

Association of 25-hydroxyvitamin D, 1,25-dihydroxyvitamin D and parathyroid hormone with mortality among middle-aged and older European men

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Abstract

Background: vitamin D deficiency has been associated with an increased risk of mortality, but whether this relationship is causal or linked to co-existent comorbidity and adverse life factors remains uncertain. Our objective was to determine whether endogenous 25-hydroxyvitamin D (25(OH)D), 1,25-dihydroxyvitamin D (1,25(OH)₂D) and parathyroid hormone (PTH)

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levels predicted all-cause, cardiovascular and cancer mortality independently of health and lifestyle factors.

Setting: prospective cohort analysis within the European Male Ageing Study.

Participants: 2,816 community-dwelling men aged 40–79 years at baseline.

Methods: Cox regression was used to examine the association of all-cause mortality with 25(OH)D, 1,25(OH)₂D and PTH; cardiovascular and cancer mortality were modelled using competing-risks regression. Results were expressed as hazard ratios (HR) and 95% confidence intervals (CIs) for Cox models; sub-hazard ratios (SHR) and 95% CIs for competing-risks models.

Results: a total of 187 men died during a median of 4.3 years of follow-up. Serum levels of 25(OH)D (per 1 SD decrease: HR = 1.45; 95% CI = 1.16, 1.81) and 1,25(OH)₂D (per 1 SD decrease: HR = 1.20; 95% CI = 1.00, 1.44) were associated with an increased risk of all-cause mortality after adjusting for age, centre, smoking, self-reported morbidities, physical activity and functional performance. Only levels of 25(OH)D <25 nmol/l predicted cancer mortality (SHR = 3.33; 95% CI = 1.38, 8.04).

Conclusion: lower 25(OH)D and 1,25(OH)₂D levels independently predicted all-cause mortality in middle-aged and older European men. Associations with cancer mortality were only observed among men with very low levels of 25(OH)D. These associations were only partially explained by the range of adverse health and lifestyle factors measured here.

Keywords: 25-hydroxyvitamin D, 1,25-dihydroxyvitamin D, parathyroid hormone, mortality, population based, older people

Introduction

Vitamin D inadequacy has been reported to be widespread among older people [1, 2]. Serum levels of 25-hydroxyvitamin D (25(OH)D) are used to establish an individual's vitamin D status, while the biologically active molecule, 1,25-dihydroxyvitamin D (1,25(OH)₂D), is produced by the hydroxylation of 25(OH)D primarily in the kidneys under the regulation of parathyroid hormone (PTH) and serum calcium [3]. Although the essential role played by the vitamin D endocrine axis in skeletal homeostasis is well recognised, low serum levels of 25(OH)D and elevated levels of PTH have also been linked to other health outcomes, including cognitive decline [4], cardiovascular diseases [5], depression [6], type 2 diabetes [7] and cancer [8].

With respect to vitamin D deficiency and mortality risk, recent systematic reviews of randomised controlled trials (RCTs) examining the potential value of vitamin D supplementation have highlighted contradictory findings. A meta-analysis of RCTs carried out by Autier and Gandini found vitamin D supplementation reduced all-cause mortality by 7% [9]. However, Bjelakovi *et al.* [10] observed that vitamin D₃ supplementation decreased mortality in elderly women, while Rejnmark *et al.* [11] found consistently reduced mortality only in elderly subjects randomised to receive vitamin D supplements in combination with calcium. Similar inconsistencies have also been seen among prospective observational studies describing associations between low 25(OH)D and all-cause [12, 13], cancer [14, 15] and cardiovascular mortality [16]. Fewer prospective studies have examined the effects of 1,25(OH)₂D and PTH. Associations have been observed between low 1,25(OH)₂D levels and all-cause and cardiovascular mortality among coronary angiography patients [17], high PTH levels and mortality among frail older people [18], and elevated PTH within the normal range and cardiovascular mortality in community-based older men [19].

While epidemiological evidence suggests a possible link between optimal vitamin D status and decreased mortality [13], there are few data describing the association of the key metabolites of the vitamin D axis with mortality among community-dwelling, middle-aged and older men. An unresolved question is whether vitamin D deficiency and optimal functioning of the vitamin D endocrine axis is an important, and potentially underestimated, cause of premature death. We utilised data from the European Male Ageing Study (EMAS) to examine whether 25(OH)D, 1,25(OH)₂D and PTH predicted all-cause, cardiovascular and cancer mortality independently of health and lifestyle factors.

Methods

Participants and study design

The design and recruitment for the EMAS have been described previously [20]. Briefly, an age-stratified sample of 3,369 men aged 40–79 (mean ± SD: 60 ± 11) years were recruited from population registers in eight European centres (Florence, Italy; Leuven, Belgium; Malmö, Sweden; Manchester, UK; Santiago de Compostela, Spain; Łódź, Poland; Szeged, Hungary; Tartu, Estonia). Participants completed a postal questionnaire (PQ) and attended a research clinic for further assessments. The men were invited to attend a follow-up assessment and completed another PQ a median of 4.3 years later (range 3.0–5.7 years). Ethical approval was obtained in accordance with each centre's institutional requirements, with all participants providing written informed consent.

Assessments

The PQ included questions concerning general health and lifestyle, including smoking and alcohol consumption. Participants were asked whether they were currently being

treated for various morbidities including heart conditions, hypertension, bronchitis, asthma, diabetes, liver disease, kidney conditions, prostate disease and thyroid disorders, and if they had ever been treated for cancer or suffered a stroke.

The assisted questionnaire included the Physical Activity Scale for the Elderly (PASE) [21], a self-report instrument to measure levels of physical activity among older individuals. Physical function was assessed during the clinic visit using Reuben's Physical Performance Test (PPT) [22], an objective measure of functional performance. Anthropometric measurements included height, weight and waist circumference.

Biochemistry

A fasting blood sample was obtained from all subjects. Processed serum was stored at -80°C and shipped frozen to central laboratories for measurement of 25(OH)D and 1,25(OH)₂D₃ (Katholieke Universiteit Leuven, Belgium) and PTH (University of Santiago de Compostela, Spain). 25(OH)D levels were determined using radioimmunoassay (RIA kit, DiaSorin, Stillwater, MN, USA). Intra- and inter-assay coefficients of variation (CVs) for 25(OH)D were 11 and 8%, respectively. The detection limit was 4.0 nmol/l. 1,25(OH)₂D₃ was measured by liquid chromatography–tandem mass spectrometry (LC–MS/MS) as a lithium adduct according to Casetta *et al.* [23] using a Shimadzu Prominence HPLC (Shimadzu, Kyoto, Japan) coupled to an AB Sciex API 5500 QTRAP tandem mass spectrometer (Sciex, Warrington, UK). Modifications to the original methodology have been described previously [24]. The limit of quantification for 1,25(OH)₂D₃ was <15.6 pmol/l, with interday CVs for pooled serum of 6% for serum with a mean concentration of 140 pmol/l and 10% for serum with a mean concentration of 18 pmol/l. PTH was assayed using a chemiluminescence immunoassay (Nichols Advantage Bio-Intact PTH, Quest Diagnostics, Madison, NJ, USA), with manufacturer's intra- and inter-assay CVs of 6 and 3%, respectively, and a detection limit of 0.2 pmol/l. Serum creatinine concentration (measure of renal function) was assayed in each centre using the Jaffe method [25]. Creatinine intra- and inter-assay CVs ranged from 0.7 to 1.9% and 1.6 to 3.5%, respectively, with detection limits between 5.3 and 18.0 $\mu\text{mol/l}$.

Mortality

Deaths that occurred during the follow-up period were determined from contact by relatives on receipt of the PQ or, if this was not returned, by further enquiries to ascertain the participant's vital status. The enquiry procedure varied between centres and included review of medical records/death registers and telephone follow-up. Deaths were verified from death certificates (28%), death registers (37%) and medical/hospital records (24%). Eleven percent of deaths were unverified and information from the family member/contact person was the only source. Deaths were categorised where possible as being due to cardiovascular diseases, cancer or other causes, preferentially using the same source

as that used to verify the death. Men who did not reply to the follow-up PQ and for whom no further information was available were classified as 'lost to follow-up'.

Statistical analysis

Analyses were conducted using STATA SE v11.2 (StataCorp, College Station, TX, USA). Smoking was categorised as current, former or never; alcohol consumption as never, intake on ≤ 2 days/week or ≥ 3 days/week; and self-reported morbidities as none, 1 or ≥ 2 . Participants contributed follow-up time from the date of admission in the baseline survey to the date of follow-up assessment or date of death. Deaths occurring up to 31 December 2008 were included in this analysis.

We used *t*-tests or Wilcoxon rank-sum tests to assess associations between vital status and continuous variables and the χ^2 -test for associations with categorical variables. Associations of 25(OH)D, 1,25(OH)₂D₃ and PTH with health and lifestyle factors that could potentially confound associations with mortality were assessed using linear regressions adjusted for baseline age, with results reported as beta-coefficients (β) and 95% CIs.

Cox proportional hazard models were used to assess the association between all-cause mortality and 25(OH)D, 1,25(OH)₂D₃ or PTH, with results reported as hazard ratios (HR) and 95% CI. To aid interpretation of HRs the values for 25(OH)D, 1,25(OH)₂D₃ and PTH as continuous variables were standardised as z scores. This provided a comparative measure of the magnitude of the association between each hormone (per SD change) and all-cause mortality, independent of the units of concentration. 25(OH)D, 1,25(OH)₂D₃ and PTH were categorised into quartiles prior to analysis, with the reference category for 25(OH)D and 1,25(OH)₂D₃ being the highest quartile and for PTH the lowest quartile. Data were also analysed after categorising 25(OH)D levels as: ≥ 75 nmol/l (reference; $n = 828$); 50.0–74.9 nmol/l ($n = 873$); 25.0–49.9 nmol/l ($n = 904$); and <25.0 nmol/l ($n = 211$). Although no current consensus on optimal levels of vitamin D exist, a recent report on dietary requirements for calcium and vitamin D from the Institute of Medicine (IOM) concluded that a serum 25(OH)D concentration of 50 nmol/l would cover the requirements of at least 97.5% of the population [26]. All Cox regressions were initially performed unadjusted, then with adjustment for baseline age and centre. To obtain parsimonious final models, additional adjustments were restricted to those health and lifestyle factors associated with both all-cause mortality and 25(OH)D, 1,25(OH)₂D₃ or PTH. Model goodness of fit was assessed using Nelson–Aalen cumulative hazard plots, and the proportional-hazards assumption tested on the basis of Schoenfeld residuals after fitting each model [27].

Associations between cause-specific mortality (cardiovascular or cancer) and 25(OH)D, 1,25(OH)₂D₃ or PTH, were modelled using competing-risks regression with results expressed as sub-hazard ratios (SHR) and 95% CIs. Competing-risks regression generalises survival analysis to situations where subjects are exposed to more than one

Table 1. Baseline characteristics of EMAS men by vital status at follow-up

Variable	Survived (<i>n</i> = 2629)	Died (<i>n</i> = 187)	<i>P</i> *
	Mean (SD)		
Age (years)	59.2 (10.7)	69.3 (8.3)	<0.001
25(OH)D (nmol/l)	64.3 (31.5)	48.3 (28.1)	<0.001
1,25(OH) ₂ D ₃ (pmol/l)	143 (39)	131 (42)	<0.001
PTH (pmol/l)	2.97 (1.22)	3.35 (1.90)	<0.001
Serum creatinine (μmol/l)	91.1 (16.2)	97.5 (21.8)	<0.001
Serum calcium (mmol/l)	2.28 (0.10)	2.31 (0.13)	<0.001
Body mass index (kg/m ²)	27.6 (4.0)	27.6 (4.9)	0.99
Waist circumference (cm)	98.3 (10.8)	99.5 (12.1)	0.15
PASE score	200 (89)	141 (91)	<0.001
PPT rating	24.1 (2.5)	21.3 (3.8)	<0.001
	Number (%)		
Smoking status			0.003
Never smoked	782 (30.4)	35 (19.2)	
Former smoker	1273 (49.4)	97 (53.3)	
Current smoker	520 (20.2)	50 (27.5)	
Frequency of alcohol consumption			<0.001
Never	402 (15.4)	53 (29.0)	
≤2 days/week	1266 (48.3)	83 (45.3)	
≥3 days/week	950 (36.3)	47 (25.7)	
Self-reported morbidities			<0.001
None	1305 (50.3)	28 (15.5)	
One	706 (27.2)	49 (27.1)	
Two or more	582 (22.5)	104 (57.4)	
Season			0.39
Winter	790 (30.1)	57 (30.5)	
Spring	598 (22.7)	33 (17.6)	
Summer	304 (11.6)	22 (11.8)	
Autumn	937 (35.6)	75 (40.1)	
Cardiovascular disease ^a	423 (16.1)	76 (40.6)	<0.001
High blood pressure ^b	848 (32.3)	105 (56.2)	<0.001
Diabetes ^c	177 (6.7)	33 (17.7)	<0.001
Cancer (ever)	140 (5.3)	28 (15.1)	<0.001
Vitamin D/Ca ²⁺ supplementation	14 (0.5)	4 (2)	0.01

**P*-value for comparison between 'survived' and 'died' groups: *t*-test or Wilcoxon rank-sum test for continuous variables, χ^2 -test for categorical variables.

^aSelf-reported heart condition and/or stroke (ever).

^bSelf-reported high blood pressure and/or using anti-hypertensives.

^cSelf-reported diabetes and/or using anti-diabetic drugs.

cause of failure, e.g. cancer mortality versus non-cancer mortality, allowing more robust estimation of cause-specific hazards [28]. Competing-risks regressions were adjusted for the same factors as in the Cox models. To minimise potential bias by reverse causality, analyses on cardiovascular mortality were repeated excluding subjects who self-reported at baseline 'heart conditions' and/or 'ever suffered a stroke', and analyses on cancer mortality were repeated excluding subjects who self-reported 'ever been treated for cancer'.

Results

From the 3,369 baseline participants, 440 men were lost to follow-up and 113 had missing serum vitamin D metabolite data. Among the remaining 2,816 men, there were 187

deaths during a median 4.3 years follow-up, an overall mortality of 6.6%. The characteristics of the analysis sample by vital status are shown in Table 1. Compared with survivors, those who died were older, had significantly lower levels of 25(OH)D and 1,25(OH)₂D₃ and higher levels of PTH and creatinine. The men who died also differed significantly from survivors in terms of their baseline physical activity (PASE), physical function (PPT), smoking status, alcohol consumption and morbidity burden. The associations of 25(OH)D, 1,25(OH)₂D₃ and PTH with a range of health and lifestyle factors in age adjusted linear regressions are summarised in the Supplementary data available in *Age and Ageing* online, Appendix 1.

Table 2 summarises the results from the Cox regression models with 25(OH)D, 1,25(OH)₂D₃ and PTH predicting all-cause mortality. Models were adjusted for age and centre [Model 1], and additionally for smoking, alcohol consumption, morbidities, PASE score, PPT rating and creatinine [Model 2]. Unadjusted Cox regressions showed HRs of 1.88 (95% CI = 1.55, 2.26) per SD decrease in 25(OH)D, 1.40 (95% CI = 1.19, 1.65) per SD decrease in 1,25(OH)₂D₃ and 1.28 (95% CI = 1.14, 1.43) per SD increase in PTH. Serial adjustment for age, centre and other confounders attenuated this relationship, such that the respective HRs were 1.45 (95% CI = 1.16, 1.81) per SD decrease in 25(OH)D, 1.24 (95% CI = 1.02, 1.51) per SD decrease in 1,25(OH)₂D₃ and 1.04 (95% CI = 0.90, 1.20) per SD increase in PTH. The association between lower 25(OH)D and increased mortality was also observed when 25(OH)D levels were classified into quartiles or categories. In fully adjusted models [Model 2], men who were in the lowest quartile of 25(OH)D (<40 nmol/l) had an HR of 2.37 (95% CI = 1.33, 4.24) compared with those in the highest quartile (>79.1 nmol/l), while those with 25(OH)D <25 nmol/l at baseline had an HR of 2.28 (95% CI = 0.20, 4.34) compared with those with a 25(OH)D level of ≥75 nmol/l. Similar associations were observed when 1,25(OH)₂D₃ was classified into quartiles, with men in the lowest quartile (<114 pmol/l) having an HR for mortality of 1.77 (95% CI = 1.05, 2.96) compared with those in the highest quartile (>164 pmol/l). The relationship between PTH quartiles and mortality was less clear, however, with only men in the third quartile of PTH (2.77–3.60 pmol/l) having a significantly reduced risk of mortality (HR = 0.56; 95% CI = 0.33, 0.95) compared with those in the lowest quartile (<2.15 pmol/l).

Results from the fully adjusted competing-risks regressions are summarised in Table 3. There was no association between 25(OH)D (per SD decrease) and cardiovascular mortality (SHR = 1.38; 95% CI = 0.95, 1.99; *P* = 0.09), nor any evidence that 25(OH)D levels (either quartiles or categories) were associated with cardiovascular mortality. The association between 25(OH)D (per SD decrease) and cancer mortality also failed to reach significance (SHR = 1.31; 95% CI = 0.92, 1.86; *P* = 0.14), although when 25(OH)D was classified into categories those men with a 25(OH)D level of <25 nmol/l at baseline had a significant SHR for cancer mortality of 3.33 (95% CI = 1.38, 8.04) compared with those with

Table 2. Cox proportional hazard models: all-cause mortality by baseline 25(OH)D, 1,25(OH)₂D₃ and PTH

	Hazard ratio (95% CI)		
	Unadjusted	Model 1	Model 2
25(OH)D (per SD decrease)	1.88 (1.55, 2.26)***	1.75 (1.42, 2.14)***	1.45 (1.16, 1.81)**
25(OH)D quartiles			
4: >79.1 nmol/l	Reference	Reference	Reference
3: 58.0–79.1 nmol/l	1.93 (1.12, 3.33)*	1.62 (0.93, 2.81)	1.66 (0.91, 3.03)
2: 40.0–57.9 nmol/l	2.41 (1.42, 4.07)**	1.77 (1.03, 3.05)*	1.52 (0.83, 2.77)
1: <40.0 nmol/l	4.33 (2.66, 7.05)***	3.43 (2.05, 5.73)***	2.37 (1.33, 4.24)**
25(OH)D categories			
≥75.0 nmol/l	Reference	Reference	Reference
50.0–74.9 nmol/l	1.92 (1.17, 3.14)**	1.44 (0.87, 2.38)	1.38 (0.80, 2.38)
25.0–49.9 nmol/l	3.20 (2.03, 5.05)***	2.55 (1.58, 4.10)***	1.99 (1.17, 3.36)*
<25.0 nmol/l	6.00 (3.57, 10.1)***	4.34 (2.51, 7.49)***	2.28 (1.20, 4.34)*
1,25(OH) ₂ D ₃ (per SD decrease)	1.40 (1.19, 1.65)***	1.33 (1.13, 1.58)**	1.24 (1.02, 1.51)*
1,25(OH) ₂ D ₃ quartiles			
4: >164 pmol/l	Reference	Reference	Reference
3: 139–164 pmol/l	1.59 (1.00, 2.52)*	1.53 (0.96, 2.43)	1.66 (0.99, 2.78)
2: 114–138 pmol/l	1.24 (0.76, 2.03)	1.13 (0.69, 1.85)	0.94 (0.52, 1.68)
1: <114 pmol/l	2.28 (1.48, 3.52)***	2.05 (1.32, 3.20)**	1.77 (1.05, 2.96)*
PTH (per SD increase)	1.28 (1.14, 1.43)***	1.15 (1.01, 1.30)*	1.04 (0.90, 1.20)
PTH quartiles			
1: <2.15 pmol/l	Reference	Reference	Reference
2: 2.15–2.76 pmol/l	0.89 (0.58, 1.36)	0.79 (0.51, 1.20)	0.84 (0.52, 1.37)
3: 2.77–3.60 pmol/l	0.65 (0.41, 1.03)	0.54 (0.34, 0.86)*	0.56 (0.33, 0.95)*
4: >3.60 pmol/l	1.53 (1.05, 2.23)*	1.10 (0.74, 1.62)	1.02 (0.65, 1.60)

Model 1: adjusted for age and centre. Model 2: adjusted for age, centre, smoking status, alcohol consumption, self-reported morbidities, PASE score, PPT rating and serum creatinine. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

Note: Including PTH in Model 2 (25(OH)D or 1,25(OH)₂D₃), or forcing BMI, waist circumference, serum calcium or season into Model 2 (25(OH)D, 1,25(OH)₂D₃ or PTH) did not markedly alter the magnitude or significance of the associations (data not shown). Using the IOM cut points for 25(OH)D [28], the HRs for Model 2 were: >50 nmol/l (reference category), 30–50 nmol/l (HR = 1.53; 95% CI = 1.03, 2.27) and <30 nmol/l (HR = 1.98; 95% CI = 1.25, 3.12).

Table 3. Competing-risks regressions: cause-specific mortality by baseline 25(OH)D

		Sub-hazard ratio (95% CI)	
		CVD death ($n = 72$)	Cancer death ($n = 72$)
25(OH)D (per SD decrease)	No.	1.38 (0.95, 1.99)	1.31 (0.92, 1.86)
25(OH)D quartiles			
4: >79.1 nmol/l	619	Reference	Reference
3: 58.0–79.1 nmol/l	609	1.29 (0.41, 4.07)	2.04 (0.89, 4.66)
2: 40.0–57.9 nmol/l	611	1.70 (0.59, 4.92)	0.55 (0.19, 1.61)
1: <40.0 nmol/l	613	2.21 (0.75, 6.51)	2.10 (0.94, 4.70)
25(OH)D categories			
≥75.0 nmol/l	728	Reference	Reference
50.0–74.9 nmol/l	759	1.25 (0.49, 3.20)	1.35 (0.61, 3.03)
25.0–49.9 nmol/l	789	1.77 (0.70, 4.45)	1.05 (0.45, 2.43)
<25.0 nmol/l	176	1.26 (0.40, 3.70)	3.33 (1.38, 8.04)**

Adjusted for age, centre, smoking status, alcohol consumption, self-reported morbidities, PASE score, PPT rating and serum creatinine. ** $P < 0.01$.

Note: Excluding subjects who reported 'ever been treated for cancer' from the cancer models attenuated the association between 25(OH)D and cancer mortality, i.e. for the <25.0 nmol/l category, SHR = 2.55 (95% CI = 1.03–6.33, $P < 0.05$).

a 25(OH)D level of ≥75 nmol/l. Excluding men who reported at baseline they had 'ever been treated for cancer' attenuated this relationship (25(OH)D <25 nmol/l: SHR = 2.55; 95% CI = 1.03, 1.99), but the association

remained significant. In contrast, those men in the lowest quartile of 25(OH)D (<40 nmol/l) had a non-significant SHR of 2.12 (95% CI = 0.96, 4.71) compared with those in the highest quartile (>79.1 nmol/l). The cumulative incidence of cancer mortality estimated from the competing-risks regression for ≥75 nmol/l, 50.0–74.9 nmol/l, 25.0–49.9 nmol/l and <25.0 nmol/l 25(OH)D categories is summarised graphically in the Supplementary data available in *Age and Ageing* online, Appendix 2. There were no associations between cause-specific mortality and 1,25(OH)₂D₃ or PTH (all $P > 0.1$, data not shown).

Discussion

In this prospective, population-based study of middle-aged and older European men lower levels of both 25(OH)D and 1,25(OH)₂D₃ were independently associated with an increased risk of all-cause mortality. This relationship was consistent whether vitamin D levels were modelled continuously or classified into quartiles or categories, such that men who were in the lowest 25% of the range of either 25(OH)D or 1,25(OH)₂D₃ had a two-fold higher risk of all-cause mortality after adjustment for potential confounders. In contrast, the relationship between PTH and all-cause mortality was broadly non-significant. For cause-specific mortality, we

observed a significant association between 25(OH)D and cancer mortality among men with a 25(OH)D level of <25 nmol/l only, i.e. more than a three-fold increased risk of cancer-related death compared with those with a 25(OH)D level of ≥ 75 nmol/l.

To our knowledge, this is the first study to simultaneously examine the associations of all of the key metabolites of the vitamin D endocrine system with all-cause and cause-specific mortality in a sample of community-dwelling individuals. Two recent meta-analyses of cohort studies examining vitamin D deficiency and mortality risk in the general population reported a non-linear decrease in mortality risk with increasing serum 25(OH)D levels, with an optimal concentration between ~ 75 and 87.5 nmol/l [13], and an 8% lower mortality in the general elderly population associated with a 20 nmol/l increase in 25(OH)D levels [12]. Our findings of a significantly increased risk of all-cause mortality at 25(OH)D concentrations considered 'insufficient' (<50 nmol/l) are in broad agreement with these meta-analyses [12, 13], and with Schottker *et al.* [29] who recently reported a significantly increased overall mortality among participants of the population-based ESTHER study (aged 50–74 years) who had a 25(OH)D level of <50 nmol/l.

There are fewer data describing the association of 1,25(OH)₂D₃ and PTH with all-cause mortality, and to the best of our knowledge ours is the first study to examine the relationship between 1,25(OH)₂D and mortality in a community-based setting. Dobnig *et al.* [17] observed a significant association of both low 1,25(OH)₂D and 25(OH)D levels with all-cause and cardiovascular mortality among male and female coronary angioplasty patients (mean age 62 years). Kendrick *et al.* [30] reported an increased risk of mortality and progression to maintenance dialysis in mainly male patients with advanced kidney disease who were in the lowest tertile of 1,25(OH)₂D. How generalisable these findings from patient populations with pre-existing disease are to community-dwelling individuals is uncertain. Given that serum 1,25(OH)₂D₃ concentrations are typically 1,000-fold lower than 25(OH)D [3], we used a sensitive LC–MS/MS method for measuring 1,25(OH)₂D₃ [23] and our data support a modest association between lower levels of 1,25(OH)₂D₃ and increased all-cause mortality.

A number of recent population-based cohort studies have examined the prospective association between 25(OH)D or PTH and cause-specific mortality. Using data from NHANES III, Freedman and colleagues observed an inverse relationship between 25(OH)D and colorectal cancer mortality during 146,578 person-years of follow-up [31]. In addition to all-cause mortality, Schottker *et al.* found strong associations between vitamin D deficiency (25(OH)D <30 nmol/l) and mortality from cardiovascular, cancer and respiratory causes among participants of the ESTHER study [29]. The Uppsala Longitudinal Study of Adult Men (ULSAM) found that higher PTH levels were associated with greater cardiovascular mortality in community-dwelling men (mean age 71 years), even among those without any sign of disturbed mineral metabolism [19]. However, we did

not observe significant associations between cardiovascular mortality and 25(OH)D, 1,25(OH)₂D₃ or PTH. It is possible that we failed to find any relationship with cardiovascular mortality due to a lack of power (number of cardiovascular deaths = 72) and/or misclassification in cause of death. The ESTHER study, in contrast, reported 350 deaths due to cardiovascular disease [29], while the ULSAM study reported 117 cardiovascular deaths defined using the Swedish Cause of Death Register [19]. Cause of death in EMAS was not validated by independent sources in $\sim 10\%$ of cases, necessitating some caution in our analysis of cause-specific mortality. Nonetheless, we did observe a significant association between cancer mortality and 25(OH)D levels <25 nmol/l, where the number of cancer deaths equalled those due to cardiovascular disease. Our observation of a three-fold increase in risk of cancer mortality only in men with 25(OH)D <25 nmol/l suggests that a threshold may exist, with an association only emerging when 25(OH)D is in the 'deficient' range.

Our previous cross-sectional data have shown that low 25(OH)D is associated with various adverse health states, including frailty [32], depression [6], decreased cognitive function [33] and metabolic syndrome [34]. While the nature of any biological mechanisms linking the vitamin D endocrine axis with adverse outcomes remain undefined, research interest in the non-skeletal effects of vitamin D has, in part, been motivated by the observation that the vitamin D receptor, and the enzyme responsible for converting 25(OH)D to 1,25(OH)₂D (1 α -hydroxylase), co-localise in a wide variety of cell types and tissues [35]. Along with *in vitro* data, this supports the notion that 1,25(OH)₂D may exert paracrine effects influencing gene expression *in vivo* [36]. However, there is no direct evidence that 25(OH)D or 1,25(OH)₂D is causally involved in non-skeletal pathophysiological mechanisms associated with mortality, and our findings do not differentiate whether low 25(OH)D/1,25(OH)₂D₃ is the cause or mediator of higher mortality or merely a non-specific marker of, or adaptive response to, poor health. Nonetheless, a key message here is that vitamin D deficiency should be recognised as not only having implications for skeletal health but may also offer a window of opportunity for improving ageing men's general health, and subsequent assessment and reduction of adverse outcomes.

The main strengths of our study are that it was prospective and population based, with detailed phenotyping of men using standardised assessments. Limitations of the EMAS study have been described in detail previously [20], as have problems pertaining to the use of single measurements of vitamin D and PTH [33]. Our overall loss to follow-up was 13% and compared with participants, those lost to follow-up had lower mean 25(OH)D (64.3 versus 55.6 nmol/l) and higher PTH (3.0 versus 3.2 pmol/l) levels, poorer PPT rating (mean 24.1 versus 23.5), a higher prevalence of two or more co-morbidities (22.5 versus 30.9%) and were more likely to smoke (20.2 versus 26.2%), and as a consequence may have had an increased mortality risk. Our data may, therefore, underestimate the true mortality of the baseline

cohort, although this should not affect the findings here which were based on an internal comparison of participants. Loss to follow-up also varied between centres (4.4–14.5%), although adjusting for centre in the analyses did not alter the main findings. Finally, we cannot discount that some associations may be due to unmeasured factors and/or residual confounding.

In summary, we found that low levels of 25(OH)D or 1,25(OH)₂D₃ are associated with an increased risk of all-cause mortality, largely independent of confounding health and lifestyle factors. We also observed a significantly increased risk of cancer mortality among men with a baseline serum 25(OH)D level of <25 nmol/l. While our data suggest that maintaining serum 25(OH)D levels >50 nmol/l may be beneficial to general health, any aetiological link between the vitamin D endocrine axis and mortality remains undefined.

Key points

- Serum levels of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D are negatively associated with all-cause mortality risk.
 - Very low levels of 25-hydroxyvitamin D were associated with an increased risk of cancer mortality.
 - Whether the link between the vitamin D endocrine axis and mortality has any causal basis remains unknown.
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Authors' contributions

D.M.L.: analysis and interpretation of data and preparation of manuscript. S.R.P., R.R., E.G., F.C.: interpretation of data and preparation of manuscript. D.V., S.B., T.W.O.: concept and design, acquisition of participants and data and preparation of manuscript. J.D.F., G.B., F.C., G.F., A.G., I.T.H., K.K., M.P.: concept and design, acquisition of participants and data and critical review of manuscript. N.P., T.S.H., M.E.J.L.: critical review of manuscript. F.C.W.: designed, instigated and led the EMAS project and contributed to the conception, interpretation of data and preparation of this manuscript.

Conflicts of interest

None declared.

Supplementary data

Supplementary data mentioned in the text are available to subscribers in *Age and Ageing* online.

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Career choices for geriatric medicine: national surveys of graduates of 1974–2009 from all UK medical schools

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Abstract

Background: numbers of elderly people are increasing worldwide. This increases the importance of the specialty of geriatric medicine. Recruitment to the specialty may not be keeping pace with need.

Objectives: to report trends in junior doctors' career choices for geriatric medicine, factors that influence career choice, and