analyse in the full cohort only). Solid line is estimated hazard ratio and dotted lines represent 95% confidence interval. Dotted vertical line indicate the cut-off value below which the risk is significantly higher (95% CI is entirely above 1.00) than the risk at 80 nmol/L

and heart failure (Figure 1D). The results were largely the same in the CVD-free cohort for all-cause death and heart failure (Figure 1A,D), but HR estimates were attenuated (closer to 1.0) for CVD death (Figure 1B).

4 | DISCUSSION

Using a prospective design with 20-year follow-up, our study provides further evidence for an independent link between vitamin D level and all-cause mortality, CVD death and heart failure in the general population. Our modelling suggested that serum 25(OH)D values below 65 nmol/L were associated with increased hazard of all-cause death, and values below 55 nmol/L with hazards of CVD death and heart failure, whereas levels above 80 nmol/L were not associated with further reduction in risk. Results were similar in participants who were free of CVD at baseline, except that associations between vitamin D and CVD mortality no longer reached statistical significance. The median 25(OH)D in our cohort was 58.8 nmol/L which means 50% participants are potentially at increased risk of mortality, CVD death and heart failure.

A general problem with evaluating the association of vitamin D status with mortality and CVD outcomes is reverse causation, as low vitamin D status could just be an indicator of poor general health and low outdoor activity level which reduces the cutaneous production of 25(OH)D. By excluding those who died in the first 2 years, our study minimized the bias in this regard. Furthermore, a population-based study reported that the association of vitamin D deficiency with all-cause and several cause-specific mortalities was independent of traditional mortality risk factors, self-rated health or frailty.[24] Our cohort had detailed baseline assessment of conventional and lifestyle CVD risk factors, and these potential confounders were adjusted for in the extended models, with most of the associations observed remained significant in Model 3 where physical activity, inflammatory markers and renal function were further adjusted.

Our results are consistent with previous studies showing that low vitamin D status is associated with increased all-cause mortality.[5] There have also been studies that suggested very high circulating 25(OH)D could be related to increased mortality risk, with a reverse J relationship.[14] This was not evident in our study, but the number of participants with very high serum 25(OH)D concentrations was small. In the whole cohort, we found a significant inverse relationship between serum 25(OH)D and CVD death, with HRs similar in magnitude to that for all-cause mortality; however, serum 25(OH)D was not significantly related to all CVD events combined. The reason for this is not known, but in a previous cohort study, the association with serum 25(OH)D was much stronger for CVD mortality than for CVD events,[25] and it has been suggested that vitamin D may be a marker of resilience to death in the setting of serious illness.[26] Consistent with this, the association of vitamin D with CVD death was significant in the whole cohort, but not in the CVD-free sub-cohort; other possible explanations for this include reverse causality or lack of statistical power, as there were fewer events in the CVD-free cohort.

Although serum 25(OH)D levels were not associated with combined CVD events, we found that lower serum 25(OH)D was consistently associated with increased risk of heart failure in the full cohort and in the CVD-free cohort. This finding is consistent with longitudinal data from NHANES III, in which participants with serum 25(OH)D levels below 50 nmol/L had increased risk for heart failure death,[4] and with results from the RECORD trial, in which vitamin D supplementation in participants aged over 70 years reduced the risk of heart failure.[11] By contrast, two studies in older adults showed that high parathyroid hormone level but not low vitamin D status was associated with increased risk of heart failure.[27,28]

There are several biological mechanisms by which vitamin D might plausibly affect cardiovascular risk.[1] In observational studies, higher serum 25(OH)D was associated with reduced blood pressure,[29] prevention of diabetes,[30] reduced inflammation[31] and reduced PTH concentrations, which in turn may affect myocardial hypertrophy and endothelial dysfunction;[32] however, meta-analyses of RCTs showed that vitamin D supplementation was effective in reducing PTH levels, but was not effective in lowering blood pressure or improving glucose homoeostasis, whereas the results on reducing inflammation have been inconsistent.[12] The association of vitamin D status with heart failure could be due to the fact that cardiomyocytes express vitamin D receptors, and their structure and function is influenced by physiological levels of active vitamin D metabolite.[1,13] One recent study showed that in patients with chronic heart failure secondary to left ventricular systolic dysfunction, 1 year of 100 μg daily vitamin D_3 supplementation had beneficial effects on left ventricular structure and function.[33] In patients with New York Heart Association (NYHA) class I-III heart failure, high-dose vitamin D significantly improved NYHA classification and ejection fraction.[34]

Our study has several strengths. Firstly, it was conducted in a well-characterized community cohort with prospective follow-up of 20 years, which is longer than most previous studies, and we evaluated the associations in both the whole cohort and the CVD-free subcohort. Secondly, baseline CVD was obtained from hospital admission data during the 15 years before the survey baseline, rather than self-report, and the use of hospital morbidity and mortality database to ascertain events removed the risk of retention bias. Thirdly, with comprehensive physical, questionnaire and biochemistry data collected in this cohort, further adjustment could be made for conventional CVD risk factors as well as other potential lifestyle and biochemical confounders. Our study also has limitations. Firstly, the observational nature of the study means we cannot assume a causal relationship between vitamin D and study outcomes. Secondly, our study, similar to most previous studies, only determined serum 25(OH)D levels at one single time-point. However, a large study of Norwegian adults showed significant tracking of serum 25(OH)D concentrations measured 14 years apart.[35] Thirdly, we had few participants with serum 25(OH)D level below 30 nmol/L; therefore, we cannot assess the association of very low levels of 25(OH)D with CVD risk and mortality. In addition, our study sample was from a small geographic area

of Western Australia and mainly of Anglo-Celtic ancestry; therefore, generalization of the study findings to other populations should be exercised with caution.

In conclusion, in a general Australian population who were followed up for 20 years, we showed that lower vitamin D status was independently associated with increased risk for all-cause mortality, CVD death and heart failure event. Our findings suggest maintaining serum 25(OH)D level at least above 55 nmol/L is associated with lower risk for mortality and heart failure. However, changes in clinical practice and health policy should await the completion of large scale RCTs designed to test the benefit of vitamin D supplementation on mortality and cardiovascular outcomes in the general population.[10]

ACKNOWLEDGEMENTS

The authors would like to thank the Busselton residents for their ongoing support of the Busselton Health Study, the Busselton Population Medical Research Institute for access to survey data, Dr Andrew St John for coordinating the vitamin D analysis, and the staff at the Western Australian Data Linkage Branch for their assistance with access to linked hospital and death records.

CONFLICT OF INTEREST

The authors state that they have no conflicts of interest.

ORCID

Kun Zhu http://orcid.org/0000-0002-8723-7574

REFERENCES

1. Norman PE, Powell JT. Vitamin D and cardiovascular disease. *Circ Res*. 2014;114:379-393.
2. Schottker B, Haug U, Schomburg L, 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;97:782-793.
3. Khaw KT, Luben R, Wareham N. Serum 25-hydroxyvitamin D, mortality, and incident cardiovascular disease, respiratory disease, cancers, and fractures: a 13-y prospective population study. *Am J Clin Nutr*. 2014;100:1361-1370.
4. Liu L, Chen M, Hankins SR, et al. Serum 25-hydroxyvitamin D concentration and mortality from heart failure and cardiovascular disease, and premature mortality from all-cause in United States adults. *Am J Cardiol*. 2012;110:834-839.
5. Schottker B, Jorde R, Peasey A, et al. Vitamin D and mortality: meta-analysis of individual participant data from a large consortium of cohort studies from Europe and the United States. *BMJ*. 2014;348:g3656.
6. Skaaby T. The relationship of vitamin D status to risk of cardiovascular disease and mortality. *Dan Med J*. 2015;62: pii: B5008.
7. Eaton CB, Young A, Allison MA, et al. Prospective association of vitamin D concentrations with mortality in postmenopausal women: results from the Women's Health Initiative (WHI). *Am J Clin Nutr*. 2011;94:1471-1478.
8. Wang L, Song Y, Manson JE, et al. Circulating 25-hydroxy-vitamin D and risk of cardiovascular disease: a meta-analysis of prospective studies. *Circ Cardiovasc Qual Outcomes*. 2012;5:819-829.
9. Veloudi P, Jones G, Sharman JE. Effectiveness of vitamin D supplementation for cardiovascular health outcomes. *Pulse (Basel)*. 2017;4:193-207.
10. Mihos CG, De La Cruz JA, Hernandez A, Santana O. Vitamin D deficiency and supplementation in cardiovascular disorders. *Cardiol Rev*. 2017;25:189-196.
11. Ford JA, MacLennan GS, Avenell A, et al. Cardiovascular disease and vitamin D supplementation: trial analysis, systematic review, and meta-analysis. *Am J Clin Nutr*. 2014;100:746-755.
12. Pilz S, Verheyen N, Grubler MR, Tomaschitz A, Marz W. Vitamin D and cardiovascular disease prevention. *Nat Rev Cardiol*. 2016;13:404-417.
13. Chen S, Law CS, Grigsby CL, et al. Cardiomyocyte-specific deletion of the vitamin D receptor gene results in cardiac hypertrophy. *Circulation*. 2011;124:1838-1847.
14. Sempos CT, Durazo-Arvizu RA, Dawson-Hughes B, et al. Is there a reverse J-shaped association between 25-hydroxyvitamin D and all-cause mortality? Results from the U.S. nationally representative NHANES. *J Clin Endocrinol Metab*. 2013;98:3001-3009.
15. Durup D, Jorgensen HL, Christensen J, et al. A reverse J-Shaped association between serum 25-Hydroxyvitamin D and cardiovascular disease mortality: the CopD study. *J Clin Endocrinol Metab*. 2015;100:2339-2346.
16. Knuiman MW, Hung J, Divitini ML, Davis TM, Beilby JP. Utility of the metabolic syndrome and its components in the prediction of incident cardiovascular disease: a prospective cohort study. *Eur J Cardiovasc Prev Rehabil*. 2009;16:235-241.
17. Knuiman MW, Jamrozik K, Welborn TA, Bulsara MK, Divitini ML, Whittall DE. Age and secular trends in risk factors for cardiovascular disease in Busselton. *Aust J Public Health*. 1995;19:375-382.
18. Holman CD, Bass AJ, Rouse IL, Hobbs MS. Population-based linkage of health records in Western Australia: development of a health services research linked database. *Aust J Public Health*. 1999;23:453-459.
19. AIHW. The Active Australia Survey: A guide and manual for implementing analysis and reporting. AIHW cat. no CVD 22. Canberra, Australia: Australian Institute of Health and Welfare, 2003.
20. Cooke DJ, Cooke BR, Bell DA, Vasikaran SD, Glendenning P. 25-Hydroxyvitamin D C3-epimer is universally present in neonatal Western Australian samples but is unlikely to contribute to diagnostic misclassification. *Ann Clin Biochem*. 2016;53:593-598.
21. Beilby J, Divitini ML, Knuiman MW, Rossi E, Hung J. Comparison of cystatin C and creatinine as predictors of cardiovascular events in a community-based elderly population. *Clin Chem*. 2010;56:799-804.
22. Institute of Medicine. *Dietary Reference Intakes for Calcium and Vitamin D*. Washington, DC: National Academy of Sciences. Institute of Medicine; 2010.
23. Holick MF, Binkley NC, Bischoff-Ferrari HA, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab*. 2011;96:1911-1930.
24. Schottker B, Saum KU, Perna L, Ordonez-Mena JM, Holleczek B, Brenner H. Is vitamin D deficiency a cause of increased morbidity and mortality at older age or simply an indicator of poor health? *Eur J Epidemiol*. 2014;29:199-210.
25. Perna L, Schottker B, Holleczek B, Brenner H. Serum 25-hydroxyvitamin D and incidence of fatal and nonfatal cardiovascular events: a prospective study with repeated measurements. *J Clin Endocrinol Metab*. 2013;98:4908-4915.
26. Schottker B, Brenner H. Vitamin D as a resilience factor, helpful for survival of potentially fatal conditions: a hypothesis Emerging from recent findings of the ESTHER Cohort Study and the CHANCES consortium. *Nutrients*. 2015;7:3264-3278.
27. Kestenbaum B, Katz R, de Boer I, et al. Vitamin D, parathyroid hormone, and cardiovascular events among older adults. *J Am Coll Cardiol*. 2011;58:1433-1441.

28. Bansal N, Zelnick L, Robinson-Cohen C, et al. Serum parathyroid hormone and 25-hydroxyvitamin D concentrations and risk of incident heart failure: the Multi-Ethnic Study of Atherosclerosis. *J Am Heart Assoc*. 2014;3:e001278.
29. Chen S, Sun Y, Agrawal DK. Vitamin D deficiency and essential hypertension. *J Am Soc Hypertens*. 2015;9:885-901.
30. Mathieu C. Vitamin D and diabetes: where do we stand? *Diabetes Res Clin Pract*. 2015;108:201-209.
31. Kalogeropoulos A, Georgiopoulou V, Psaty BM, et al. Inflammatory markers and incident heart failure risk in older adults: the Health ABC (Health, Aging, and Body Composition) study. *J Am Coll Cardiol*. 2010;55:2129-2137.
32. Fitzpatrick LA, Bilezikian JP, Silverberg SJ. Parathyroid hormone and the cardiovascular system. *Curr Osteoporos Rep*. 2008;6:77-83.
33. Witte KK, Byrom R, Gierula J, et al. Effects of vitamin D on cardiac function in patients with chronic HF: the VINDICATE study. *J Am Coll Cardiol*. 2016;67:2593-2603.
34. Amin A, Minaee S, Chitsazan M, Naderi N, Taghavi S, Ardeshiri M. Can vitamin D supplementation improve the severity of congestive heart failure? *Congest Heart Fail*. 2013;19:E22-E28.
35. 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;171:903-908.

How to cite this article: Zhu K, Knuiman M, Divitini M, et al. Serum 25-hydroxyvitamin D as a predictor of mortality and cardiovascular events: A 20-year study of a community-based cohort. *Clin Endocrinol (Oxf)*. 2018;88:154–163. https://doi.org/10.1111/cen.13485