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A Reverse J-Shaped Association of All-Cause Mortality with Serum 25-Hydroxyvitamin D in General Practice: The CopD Study

D. Durup, H. L. Jørgensen, J. Christensen, P. Schwarz, A. M. Heegaard ✉, B. Lind

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Abstract

Context:
Optimal levels of vitamin D have been a topic of heavy debate, and the correlation between 25-hydroxyvitamin D [25(OH)D] levels and mortality still remains to be established.

Objective:
The aim of the study was to determine the association between all-cause mortality and serum levels of 25(OH)D, calcium, and PTH.

Design and Setting:
We conducted a retrospective, observational cohort study, the CopD Study, in a single laboratory center in Copenhagen, Denmark.

Participants:
Serum 25(OH)D was analyzed from 247,574 subjects from the Copenhagen general practice sector. In addition, serum levels of calcium, albumin-adjusted calcium, PTH, and creatinine were measured in 111,536; 20,512; 34,996; and 189,496 of the subjects, respectively.

Main Outcome Measures:
Multivariate Cox regression analysis was used to compute hazard ratios for all-cause mortality.

Results:
During follow-up (median, 3.07 yr), 15,198 (6.1%) subjects died. A reverse J-shaped association between serum level of 25(OH)D and mortality was observed. A serum 25(OH)D level of 50–60 nmol/liter was associated with the lowest mortality risk. Compared to 50 nmol/liter, the hazard ratios (95% confidence intervals) of all-cause mortality at very low (10 nmol/liter) and high (140 nmol/liter) serum levels of 25(OH)D were 2.13 (2.02–2.24) and 1.42 (1.31–1.53), respectively. Similarly, both high and low levels of albumin-adjusted serum calcium and serum PTH were associated with an increased mortality, and secondary hyperparathyroidism was associated with higher mortality ($P < 0.0001$).

Conclusion:
In this study from the general practice sector, a reverse J-shaped relation between the serum level of 25(OH)D and all-cause mortality was observed, indicating not only a lower limit but also an upper limit. The lowest mortality risk was at 50–60 nmol/liter. The study did not allow inference of causality, and further studies are needed to elucidate a possible causal relationship between 25(OH)D levels, especially higher levels, and mortality.

Issue Section: Endocrine Care

Vitamin D insufficiency (25-hydroxyvitamin D [25(OH)D] < 50 nmol/liter) is prevalent (1–3) and has been suggested to be involved in various diseases such as diabetes, cardiovascular disease, depression, immune system diseases, and certain cancers (4–8). The biomarker used for the determination of vitamin D status is 25(OH)D, rather than the biologically active hormone 1,25-dihydroxyvitamin D (9).

The association between 25(OH)D levels and all-cause mortality has been estimated in several studies (3, 10–23). Most of the studies have investigated the association between low levels of 25(OH)D and mortality and found an increased risk (12–15, 19, 20, 22, 23); some studies suggested an inverse relation (3, 11, 21), whereas other studies did not find any association with low levels of 25(OH)D and mortality risk (10, 16, 18). Although the preponderance of focus has been directed toward vitamin D insufficiency, the U.S. Food and Nutrition Board at the Institute of Medicine (IOM) has indicated that higher concentrations of 25(OH)D (below toxicity levels) may also be of concern; this was based on a reevaluation of data in the literature, including some of the above-mentioned studies (24). Two studies have investigated the association between mortality and higher levels of 25(OH)D and found both high and low levels of 25(OH)D to be associated with increased risk of overall mortality (14, 17).

An optimal level of vitamin D is a topic of heavy debate among health care professionals, and further investigations are needed to provide evidence of how 25-hydroxyvitamin D levels relate to mortality. In this context, there is a special need for studies that also evaluate higher levels of 25(OH)D. To address this issue, a database containing serum 25(OH)D measurements from 247,574 subjects from the general practice sector was used to determine the association between serum levels of 25(OH)D and mortality, including higher as well as lower levels. Additionally, the study investigated the association between mortality and serum levels of albumin-adjusted serum calcium and PTH because abnormal serum levels of PTH and calcium, as well as vitamin D, have been reported to be associated with increased mortality (16, 18, 25–28).

Subjects and Methods

Study subjects

The Copenhagen General Practitioners Laboratory (CGPL) serves physicians in the primary care sector of the greater Copenhagen area mainly by conducting a wide range of blood tests. In this study, the CGPL database was accessed, and subjects with a serum 25(OH)D measurement were included.

The first and the last subject included in the study had their blood measurements on April 29, 2004, and January 22, 2010, respectively. If a subject had more than one serum 25(OH)D measurement, only the first measurement was used. During the study period, the CGPL has analyzed blood samples from a total of 776,954 subjects, of which 247,574 subjects (32%) had serum 25(OH)D analyzed. The CGPL is the sole laboratory that serves the general practitioners in the greater Copenhagen area, and it covers approximately 1.1 million inhabitants. In addition to serum 25(OH)D, serum levels of calcium, albumin-adjusted calcium, PTH, and creatinine were measured in 111,536; 20,512; 34,996; and 189,496 of the subjects, respectively. A total of 92.8% of the subjects had their blood collected either at the CGPL or at the office of their primary health care physician, who then sent the blood samples to the CGPL for further analysis. The rest of the blood samples (7.2%) were taken by the CGPL at institutions/residential care or home visits.

The personal identification number (CPR number) is unique to every citizen in Denmark and enables matching of individuals to registers. All mortality information was extracted from the civil registration database on the same day (August 25, 2011) corresponding to end of study. All subjects were followed from the date of blood measurement until the date of emigration, date of death, or August 25, 2011, whichever came first; the median follow-up for all subjects was 3.07 yr (1.59; 5.96; 5th and 95th percentiles).

All necessary approvals from the Danish Data Protection Agency were obtained before collecting data (no. 2010-41-4826).

Biochemical analyses

25(OH)D assays

25(OH)D was assessed in serum by two commercially available assays, LIAISON 25(OH)D assay (Diasorin, Saluggia, Italy) and OCTEIA 25(OH)D_3 and 25(OH)D_2 (Immunodiagnostic Systems, Ltd., Boldon, UK) according to the instructions of the manufacturers. Both assays determine the sum of 25(OH)D_3 and 25(OH)D_2. For the LIAISON assay, the interserial coefficient of variation percentage (CV%) was 12.5% (at level 41 nmol/liter). For the OCTEIA assay, the interserial CV% was 9% (at level 32 nmol/liter). Results obtained by the OCTEIA assay were adjusted to results obtained by LIAISON using the equation: LIAISON = 0.893 × OCTEIA + 0.48. The equation was determined by parallel analysis of 59 human serum samples during a period of 5 d in August 2007. The LIAISON assay was used after August 19, 2007 and until the end of study. Both assays were subject to external quality control through participation in the Vitamin D External Quality Assessment Scheme (DEQAS; Charing Cross Hospital, London, UK). The assessment scheme included four distributions annually. Each distribution comprised five samples. The results from DEQAS through the entire study period (from 2004 to 2010) confirmed the reliability of the assays, and the results from CGPL deviated less than 15% from the method mean.

PTH assay

PTH was determined in serum by the commercially available ADVIA Centaur iPTH kit (Bayer/Siemens, Tarrytown, NY) according to the instructions of the manufacturer (upper limit of the normal range, 7.6 pmol/liter). The interserial CV% was 9.1% (at level 2.76 pmol/liter) and 5.6% (at level 26.2 pmol/liter). The assay is specific for intact PTH (amino acid 1–84).

Calcium assay

Total calcium was determined in serum by the Advia Chemistry System (Bayer/Siemens) using Arsenazo III reagents. Results were traceable to and adjusted to the target values of Reference Serum X, Nordic Society of Clinical Chemistry. The interserial CV% was 1.6% (at level 2.43 mmol/liter) and 1.2% (at level 3.13 mmol/liter).

Albumin assay

Albumin was determined in serum by the commercially available Advia Chemistry System (Bayer/Siemens) albumin kit according to the instructions of the manufacturer. The interserial CV% was 2.1% (at level 31.6 g/liter) and 1.7% (at level 40.6 g/liter).

Albumin-adjusted calcium

Albumin-adjusted calcium was calculated as: total calcium (in mmol/liter) + 0.020*(41.3 − albumin (g/liter)).

Creatinine assay

Creatinine was determined in serum by the commercially available ADVIA Chemistry System (Bayer/Siemens) creatinine (Jaffe) kit according to the instructions of the manufacturer. The interserial CV% was 2.0% (at level 92 μmol/liter and level 527 μmol/liter).

Statistical analyses

Normally distributed variables were shown as mean (SD), and differences between groups were analyzed using unpaired *t* tests. Nonnormally distributed variables were shown as medians with 5 and 95% percentiles, and Mann–Whitney *U* tests were used to test for differences. Categorical variables were shown as proportions, and the differences were analyzed using χ^2 tests. *P* values less than 0.05 were considered statistically significant.

The nonlinear association between all-cause mortality and serum level of 25(OH)D, albumin-adjusted serum calcium, and serum PTH was analyzed using a Cox proportional hazards model, where serum levels of 25(OH)D, albumin-adjusted serum calcium, and serum PTH were entered in the model as a restricted cubic spline with five knots placed at the 5th, 27th, 50th, 73rd, and 95th percentiles of serum 25(OH)D, albumin-adjusted serum calcium, and serum PTH. A serum 25(OH)D concentration of 50 nmol/liter was used as a reference. For albumin-adjusted serum calcium and serum PTH, the reference levels were set at 2.25 mmol/liter and 4.5 pmol/liter, respectively. The models were adjusted for season of blood sampling (January–March, April–June, July–September, and October–December), age, and gender.

All statistical analyses were performed using SAS statistical software (SAS Institute, Inc., Cary, NC) or the computing environment R (R Development Core Team, 2005).

Results

A total of 247,574 subjects with a serum 25(OH)D measurement were included in the CopD Study. Table 1 shows the characteristics of the study population by serum levels of 25(OH)D. Measures of serum 25(OH)D were obtained in almost twice as many women as men, and in the total study population, an average of 54.4% suffered from vitamin D insufficiency [25(OH)D < 50 nmol/liter]. The study included a wide age range, and vitamin D insufficiency existed within all age groups. Furthermore, the study subjects were also well represented in the very lower and higher levels of 25(OH)D: 13,885 subjects had 25(OH)D levels below or equal to 12.5 nmol/liter, and 1,365 had 25(OH)D levels equal to or higher than 150 nmol/liter. More than 90% of the subjects who had a creatinine measurement, had a creatinine level below 110 nmol/liter, both at low and high serum levels of 25(OH)D, indicating functional kidneys. Finally, from February to April, the prevalence of vitamin D insufficiency was significantly higher than from July to September ($P < 0.0001$), both among women (62 *vs.* 38%) and among men (74 *vs.* 39%).

Table 1.

Characteristics of the CopD study population by serum levels of 25(OH)D

	All	Serum level of 25(OH)D (nmol/liter)							*P* value
		≤12.5	12.5–25	25–50	50–75	75–100	100–125	125–150	≥150	
n	247,574	13,885	41,804	82,442	67,462	29,680	8,539	2,397	1,365	<0.0001
Age (yr), mean (SD)	51.0 (20.4)	46.6 (20.3)	47.9 (20.1)	51.1 (20.1)	53.0 (20.2)	52.9 (20, 7)	51.6 (21.5)	50.3 (21.5)	48.9 (20.8)	<0.0001
Gender (female/male)	65.2%/34.8%	60.6%/39.4%	61.6%/38.4%	62.6%/37.4%	67.5%/32.5%	71.2%/28.8%	73.0%/27%	74.6%/26%	73.7%/26.3%	<0.0001
Dead	6.1%	8.1%	6.9%	5.9%	5.3%	5.9%	8.0%	8.2%	8.4%	<0.0001
Age groups, n (%)										
0–15 yr	7,756 (3.1)	539 (3.8)	1,462 (3.6)	2,742 (3.3)	1,927 (2.9)	764 (2.6)	221 (2.6)	52 (2.2)	29 (2.1)	<0.0001
15–30 yr	35,670 (14.4)	2,652 (19.1)	7,038 (16.8)	11,150 (13.5)	8,338 (12.4)	4,212 (14.2)	1,499 (17.6)	490 (20.5)	291 (21.3)	<0.0001
30–45 yr	55,919 (22.6)	3,963 (28.6)	11,606 (27.8)	18,813 (22.8)	13,478 (20.0)	5,635 (19.0)	1,669 (19.6)	478 (19.9)	277 (20.3)	<0.0001
45–60 yr	59,088 (23.9)	3, 125 (22, 5)	10,004 (23.9)	20,473 (24.9)	16,153 (23.9)	6,721 (22.6)	1,771 (20.7)	499 (20.8)	322 (23.6)	<0.0001
60–75 yr	54,530 (22.0)	2,003 (14.4)	6,756 (16.2)	17,896 (21.7)	17,275 (25.6)	7,770 (26.2)	2,034 (23.8)	516 (21.5)	280 (20.5)	<0.0001
>75 yr	34,611 (14.0)	1,613 (11.6)	4,908 (11.7)	11,368 (13.8)	10.291 (15.2)	4,578 (15.4)	1,345 (15.7)	362 (15.1)	166 (12.2)	<0.0001
PTH (n)	34,996	2,272	6,560	11,543	8,965	3,960	1,169	344	183	<0.0001
Serum PTH (pmol/liter), median [5th; 95th percentile]	4.5 [1.3; 11.2]	7.0 [2.9; 23.5]	5.7 [2.4; 14.2]	4.6 [2.0; 10.3]	4.0 [1.8; 8.5]	3.7 [1.4; 7.7]	3.5 [1.5; 7.4]	3.2 [1.4; 7.0]	3.1 [1.1; 6.8]	<0.0001
Ca (n)	111,536	6,487	18,855	36,919	30,258	13,379	3,921	1,119	598	<0.0001
Serum Ca (mmol/liter), mean (SD)	2.31 (0.10)	2.32 (0.11)	2.34 (0.11)	2.36 (0.10)	2.36 (0.10)	2.36 (0.10)	2.37 (0.11)	2.37 (0.11)	2.39 (0.13)	<0.0001

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	Mortality	*P*-value
25(OH)D >75 nmol/liter AND		
PTH >7.6 nmol/liter [n = 23,905]	4.99%	<0.0001
PTH <7.6 nmol/liter [n = 5,217]	11.31%	
25(OH)D >50 nmol/liter AND		
PTH >7.6 nmol/liter [n = 15,589]	5.02%	<0.0001
PTH <7.6 nmol/liter [n = 4,473]	10.88%	
25(OH)D <25 nmol/liter AND		
PTH >7.6 nmol/liter [n = 5,769]	4.70%	<0.0001
PTH <7.6 nmol/liter [n = 2,755]	10.31%	

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