Circulating 25-hydroxyvitamin D concentration and cause-specific mortality in the Melbourne Collaborative Cohort Study

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ABSTRACT

Vitamin D deficiency is associated with higher all-cause mortality, but associations with specific causes of death are unclear. We investigated the association between circulating 25-hydroxyvitamin D (25(OH)D) concentration and cause-specific mortality using a case-cohort study within the Melbourne Collaborative Cohort Study (MCCS). Eligibility for the case-cohort study was restricted to participants with baseline dried blood spot samples and no pre-baseline diagnosis of cancer. These analyses included participants who died (*n* = 2307) during a mean follow-up of 14 years and a sex-stratified random sample of eligible cohort participants ('subcohort', *n* = 2923). Concentration of 25(OH)D was measured using liquid chromatography-tandem mass spectrometry. Cox regression, with Barlow weights and robust standard errors to account for the case-cohort design, was used to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) for cause-specific mortality in relation to 25(OH)D concentration with adjustment for confounders.

Circulating 25(OH)D concentration was inversely associated with risk of death due to cancer (HR per 25 nmol/L increment = 0.88, 95% CI 0.78–0.99), particularly colorectal cancer (HR = 0.75, 95% CI 0.57–0.99). Higher 25(OH)D concentrations were also associated with a lower risk of death due to diseases of the respiratory system (HR = 0.62, 95% CI 0.43–0.88), particularly chronic obstructive pulmonary disease (HR = 0.53, 95% CI 0.30–0.94), and diseases of the digestive system (HR = 0.44, 95% CI 0.26–0.76). Estimates for diabetes mortality (HR = 0.64, 95% CI 0.33–1.26) and cardiovascular disease mortality (HR = 0.90, 95% CI 0.76–1.07) lacked precision.

The findings suggest that vitamin D might be important for preventing death due to some cancers, respiratory diseases, and digestive diseases.

1   Introduction

Many cohort studies have found that plasma 25-hydroxyvitamin D [25(OH)D] concentration, which is currently considered to provide the best measure of vitamin D status, is inversely associated with all-cause mortality [1-5]. A Mendelian randomisation study reaffirmed this association by demonstrating an inverse association between genetically-determined 25(OH)D and all-cause mortality of similar magnitude to that found for plasma 25(OH)D [6]. Complementing these findings, meta-analyses of randomised controlled trials (RCTs) have shown that vitamin D_3 supplementation is associated with lower all-cause mortality, although the magnitude of benefit appears to be small [2, 7]. With mounting evidence that vitamin D might be important for reducing mortality, it is important to investigate which specific causes of death are associated with vitamin D status.

The most commonly investigated causes of death in relation to 25(OH)D have been cancer and cardiovascular disease, with most studies reporting inverse associations, but data on specific cancers and cardiovascular diseases are lacking [1]. Associations with other causes of death, particularly less common causes, are unclear due to limited data [1].

In light of the lack of data for many causes of death, we sought to investigate the association between circulating concentration of 25(OH)D and various cause-specific mortality outcomes using a case-cohort study nested within a population-based prospective cohort study. To explore the potential influence of reverse causation, we performed sensitivity analyses excluding the first two years of follow-up and, for the main causes of death, evaluated associations for participants who reported being in good to excellent health about four years after baseline blood sample collection.

2   Methods

2.1   Participants

The Melbourne Collaborative Cohort Study (MCCS) is a prospective cohort study of 41,513 residents (24,469 women and 17,044 men) in Melbourne, Victoria, Australia, who were aged 27–76 (99% aged 40–69) years when recruited between 1990 and 1994. Details of the study have been published [8]. Briefly, at recruitment participants attended study centres where they completed a 121-item food frequency questionnaire, were interviewed about lifestyle and medical history, and had anthropometric measurements and blood samples collected. For participants recruited from the second year of recruitment onwards (i.e. for approximately 75% of participants), a whole blood sample was spotted onto Guthrie cards; these dried blood spot samples were stored at room

temperature in dark conditions. Approximately four years after recruitment, participants were mailed a self-administered questionnaire (wave 2), which included a question on general health status, rated as excellent, very good, good, fair, or poor. Participants are regularly sent study newsletters and asked to update contact details. This information, plus alternative contacts and searching the electoral roll was used to identify participants who had left Australia.

A case-cohort study was set up to investigate circulating concentration of 25(OH)D and risk of incident breast, prostate, and colorectal cancers, diabetes, and mortality. By the time it began, the baseline plasma samples for many of the participants diagnosed with cancer and those who had died from cardiovascular disease had been used. We, therefore, used archived dried blood spots for measurement of 25(OH)D. Eligibility for the case-cohort study was restricted to participants who had dried blood spot samples available from baseline and no pre-baseline diagnosis of cancer ($n =$ 29,205). Results pertaining to incident breast, prostate, and colorectal cancers [9], diabetes [10], and all-cause mortality [11] have been published. As per the investigation of all-cause mortality, the cause-specific mortality analyses herein included all eligible participants who died by 31 December 2007 (n = 2410) and the subcohort, a sex-stratified random sample of 2996 participants. Participants for whom 25(OH)D measurements were not performed, with missing data for key confounding variables included in all models, or with extreme values for total daily energy intake (<1st and >99th sex-specific percentiles) were excluded. In analyses of causes of death adjusted for additional confounders, participants with missing data for the relevant covariates were excluded.

The Cancer Council Victoria's Human Research Ethics Committee approved the study protocol and participants provided written consent to participate and for the investigators to obtain access to their medical records.

2.2   *Measurement of 25-hydroxyvitamin D*

Concentrations of 25(OH)D$_2$ and 25(OH)D$_3$ in baseline dried blood spot samples were measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS) in the laboratory of D.W.E and summed to yield total 25(OH)D as previously described [12-14]. Samples were processed in random order and laboratory analysts were blind to outcome status of participants. The laboratory uses National Institute of Standards and Technology (NIST) Standard Reference Materials and participates in the Vitamin D External Quality Assessment Scheme (DEQAS). The relative assay inaccuracies using four NIST sera calibrants were +2.3, +3.4, +2.0 and −0.5% at concentrations of 17.7, 32.2, 49.6, and 61.85 nmol/L, respectively, with an overall inaccuracy of 2.0% (international standards for dried

blood spots do not yet exist). A repeatability study of 493 duplicates demonstrated that the dried blood spot 25(OH)D measurements have high reliability [13]. Methods for removing batch effects and seasonal variation, and conversion of 25(OH)D measured in dried blood spots to plasma-equivalent concentrations have been described [10, 11, 13]. All results presented are for batch- and season-adjusted plasma-equivalent 25(OH)D concentrations.

2.3 Ascertainment of deaths

Vital status and cause of death were identified through record linkage to death certificates issued in Victoria by the Registry of Births, Deaths and Marriages, and linkage to the National Death Index compiled by the Australian Institute of Health and Welfare. For cancer mortality, cause of death was ascertained by linkage to the Victorian Cancer Registry (VCR), which in some cases provided a more accurate indication of the primary site of the cancer. Causes of death were coded according to the WHO International Classification of Diseases, 9th revision (ICD-9) and 10th revision (ICD-10) and grouped based on ICD-10 chapters (**Supplementary Table 1**). For the purposes of analysis, only malignant neoplasms (ICD-9 140–209.3 or ICD-10 C00–C97) were included in the classification of cancer. Colorectal cancer included malignant neoplasm of the colon, rectosigmoid junction, or rectum (ICD-9 153–154.1 or ICD-10 C18–C20). Analyses of breast cancer mortality were restricted to women (ICD-9 174 malignant neoplasm of female breast or ICD-10 C50). Cardiovascular disease included all diseases of the circulatory system (ICD-9 390–459 or ICD-10 I00–I99).

2.4 Statistical analysis

All statistical analyses were conducted using Stata 13.1 (Stata Corporation, College Station, TX, USA). Analyses were performed for all causes of death or disease groups for which there were at least 50 deaths (except for external causes, for which analyses were not performed). Cox regression was used to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) for each cause of death per 25 nmol/L increment in 25(OH)D by modelling 25(OH)D continuously, and according to sex-specific quartiles of 25(OH)D (relative to the lowest quartile) based on the distribution of batch- and season-adjusted plasma-equivalent concentrations of 25(OH)D in the subcohort. We also performed analyses for 25(OH)D$_3$ separately, but results were similar since few participants had detectable circulating 25(OH)D$_2$ (n = 62) and only results for total 25(OH)D are presented. Barlow's method and robust standard errors were used to account for the case-cohort design [15]. Age was the timescale and all models were stratified by country of birth (Australia/New Zealand/northern Europe versus southern Europe) and sex (except for breast cancer mortality and prostate cancer mortality). Follow-up began at baseline attendance and ended at the earliest of: date of death, date a participant left

Australia, or 31 December 2007. In the analyses for each cause of death, subcohort participants who died due to other causes were censored on the day of death, while non-subcohort participants who died due to other causes were excluded.

For each cause of death, potential confounders were selected using a causal diagram based on existing literature. The set of confounders included in analyses differed for each cause of death (**Supplementary Table 2**), but all models were adjusted for: educational attainment (primary school or less, some secondary/technical school, completed secondary school, tertiary qualification), socioeconomic status (quintiles of an area-based measure of relative socioeconomic disadvantage, based on residential postcodes at baseline), smoking status (never, former, current smoker), alcohol intake (lifetime abstainer, former drinker, current low intake, current medium intake, current high intake; current drinkers were grouped into sex-specific tertiles of alcohol consumed per day based on the subcohort), physical activity (four categories reflecting the intensity and frequency of recreational activity over the last 6 months: none, low, moderate, high), waist circumference (sex-specific quartiles based on the distribution of measurements in the subcohort), Mediterranean diet score (grouped into 3 categories: low, moderate, high adherence to a Mediterranean dietary pattern), and total daily energy intake (sex-specific quartiles based on the subcohort). Analyses of death due to strongly smoking-related causes—i.e. lung cancer, cardiovascular disease (including all subgroupings), and respiratory diseases (including subgroupings)—were adjusted for smoking status and amount smoked (never, former light smoker [<20 cigarettes/day], former heavy smoker [≥20 cigarettes/day], current light smoker [<20 cigarettes/day], current heavy smoker [≥20 cigarettes/day]; former and current smokers were divided into light/heavy smokers based on the median number of cigarettes smoked per day). For these causes of death, in exploratory analyses we also evaluated adjustment for smoking duration or pack-years of smoking (instead of amount smoked), but results were similar so only the results for smoking adjusted by smoking status and amount are presented. Models for breast cancer mortality included additional adjustment for parity (no children, any children). Menopausal status was not included in the models because after accounting for age at baseline there was no association between menopausal status and 25(OH)D. Use of oral contraceptives and hormone replacement therapy were not associated with 25(OH)D and were therefore not included as confounders. Data on family history of breast cancer was not available. Analyses of death due to endocrine, nutritional and metabolic diseases and cardiovascular disease were additionally adjusted for history of hypertension, heart attack, stroke, angina, and diabetes. Models for respiratory disease mortality were additionally adjusted for history of hypertension, heart attack, stroke, angina, and asthma. There was no evidence of violation of the

proportional hazards assumption, which was assessed by fitting interactions between each covariate separately (modelled as a time varying effect) and attained age.

2.5 Subgroup analyses

Because we had previously found that 25(OH)D was inversely associated with incident colorectal cancer for women but not men in the MCCS [9], for colorectal cancer mortality we tested an interaction between 25(OH)D (modelled continuously) and sex.

To investigate possible effect modification by baseline disease status, for cardiovascular disease mortality we assessed an interaction between continuous 25(OH)D and history of cardiovascular disease (heart attack, stroke, or angina).

2.6 Sensitivity analyses

To mitigate possible reverse causation, sensitivity analyses were undertaken in which follow-up began two years after baseline.

For cancer mortality and cardiovascular mortality (the most common causes of death), we performed analyses restricted to participants who completed the wave 2 questionnaire approximately four years after baseline and reported being in good, very good, or excellent health. For these analyses, follow-up began on the date of completion of the wave 2 questionnaire. These sensitivity analyses were not possible for the other causes of death presented here because there were too few deaths in this restricted subsample of healthy participants (44 due to endocrine, nutritional and metabolic diseases, 51 due to diseases of the nervous system, 38 due to diseases of the respiratory system, 21 due to diseases of the digestive system).

3   Results

After excluding participants for whom 25(OH)D measurements were not performed (n = 23), with missing data for key confounding variables (n = 44), or with extreme values for total daily energy intake (n = 100), a total of 2307 participants who died and 2923 subcohort participants (of whom 266 died) remained for analysis (**Supplementary Figure 1**).

Of the 2307 participants who died during a mean follow-up of 13.7 (SD 2.2) years, 1082 died due to cancer, 618 due to cardiovascular disease, and 119 due to respiratory diseases. Participants who

died were on average aged 61.3 (SD 7.2) years at baseline and 70.2 (SD 8.1) years at death. The number of deaths due to specific causes are shown in **Supplementary Table 1**. Characteristics of participants at the time of blood collection are shown in **Table 1**. Participants who died were more likely to be older, current or former smokers, have greater socioeconomic disadvantage, lower educational attainment, higher waist circumference, and to have a history of hypertension, history of cardiovascular disease, or diabetes at baseline. The median batch- and season-adjusted plasma-equivalent 25(OH)D concentrations for subcohort participants and those who died were 49.1 (interquartile range [IQR] 38.1–61.9) nmol/L and 47.6 (IQR 36.7–60.1) nmol/L, respectively. Within the subcohort, median 25(OH)D concentrations for women and men were 42.9 (IQR 34.8–53.0) and 54.9 (IQR 43.0–68.8), respectively.

Table 1: Characteristics of participants at recruitment.

	Subcohort[a]	Deaths
	n (%)	*n* (%)
n	2923	2307
25(OH)D (nmol/L), sex-specific quartiles[b]		
1	730 (25.0)	659 (28.6)
2	731 (25.0)	574 (24.9)
3	732 (25.0)	562 (24.4)
4	730 (25.0)	512 (22.2)
Sex		
Women	1309 (44.8)	965 (41.8)
Men	1614 (55.2)	1342 (58.2)
Age (years)		
<45	592 (20.3)	88 (3.8)
45–54	988 (33.8)	341 (14.8)
55–64	907 (31.0)	932 (40.4)
≥65	436 (14.9)	946 (41.0)
Country of birth		
Australia/New Zealand/Northern Europe	2443 (83.6)	1924 (83.4)
Southern Europe	480 (16.4)	383 (16.6)
Socioeconomic disadvantage		
1st quintile (most disadvantage)	383 (13.1)	407 (17.6)
2nd quintile	478 (16.4)	482 (20.9)
3rd quintile	483 (16.5)	379 (16.4)
4th quintile	676 (23.1)	465 (20.2)
5th quintile (least disadvantage)	903 (30.9)	574 (24.9)
Educational attainment		
Primary school or less	331 (11.3)	413 (17.9)
Some secondary school	1100 (37.6)	948 (41.1)
Secondary school	693 (23.7)	532 (23.1)
Tertiary qualification	799 (27.3)	414 (17.9)

	Subcohort[a] n (%)	Deaths n (%)
Waist circumference (cm), sex-specific quartiles[c]		
1	718 (24.6)	437 (18.9)
2	733 (25.1)	458 (19.9)
3	742 (25.4)	582 (25.2)
4	730 (25.0)	830 (36.0)
Physical activity		
None	596 (20.4)	476 (20.6)
Low	571 (19.5)	419 (18.2)
Moderate	998 (34.1)	958 (41.5)
High	758 (25.9)	454 (19.7)
Smoking status		
Never	1583 (54.2)	977 (42.3)
Former	1017 (34.8)	918 (39.8)
Current	323 (11.1)	412 (17.9)
Alcohol intake (g/day)[d]		
Never drinker	656 (22.4)	625 (27.1)
Former drinker	128 (4.4)	125 (5.4)
Current low	687 (23.5)	504 (21.8)
Current medium	699 (23.9)	418 (18.1)
Current high	753 (25.8)	635 (27.5)
Mediterranean diet score		
0–3 (low)	715 (24.5)	625 (27.1)
4–6 (moderate)	1830 (62.6)	1435 (62.2)
7–9 (high)	378 (12.9)	247 (10.7)
Energy intake (kJ/day), sex-specific quartiles[e]		
1	730 (25.0)	665 (28.8)
2	731 (25.0)	570 (24.7)
3	732 (25.0)	508 (22.0)
4	730 (25.0)	564 (24.5)
History of diabetes mellitus	78 (2.7)	188 (8.2)
History of hypertension	532 (18.2)	754 (32.7)
History of cardiovascular disease[f]	184 (6.3)	385 (16.7)
History of asthma	354 (12.1)	299 (13.0)

All values are n (%). Sex-specific quartiles were formed from the distribution in the subcohort.

[a]266 subcohort participants died.

[b]Quartiles of batch- and season-adjusted plasma-equivalent 25(OH)D concentration in nmol/L:

 1, Women 13.9–34.7, Men 8.2–43.0

 2, Women 34.8–42.9, Men 43.1–54.8

 3, Women 43.0–53.0, Men 54.9–68.8

 4, Women 53.1–121.3, Men 68.9–201.8

[c]Quartiles of waist circumference in cm:

 1, Women 52.0–70.6, Men 62.0–85.9

 2, Women 70.7–76.9, Men 86.0–91.9

 3, Women 77.0–85.9, Men 92.0–98.4

 4, Women 86.0–153.6, Men 98.5–143.0

[d]Categories of alcohol intake for current drinkers in grams/day:

 Low: Women 0.1–4.2, Men 0.1–9.2

 Medium: Women 4.3–14.9, Men 9.3–25.7

 High: Women 15.0–157.9, Men 25.8–232.2

Circulating 25(OH)D concentration was inversely associated with cancer mortality, with a 12% lower risk for each 25 nmol/L increment in 25(OH)D (HR = 0.88, 95% CI 0.78–0.99) and 24% lower risk comparing the highest with the lowest quartile (HR = 0.76, 95% CI 0.60–0.95) (**Table 2**). An inverse association was found for death due to colorectal cancer (HR per 25 nmol/L increment = 0.75, 95% CI 0.57–0.99), with little evidence that this association differed by sex (*P* for interaction = 0.39), although it appeared to be slightly stronger for women (HR = 0.63, 95% CI 0.40–1.01) than for men (HR = 0.82, 95% CI 0.58–1.14). The HR for lung cancer mortality was similar in magnitude to that for overall cancer mortality but the estimate was accompanied by substantial uncertainty (HR = 0.90, 95% CI 0.67–1.19). 25(OH)D was not associated with risk of death due to pancreatic cancer (HR = 0.96, 95% CI 0.69–1.33), breast cancer (HR = 1.27, 95% CI 0.76–2.13), or prostate cancer (HR = 0.95, 95% CI 0.67–1.35) (**Figure 1**).
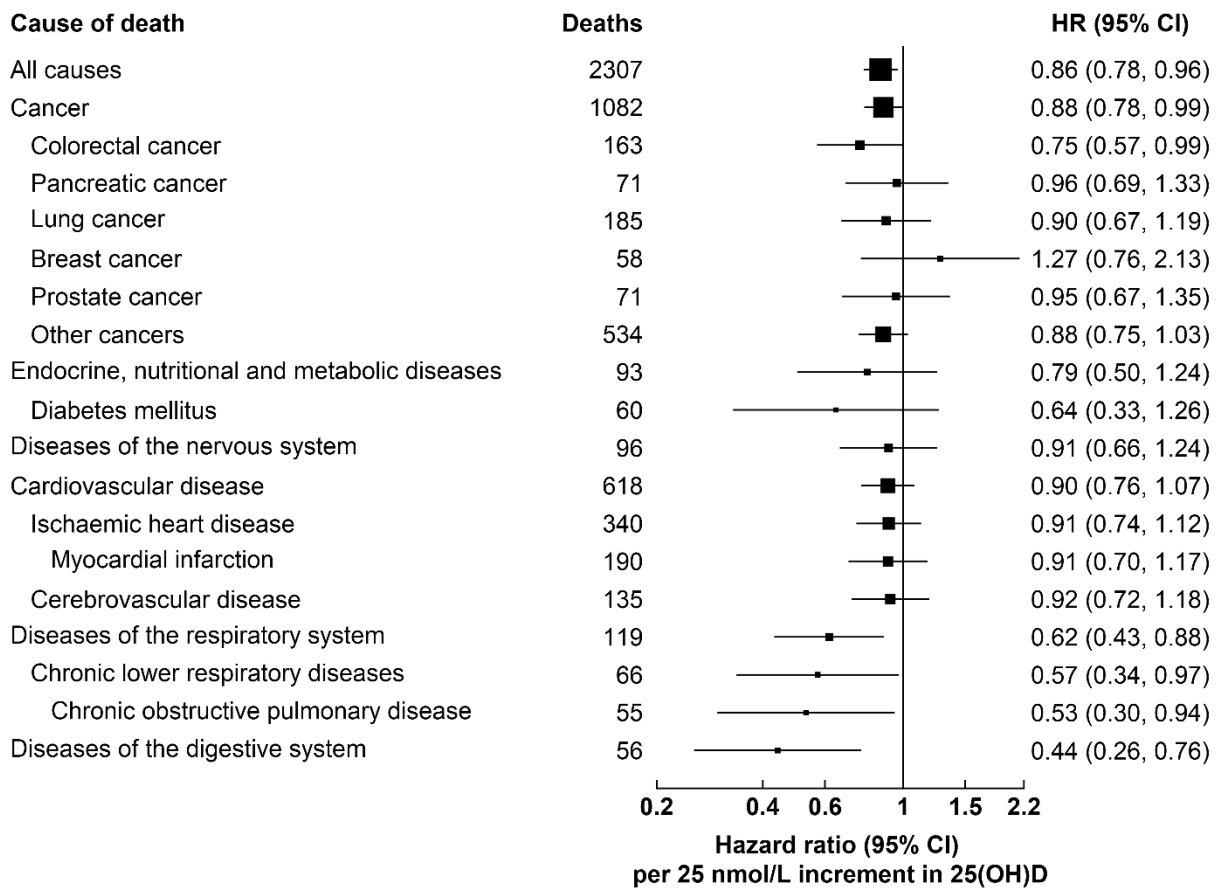
For death due to diseases of the respiratory system, there was a strong inverse association, with each 25 nmol/L increment in 25(OH)D associated with a 38% lower risk (HR = 0.62, 95% CI 0.43–0.88). Considering subgroupings of respiratory diseases, strong inverse associations were evident for death due to chronic lower respiratory diseases, and specifically chronic obstructive pulmonary disease (COPD), with 43% (HR = 0.57, 95% CI 0.34–0.97) and 47% (HR = 0.53, 95% CI 0.30–0.94) lower risks per 25 nmol/L increment in 25(OH)D, respectively. 25(OH)D concentration was also strongly associated with risk of death due to diseases of the digestive system; each 25 nmol/L increment in 25(OH)D was associated with a 56% lower risk (HR = 0.44, 95% CI 0.26–0.76).

The magnitude of the association for death due to endocrine, nutritional and metabolic diseases, and specifically diabetes mellitus, was suggestive of a moderate inverse association but estimates were imprecise due to the limited number of deaths due to these diseases (93 and 60, respectively) (**Figure 1** and **Table 2**).

The HRs for cardiovascular disease mortality were similar to those for all-cause mortality [11] and cancer mortality, but the CIs were wide (**Figure 1** and **Table 2**). For death due to myocardial

infarction and cerebrovascular disease, estimates by 25(OH)D quartiles did not show any dose-response relationship. There was little evidence that diseases of the nervous system were related to 25(OH)D concentration (**Table 2**).

Figure 1: Cause-specific mortality in relation to a 25 nmol/L increment in batch- and season-adjusted plasma-equivalent 25-hydroxyvitamin D concentration. Hazard ratios (HRs) are plotted as squares, with the area of each square inversely proportional to the variance of the log HR, and corresponding 95% confidence intervals (CIs) are plotted as lines. Models were adjusted for the covariates listed in Supplementary Table 2.

Cause of death	Deaths	HR (95% CI)
All causes	2307	0.86 (0.78, 0.96)
Cancer	1082	0.88 (0.78, 0.99)
Colorectal cancer	163	0.75 (0.57, 0.99)
Pancreatic cancer	71	0.96 (0.69, 1.33)
Lung cancer	185	0.90 (0.67, 1.19)
Breast cancer	58	1.27 (0.76, 2.13)
Prostate cancer	71	0.95 (0.67, 1.35)
Other cancers	534	0.88 (0.75, 1.03)
Endocrine, nutritional and metabolic diseases	93	0.79 (0.50, 1.24)
Diabetes mellitus	60	0.64 (0.33, 1.26)
Diseases of the nervous system	96	0.91 (0.66, 1.24)
Cardiovascular disease	618	0.90 (0.76, 1.07)
Ischaemic heart disease	340	0.91 (0.74, 1.12)
Myocardial infarction	190	0.91 (0.70, 1.17)
Cerebrovascular disease	135	0.92 (0.72, 1.18)
Diseases of the respiratory system	119	0.62 (0.43, 0.88)
Chronic lower respiratory diseases	66	0.57 (0.34, 0.97)
Chronic obstructive pulmonary disease	55	0.53 (0.30, 0.94)
Diseases of the digestive system	56	0.44 (0.26, 0.76)

Hazard ratio (95% CI)
per 25 nmol/L increment in 25(OH)D

Table 2: Hazard ratios (HRs) and 95% confidence intervals (CIs) for cause-specific mortality in relation to circulating batch- and season-adjusted plasma-equivalent 25(OH)D concentrations. Models for each cause of death were adjusted for the covariates listed in Supplementary Table 2.

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P trend	Per 25 nmol/L increment	P
		25(OH)D concentration (nmol/L), range[a]					
Women	13.9–34.7	34.8–42.9	43.0–53.0	53.1–121.3			
Men	8.2–42.9	43.0–54.8	54.9–68.8	68.9–201.8			
Cause of death							
All causes[b]							
N	1318	1223	1239	1184		4964	
Deaths	659	574	562	512		2307	
HR (95% CI)	1.00 (ref)	0.87 (0.72–1.06)	0.85 (0.70–1.04)	0.74 (0.60–0.91)	0.005	0.86 (0.78–0.96)	0.007
Cancer							
N	996	962	984	942		3884	
Deaths	293	267	281	241		1082	
HR (95% CI)	1.00 (ref)	0.90 (0.72–1.12)	0.90 (0.72–1.13)	0.76 (0.60–0.95)	0.03	0.88 (0.78–0.99)	0.03
Colorectal cancer							
N	775	769	768	753		3065	
Deaths	46	48	39	30		163	
HR (95% CI)	1.00 (ref)	1.05 (0.68–1.63)	0.79 (0.50–1.26)	0.60 (0.36–0.98)	0.02	0.75 (0.57–0.99)	0.04
Pancreatic cancer							
N	750	745	748	748		2991	
Deaths	20	14	18	19		71	
HR (95% CI)	1.00 (ref)	0.70 (0.33–1.47)	1.05 (0.51–2.17)	1.06 (0.50–2.22)	0.69	0.96 (0.69–1.33)	0.80
Lung cancer							
N	781	777	763	768		3089	
Deaths	57	52	34	42		185	
HR (95% CI)	1.00 (ref)	1.05 (0.64–1.71)	0.66 (0.39–1.12)	0.85 (0.51–1.43)	0.28	0.90 (0.67–1.19)	0.45
Breast cancer[c]							
N	337	334	347	341		1359	
Deaths	12	10	20	16		58	
HR (95% CI)	1.00 (ref)	0.73 (0.29–1.81)	1.45 (0.64–3.30)	1.08 (0.47–2.46)	0.45	1.27 (0.76–2.13)	0.36

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P trend	Per 25 nmol/L increment	P
Prostate cancer							
N	413	425	423	414		1675	
Deaths	13	24	21	13		71	
HR (95% CI)	1.00 (ref)	1.82 (0.83–4.00)	1.38 (0.61–3.11)	0.75 (0.31–1.78)	0.29	0.95 (0.67–1.35)	0.79
Other cancers[d]							
N	860	836	863	838		3397	
Deaths	145	119	149	121		534	
HR (95% CI)	1.00 (ref)	0.79 (0.59–1.04)	0.93 (0.70–1.22)	0.73 (0.55–0.98)	0.10	0.88 (0.75–1.03)	0.10
Endocrine, nutritional and metabolic diseases							
N	760	755	751	742		3008	
Deaths	33	27	20	13		93	
HR (95% CI)	1.00 (ref)	0.96 (0.47–1.93)	0.88 (0.44–1.78)	0.51 (0.21–1.24)	0.16	0.79 (0.50–1.24)	0.31
Diabetes mellitus							
N	756	747	741	736		2980	
Deaths	28	17	9	6		60	
HR (95% CI)	1.00 (ref)	0.92 (0.39–2.16)	0.64 (0.24–1.73)	0.36 (0.09–1.46)	0.13	0.64 (0.33–1.26)	0.20
Diseases of the nervous system							
N	749	754	754	754		3011	
Deaths	19	30	23	24		96	
HR (95% CI)	1.00 (ref)	1.56 (0.84–2.90)	1.07 (0.55–2.07)	1.07 (0.55–2.08)	0.79	0.91 (0.66–1.24)	0.55
Diseases of the circulatory system							
N	884	864	866	856		3470	
Deaths	176	155	146	141		618	
HR (95% CI)	1.00 (ref)	0.86 (0.63–1.18)	0.88 (0.64–1.20)	0.80 (0.58–1.11)	0.21	0.90 (0.76–1.07)	0.24
Ischaemic heart disease							
N	816	803	797	804		3220	
Deaths	99	88	72	81		340	
HR (95% CI)	1.00 (ref)	0.89 (0.61–1.31)	0.79 (0.53–1.17)	0.84 (0.56–1.26)	0.32	0.91 (0.74–1.12)	0.37

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	*P* trend	Per 25 nmol/L increment	*P*
Myocardial infarction							
N	770	777	771	774		3092	
Deaths	48	53	41	48		190	
HR (95% CI)	1.00 (ref)	1.11 (0.69–1.78)	0.92 (0.55–1.53)	1.03 (0.62–1.73)	0.93	0.91 (0.70–1.17)	0.44
Cerebrovascular disease							
N	758	762	765	760		3045	
Deaths	32	33	37	33		135	
HR (95% CI)	1.00 (ref)	0.99 (0.56–1.76)	1.15 (0.65–2.03)	0.91 (0.51–1.62)	0.87	0.92 (0.72–1.18)	0.51
Diseases of the respiratory system							
N	772	755	755	742		3024	
Deaths	47	28	29	15		119	
HR (95% CI)	1.00 (ref)	0.67 (0.37–1.20)	0.63 (0.36–1.11)	0.34 (0.17–0.66)	0.002	0.62 (0.43–0.88)	0.007
Chronic lower respiratory diseases							
N	754	743	742	735		2974	
Deaths	28	15	16	7		66	
HR (95% CI)	1.00 (ref)	0.57 (0.22–1.50)	0.61 (0.26–1.43)	0.30 (0.11–0.82)	0.03	0.57 (0.34–0.97)	0.04
Chronic obstructive pulmonary disease							
N	750	741	741	733		2965	
Deaths	23	13	15	4		55	
HR (95% CI)	1.00 (ref)	0.61 (0.22–1.70)	0.70 (0.27–1.81)	0.22 (0.06–0.76)	0.04	0.53 (0.30–0.94)	0.03
Diseases of the digestive system							
N	748	744	744	737		2973	
Deaths	21	15	13	7		56	
HR (95% CI)	1.00 (ref)	0.66 (0.31–1.40)	0.53 (0.23–1.25)	0.27 (0.11–0.71)	0.008	0.44 (0.26–0.76)	0.003

[a]Concentration range of batch- and season-adjusted plasma-equivalent 25(OH)D in each quartile.

[b]All-cause mortality results have been published previously [11], and are reported here to facilitate comparisons with specific causes of death.

[c]Female breast cancer.

[d]Other cancers included all malignant neoplasms other than colorectal cancer, pancreatic cancer, lung cancer, female breast cancer, and prostate cancer.

3.1 Effect modification by baseline disease status

There was suggestion of an inverse association between 25(OH)D and cardiovascular disease mortality for participants with a history of cardiovascular disease (*n* = 181 deaths; HR per 25 nmol/L increment = 0.82, 95% CI 0.58–1.15), but no association for those without cardiovascular disease at baseline (*n* = 437 deaths; HR = 1.00, 95% CI 0.84–1.18); however, the test of interaction had a large *p*-value (*P* for interaction = 0.30).

3.2 Analyses excluding the first two years of follow-up

Results were similar after excluding the first two years of follow-up, but there was no association for death due to myocardial infarction, while the associations for death due endocrine, nutritional and metabolic diseases and diabetes mellitus were slightly stronger (**Supplementary Figure 2** and **Supplementary Table 3**).

3.3 Participants in good, very good, or excellent health

The association for cancer mortality was similar to that for all participants when restricted to those who rated their general health as good, very good, or excellent approximately four years after baseline blood collection (2681 participants, of whom 600 died due to cancer); HRs per 25 nmol/L increment in 25(OH)D and comparing the highest and lowest 25(OH)D quartiles were 0.86 (95% CI 0.73–1.00) and 0.72 (95% CI 0.54–0.94), respectively. There was also suggestion of an inverse association between 25(OH)D and cardiovascular disease mortality for those who reported being in good, very good, or excellent health (2384 participants, of whom 270 died due to cardiovascular disease), but the CI included associations in either direction; HRs per 25 nmol/L increment in 25(OH)D and comparing the highest and lowest 25(OH)D quartiles were 0.82 (95% CI 0.63–1.05) and 0.69 (95% CI 0.44–1.08) respectively.

4 Discussion

In this cohort of middle-aged Australians without pre-baseline cancer, plasma-equivalent 25(OH)D concentration was inversely associated with death due to cancer (all malignant neoplasms combined), colorectal cancer, diseases of the respiratory system (particularly chronic lower respiratory diseases or COPD), and diseases of the digestive system. There was suggestion of an inverse association for death due to endocrine, nutritional and metabolic diseases (and specifically diabetes mellitus), but there were few deaths attributed to these diseases and estimates were

accompanied by substantial uncertainty. Similarly, estimates for an association with cardiovascular disease mortality lacked precision. We found little evidence for an association between circulating 25(OH)D and death due to diseases of the nervous system. Results were similar in analyses excluding the first two years of follow-up. For participants who reported being in good, very good, or excellent health several years after baseline blood sample collection, the association for cancer mortality was similar to that for all participants, while for cardiovascular disease mortality there was suggestion of an inverse association that was slightly stronger than that for all participants, but estimates were imprecise.

There are abundant data on the association between circulating 25(OH)D and all-cause mortality, but associations with specific causes of death remain uncertain [1]. Although numerous observational studies have investigated cancer and cardiovascular disease mortality, most existing studies have had insufficient cases to be able to examine specific causes of death beyond aggregate disease groupings. This may be problematic because these groupings can represent a heterogeneous set of diseases, which might be differently associated with vitamin D. A strength of this study was the relatively large number of deaths, which enabled investigation of several causes of death for which existing evidence is sparse. By evaluating specific cancer types, we found that higher circulating 25(OH)D might reduce mortality from colorectal cancer, but might not have any benefit for reducing mortality from pancreatic, breast, or prostate cancers. A limitation of our study was that there were insufficient deaths to investigate other cancers and we could not examine death due to specific types of diseases within some groups, such as diseases of the digestive system or nervous system. Substantial advantages of this study were the availability of extensive information on potential confounding variables, recruitment from one city (minimising any potential confounding effect of geographical location, including latitude), accurate and complete mortality data, and use of a highly sensitive and reliable LC-MS/MS assay for measurement of 25(OH)D. Nevertheless, the adjustments made for batch and seasonal effects, and the conversion from dried blood spot 25(OH)D concentrations to plasma-equivalent 25(OH)D concentrations mean caution is necessary when interpreting the absolute 25(OH)D values. A further limitation was that concentration of 25(OH)D was determined at only one time point; however, studies have shown consistency in 25(OH)D measurements from individuals several years apart [16-18], suggesting a single measurement at baseline provides a reasonable indication of long-term vitamin D status in epidemiological studies.

The inverse association between 25(OH)D concentration and cancer mortality found in our study is consistent with the findings from two meta-analyses of cohort studies [2, 19], but there was no association reported by an individual participant data meta-analysis of standardised 25(OH)D [3]. In an individual participant data meta-analysis of cohort studies from Europe and the United States, there was no association between circulating 25(OH)D and cancer mortality for participants without a history of cancer, whereas there was an inverse association for participants with a history of cancer [5]. While this could reflect that vitamin D is perhaps more important for cancer progression and survival, it also raises the possibility that the association between 25(OH)D and cancer mortality might be due to reverse causation, whereby low 25(OH)D concentrations are the result of prevalent cancer and suboptimal health. Since our study was restricted to participants without a pre-baseline diagnosis of cancer, and 25(OH)D concentrations were ascertained before the diagnosis of cancer, we excluded this possibility. Further, the association was unchanged in sensitivity analyses excluding the first two years of follow-up or when restricted to participants in good to excellent health several years after baseline. Thus, reverse causation due to poor health is unlikely. Two Mendelian randomisation studies have yielded conflicting results; one found an inverse association between genetically-predicted 25(OH)D and cancer mortality [6], while an analysis of UK Biobank data did not find any association [20]. Meta-analyses of RCTs have shown that vitamin D supplementation was associated with reduced cancer mortality [7, 21, 22]. Subsequent to these meta-analyses, the results from two large-scale RCTs have also become available. In the Vitamin D Assessment (ViDA) trial involving 5108 New Zealanders, monthly supplementation with 100,000 IU vitamin D_3 did not reduce cancer mortality, but only 60 deaths due to cancer accrued during follow-up in those without cancer before randomisation [23]. In the Vitamin D and Omega-3 Trial (VITAL) of 25,871 participants in the United States, daily 2000 IU vitamin D_3 supplementation was associated with lower cancer mortality [24].

Few studies have investigated the association between 25(OH)D concentration and cancer-specific mortality [1]. For colorectal cancer mortality, no association was found in a combined analysis of the Copenhagen City Heart Study (CCHS) and Copenhagen General Population Study (CGPS) [6], while separate analyses of NHANES III data have yielded results consistent with an inverse association, but with few cases [25-28]. To our knowledge, no other studies have investigated circulating 25(OH)D in relation to pancreatic cancer mortality. For lung cancer mortality, an inverse association was found in the CCHS and CGPS (combined) (HR per 20 nmol/L decrease in 25(OH)D = 1.28, 95% CI 1.06–1.54, which is equivalent to HR = 0.78, 95% CI 0.65–0.94 for a 20 nmol/L increment) [6], while in the Calcium Intake Fracture Outcome Study (CAIFOS), the estimate was imprecise with only 13 deaths

due to lung cancer (HR per 30 nmol/L decrease = 1.30, 95% CI 0.70–2.43; equivalent to 0.77, 95% CI 0.41–1.43 per 30 nmol/L increment) [29]. There was little evidence of an association with lung cancer mortality in NHANES III (RR for 25(OH)D 80 to <100 nmol/L compared with <50 nmol/L = 0.99, 95% CI 0.58–1.70) [27]. Two previous studies (CAIFOS and NHANES III) have investigated 25(OH)D in relation to breast cancer mortality, with no association found in CAIFOS based on 6 cases (HR per 30 nmol/L decrease in 25(OH)D = 1.06, 95% CI 0.43–2.63; equivalent to 0.94, 95% CI 0.38–2.33 per 30 nmol/L increment) [29], while in NHANES III there was suggestion of lower breast cancer mortality for higher 25(OH)D, but with only 53 cases the estimate had a wide CI (RR for 25(OH)D >80 versus <50 nmol/L = 0.65, 95% CI 0.18–2.38) [27]. Consistent with our findings for prostate cancer mortality, evidence for an association was lacking in NHANES III [27, 28]. There is a paucity of data on pre-diagnostic 25(OH)D concentration and death due to cancers of other sites, necessitating further large studies with long follow-up durations and adequate power to evaluate these associations. In patients with certain cancers (including colorectal, pancreatic, breast, and prostate cancers), higher circulating 25(OH)D concentration has been associated with better survival [30-34]. It is therefore possible that vitamin D is more important for disease progression and prognosis than for prevention of cancer, warranting further investigations to disentangle the association of vitamin D with cancer incidence, mortality and survival.

While many studies have investigated an association between circulating 25(OH)D and the risk of incident diabetes [35, 36], data on the relationship of 25(OH)D with diabetes mortality are lacking. We found a moderate inverse association between 25(OH)D concentration and death due to diabetes mellitus, as well as the collective group of endocrine, nutritional and metabolic diseases (which was reaffirmed in analyses excluding the first two years of follow-up), but there were relatively few deaths attributed to these causes and estimates were imprecise. It is possible that deaths due to diabetes mellitus are underestimated in death statistics because diabetes is often not recorded as the underlying cause of death, and this limits the ability to assess whether factors such as vitamin D status are associated with diabetes mortality.

We did not find compelling evidence of an association between 25(OH)D concentration and cardiovascular mortality or death due to ischaemic heart disease. Considering results of both continuous and categorical analyses of 25(OH)D, there was little evidence that 25(OH)D concentration was associated with risk of death due to myocardial infarction or stroke. In another Australian cohort study, comprising participants of similar age but with higher 25(OH)D concentrations (mean 61 nmol/L) than the MCCS, and excluding those who died within the first two

years of follow-up, 25(OH)D was inversely associated with cardiovascular disease mortality [37]. Likewise, inverse associations between circulating 25(OH)D concentration and cardiovascular mortality have been reported in most observational studies [1]. However, the findings of these studies are not supported by data from a Mendelian randomisation study [6] or RCTs [7, 38], raising the possibility that results from observational studies might be biased by confounding and reverse causation [1]. Since people may change their diet and lifestyle when diagnosed with cardiovascular disease or after suffering a cardiovascular event such as a heart attack or stroke, we evaluated whether the association for cardiovascular disease mortality was modified by baseline disease status. While the test of interaction did not indicate a substantial difference, there was suggestion of an inverse association for participants with a history of cardiovascular disease but little evidence of an association for those without prior cardiovascular disease, suggesting that vitamin D might only have an effect on cardiovascular mortality in people with pre-existing disease. Nevertheless, 25(OH)D concentration was inversely associated with cardiovascular disease mortality (albeit with imprecise estimates) for participants who rated their general health as good, very good, or excellent approximately four years after baseline blood sample collection. It is therefore not possible to rule out a potential association between 25(OH)D and cardiovascular mortality, particularly of small magnitude, or a role for vitamin D in cardiovascular disease survival post-diagnosis, and further research is required to examine these possibilities and elucidate whether vitamin D has any benefit for reducing mortality due to specific types of cardiovascular disease.

Vitamin D status has been linked to several diseases of the nervous system, including multiple sclerosis [39] and Alzheimer's disease [40], but associations with death due to these diseases remain unexplored. We did not find a lower risk of nervous system disease mortality for higher 25(OH)D concentrations. One other study has investigated the association between circulating 25(OH)D and death due to diseases of the nervous system, but with only 20 cases the estimate was imprecise (HR for highest versus lowest 25(OH)D quartile = 0.75, 95% CI 0.21–2.70) [41].

Six prospective cohort studies have assessed the association between 25(OH)D concentration and respiratory disease mortality, with all [6, 41-44] but one [45] showing a lower risk for higher 25(OH)D, but these associations were based on relatively sparse data. In the MCCS, the inverse association for death due to diseases of the respiratory system appeared to be attributable to the relatively strong association for death due to COPD. A meta-analysis found that low 25(OH)D concentrations were associated with COPD severity and exacerbations [46], while a meta-analysis of RCTs found that vitamin D supplementation reduced the rate of moderate/severe COPD

exacerbations in those with low baseline 25(OH)D concentrations [47]. It is plausible that vitamin D sufficiency might be associated with improved COPD-related survival and further studies are required to confirm whether vitamin D can reduce mortality due to COPD and other respiratory diseases.

The strong inverse association between 25(OH)D concentration and death due to diseases of the digestive system should be interpreted cautiously due to the limited number of cases, which prevented any appraisal of the relationship between 25(OH)D and specific diseases of the digestive system. Similar associations have been found by other studies, but these were also constrained by having few cases [16, 41]. While all studies thus far have found a strong inverse association, diseases of the digestive system are a diverse group of diseases with different aetiologies, and it remains unclear whether vitamin D is beneficial for reducing mortality due to all of these diseases.

Observational studies are limited by the possibility of confounding and reverse causation and therefore cannot ascertain whether vitamin D is causally associated with mortality. Several large RCTs which were initiated over the last decade have so far found no effect of vitamin D_3 supplementation for cardiovascular disease mortality [23], while for cancer mortality, there was suggestion of a benefit in VITAL [24] but no effect in ViDA [23]. It is possible that the follow-up time in these trials is not yet adequate for sufficient cases to accrue. In addition, existing trials are unlikely to have sufficient power to examine specific causes of death other than cardiovascular disease and cancer, both of which comprise heterogeneous groups of diseases. Further, these trials included a majority of participants who already had adequate 25(OH)D concentrations at baseline, and it is suggested that vitamin D supplementation will confer little benefit to these individuals [48, 49]. While future results from other large RCTs (such as D-Health [50]) might help elucidate whether vitamin D can reduce mortality due to certain diseases, further large long-term cohort studies are required to provide information about the relationship between vitamin D and mortality, particularly for less common causes of death.

5   Conclusions

Overall, our results suggest that vitamin D might be important for preventing death due to some cancers, particularly colorectal cancer, as well as death due diseases of the respiratory system and diseases of the digestive system. Diabetes mortality appeared to be inversely related to 25(OH)D concentration in the MCCS. The relationship between circulating 25(OH)D and cardiovascular disease mortality remains uncertain. Further large long-term cohort studies and RCTs are required to

confirm these findings and explore associations with rarer causes of death so that appropriate clinical guidelines for vitamin D requirements in chronic diseases can be established.

Declarations of interest: none.

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