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Serum 25-hydroxyvitamin D level, chronic diseases and all-cause mortality in a population-based prospective cohort: the HUNT Study, Norway

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ABSTRACT

Objective To investigate the association of vitamin D status with all-cause mortality in a Norwegian population and the potential influences of existing chronic diseases on the association.

Design A population-based prospective cohort study.

Setting Nord-Trøndelag County, Norway.

Participants A random sample (n=6613) of adults aged 20 years or older in a cohort.

Methods Serum 25-hydroxyvitamin D (25(OH)D) levels were measured in blood samples collected at baseline (n=6377). Mortality was ascertained from the Norwegian National Registry. Cox regression models were applied to estimate the HRs with 95% CIs for all-cause mortality in association with serum 25(OH)D levels after adjustment for a wide spectrum of confounding factors as well as chronic diseases at baseline.

Results The median follow-up time was 18.5 years, during which 1539 subjects died. The HRs for all-cause mortality associated with the first quartile level of 25(OH)D (<34.5 nmol/L) as compared with the fourth quartile (≥58.1 nmol/L) before and after adjustment for chronic diseases at baseline were 1.30 (95% CI 1.11 to 1.51) and 1.27 (95% CI 1.09 to 1.48), respectively. In the subjects without chronic diseases at baseline and with further exclusion of the first 3 years of follow-up, the corresponding adjusted HR was 1.34 (95% CI 1.09 to 1.66).

Conclusions Low serum 25(OH)D level was associated with increased all-cause mortality in a general Norwegian population. The association was not notably influenced by existing chronic diseases.

Strengths and limitations of this study

- Data were from the Nord-Trøndelag Health Study 2 (HUNT2): a large population-based Norwegian cohort with long follow-up time (median 18.5 years).
- The potential influences of existing chronic diseases on the association of serum 25-hydroxyvitamin D levels with all-cause mortality were investigated with two definitions of chronic illness respectively.
- The observed associations remained when the analyses were performed in the subjects without chronic diseases at baseline, and with further exclusion of the first 3 years of follow-up.
- Secondary analyses based on the imputed data for missing values of covariates produced similar association estimates.
- Non-participation in HUNT2 (30%) may influence the generalisability of our findings.

INTRODUCTION

All-cause mortality is an outcome with high public health relevance. Globally, life expectancy in elderly has steadily increased over the past 30 years.[1] Decline in older age mortality has been mainly the result of decreases in smoking for men and in cardiovascular disease mortality for both genders.[1] Findings of new risk factors for mortality and subsequent prevention could improve life expectancy further.

Vitamin D status has been recognised as a public health issue since low vitamin D levels are very common and may lead to the development of a wide spectrum of diseases.[2–5] The pleiotropic effect of vitamin D was suggested by the presence of vitamin D synthesis enzymes and vitamin D receptors in many tissues.[6 7] In addition, vitamin D is estimated to regulate 1%–3% of all gene expressions in human.[8 9]

All latest meta-analyses of epidemiological studies have documented that individuals with low serum 25-hydroxyvitamin D (25(OH)D) levels are at increased risk of all-cause mortality.[5 10–15] Among the meta-analysis studies a few included chronic diseases as potential confounders and found that the association estimates were similar before and after the adjustment for these variables.[12 13] Nevertheless, there has been a growing concern on the possibility of reverse association between low vitamin D and existing chronic diseases leading to increased all-cause mortality.[16] Thus, the aim of the current study was to investigate

the association of serum 25(OH)D levels with all-cause mortality in a long-term follow-up of a Norwegian population, and to especially study the potential influences of chronic diseases as a possible confounder, effect modifier or reverse causal factor on the relationship between serum 25(OH)D levels and all-cause mortality.

METHODS

Study design and population

The Nord-Trøndelag Health Study (the HUNT Study) is one of the largest population-based health surveys conducted in Norway. The adult part of HUNT invited all inhabitants aged 20 years or older in the county of Nord-Trøndelag in the three separate surveys: HUNT1 (1984–1986), HUNT2 (1995–1997) and HUNT3 (2006–2008). In the current study, we used data from HUNT2 in which 65 229 subjects participated (response rate 70%). All participants in HUNT2 were invited to complete a general questionnaire including lifestyle questions, social economic status and history of chronic diseases. At the clinical examination, body weight and height were measured and blood samples were drawn for later measurement of biomarkers. We established a subcohort population (n=6613) including a 10% random sample of the HUNT2 population. Baseline serum 25(OH)D levels were measured in 6377 individuals whose blood samples were available from HUNT2. The 6377 adults (96.4% of the 6613 subjects) made up our analysis cohort.

Measurement of serum 25(OH)D levels

Blood samples collected in HUNT2 were stored at −70°C. Serum 25 (OH)D levels were measured using LIAISON 25-OH Vitamin D TOTAL (DiaSorin, Saluggia, Italy), a fully automated antibody-based chemiluminescence assay. The detection range of the assay is 10–375 nmol/L. The assay has an intra-assay coefficient of variation of 4% and an interassay coefficient of variation of 8%.

Ascertainment of all-cause mortality

The HUNT Research Centre receives updated information about deaths of all causes and emigration of the HUNT participants from the Norwegian National Registry. The Norwegian National Registry records the date of death for all people living in Norway. In the current study, the HUNT2 participants were followed up from their participation date until 15 April 2015 or the date of death.

Information on covariates

Baseline variables were collected by questionnaires or at clinical examination. These covariates were categorised as: age (<35, 35–44, 45–54, 55–64, 65–74 and ≥75 years), sex (female, male), season of blood draw (spring: March–May, summer: June–August, fall: September–November, winter: December–February), daily smoker (never, former, current), alcohol consumption (never, 1–4 times per month, ≥5 times per month), physical activity (inactive or very low, low, moderate, high), education (<10, 10–12, ≥13 years) and economic difficulties (During the last year, has it at any time been difficult to meet the costs of food, transportation, housing and such? yes/no). Body mass index (BMI, kg/m^2) was grouped into <25.0, 25.0–29.9 and ≥30.0 kg/m^2 categories according to the recommendations of the WHO.[17] Chronic illness at baseline (first definition) was a variable generated from responses to a number of questions on major somatic diseases (Have you had or do you have any of the following diseases: myocardial infarction (heart attack)/angina pectoris (chest pain)/stroke (brain haemorrhage)/diabetes/cancer? yes/no). Chronic illness at baseline (second definition) was a direct variable extracted from the HUNT2 questionnaire data (Do you suffer from any long-term illness or injury of a physical or psychological nature that impairs your functioning in your everyday life (long term means at least 1 year)? yes/no). People with missing information on BMI, smoking, alcohol consumption, physical activity, education years, economic difficulties or chronic illness were regarded as an 'unknown' category for each variable and included in the primary analyses. The classification of each covariate has been widely used in the previous HUNT studies.[18 19]

Statistical analyses

We first tested the linearity of serum 25(OH)D levels in relation to all-cause mortality using restricted cubic spline model, which showed evidence of departure from a linear relationship (p=0.03). Therefore, 25(OH)D levels were treated as a categorical variable classified by quartiles and cut-off points (<25.0, 25.0–49.9, 50.0–74.9, ≥75.0 nmol/L) for presentation of results. The fourth quartile (≥58.1 nmol/L) and the level 50.0–74.9 nmol/L were used as the reference groups respectively since the level 50.0–74.9 nmol/L was suggested as sufficient according to the National Academy of Sciences report.[20]

Cox proportional hazards regression models were applied to estimate the HRs with 95% CIs for all-cause mortality in association with serum 25(OH)D levels. Person-years were calculated from the date of participation in HUNT2 to the date when death occurred, the person emigrated out of Norway or follow-up ended (15 April 2015), whichever occurred the first. We tested proportional hazards assumption by Schoenfeld residuals for 25(OH)D levels and all covariates. Apart from sex, physical activity and economic difficulties, other variables did not show evidence against proportional hazards assumption. We therefore used the *tvc* option of the *stcox* command in Stata to model the non-proportional hazards for sex, physical activity and economic difficulties. Four multivariable models were presented to adjust confounding: model 1 adjusted for season of blood draw since serum 25(OH)D levels vary by season; model 2 adjusted for age, sex, BMI, smoking, alcohol consumption, physical activity, education and economic difficulties as potential confounders in addition to season of blood draw; model 3 adjusted for chronic illness (first definition) in addition to the variables included in model 2;

Table 1 Baseline characteristics of subjects in random subcohort and analysis cohort in the HUNT2 study, 1995–1997

	Subcohort (n=6613)	Analysis cohort (n=6377)
Age (years)		
<65	5028 (76.0%)	4878 (76.5%)
≥65	1585 (24.0%)	1499 (23.5%)
Sex		
Female	3493 (52.8%)	3395 (53.2%)
Male	3120 (47.2%)	2982 (46.8%)
Season of blood draw		
Spring	1523 (23.0%)	1474 (23.1%)
Summer	828 (12.5%)	800 (12.5%)
Fall	2303 (34.8%)	2225 (34.9%)
Winter	1959 (29.6%)	1878 (29.4%)
Body mass index (kg/m^2)		
Normal/underweight (<25.0)	2637 (39.9%)	2558 (40.1%)
Overweight (25.0–29.9)	2822 (42.7%)	2744 (43.0%)
Obese (≥30.0)	1064 (16.1%)	1022 (16.0%)
Unknown	90 (1.4%)	53 (0.8%)
Daily smoker		
Never	2798 (42.3%)	2720 (42.7%)
Former	1752 (26.5%)	1688 (26.5%)
Current	1896 (28.7%)	1822 (28.6%)
Unknown	167 (2.5%)	147 (2.3%)
Alcohol consumption (times per month)		
Never	2295 (34.7%)	2190 (34.3%)
1–4	3010 (45.5%)	2937 (46.1%)
≥5	726 (11.0%)	704 (11.0%)
Unknown	582 (8.8%)	546 (8.6%)
Physical activity		
Inactive or very low	1419 (21.5%)	1367 (21.4%)
Low	1155 (17.5%)	1121 (17.6%)
Moderate	1424 (21.5%)	1378 (21.6%)
High	557 (8.4%)	544 (8.5%)
Unknown	2058 (31.1%)	1967 (30.8%)
Education (years)		
<10	2271 (34.3%)	2180 (34.2%)
10–12	2150 (32.5%)	2085 (32.7%)
≥13	1824 (27.6%)	1774 (27.8%)
Unknown	368 (5.6%)	338 (5.3%)
Economic difficulties		
No	3197 (48.3%)	3125 (49.0%)
Yes	1351 (20.4%)	1304 (20.4%)
Unknown	2065 (31.2%)	1948 (30.5%)
Chronic illness (first definition)		
No	5418 (81.9%)	5256 (82.4%)
Yes	906 (13.7%)	856 (13.4%)
Unknown	289 (4.4%)	265 (4.2%)
Chronic illness (second definition)		
No	4115 (62.2%)	4007 (62.8%)
Yes	2228 (33.7%)	2125 (33.3%)
Unknown	270 (4.1%)	245 (3.8%)

Data are given as number of subjects (percentage).
HUNT2, the Nord-Trøndelag Health Study 2.

model 4 adjusted for chronic illness (second definition) in addition to the variables in model 2.

To address the issue of potential effect modification by existing chronic diseases, the association of serum 25(OH)D levels with all-cause mortality was evaluated in subgroups stratified by chronic illness (first and second definitions). To further address possible reverse association, we restricted the analyses to subjects without chronic diseases at baseline, and with additional exclusion of the first 3 years of follow-up.

For secondary analyses, based on assumption of missing at random, missing values ('unknown' in tables 1 and 2) in covariates BMI, daily smoker, alcohol consumption, physical activity, education, economic difficulties and chronic illness (first and second definitions) were imputed using multivariable chained imputation with fully conditional specification (command *mi impute chained* in Stata). Cox proportional hazards regression was executed on 10 imputed datasets to obtain 10 sets of coefficients and SEs, and the averaged estimates were given as inferential statistics.

All statistical analyses were performed with Stata/SE V.13.1 (College Station, Texas, USA).

RESULTS

Table 1 shows that the analysis cohort (n=6377) and the random subcohort (n=6613) had similar distributions of the covariates. The median follow-up time among the 6377 adults was 18.5 years, and during the study period 1539 subjects died and 26 emigrated out of the country.

As shown in table 2, the mean level of serum 25(OH)D in the analysis cohort was 47.3 nmol/L, with a lower level in the subjects with chronic illness (first definition) than those without chronic illness (45.2 vs 47.8 nmol/L respectively, p<0.001). In both the total cohort and the subgroup without chronic illness, multiple linear regression analysis revealed that older age, summer or fall season, more frequent alcohol consumption and higher level of physical activity were associated with higher 25(OH)D levels, whereas people with high BMI, current smoking and socioeconomic difficulties had lower levels

Table 2 Overall and chronic illness-stratified distributions of serum 25(OH)D level according to baseline covariates

	25(OH)D level (nmol/L)					
			Chronic illness (first definition)†			
	Total (n=6377)		No (n=5256)		Yes (n=856)	
	Mean	SD	Mean	SD	Mean	SD
Total	47.3	17.8	47.8	18.0	45.2	16.6
Age (years)						
<65	47.5	18.0	47.6	18.1	45.5	16.9
≥65	46.8***	17.0	48.5***	17.4	45.1	16.5
Sex						
Female	47.0	17.6	47.9	17.9	42.2	14.9
Male	47.7	18.0	47.6	18.0	48.4**	17.7
Season of blood draw						
Spring	44.2	16.9	44.5	17.1	47.2	15.2
Summer	53.7***	17.9	54.7***	17.9	50.1***	17.1
Fall	50.8***	17.6	51.8***	17.6	46.7***	16.3
Winter	43.0	17.1	42.9	17.1	43.0	17.2
Body mass index (kg/m^2)						
Normal/underweight (<25.0)	51.1	18.3	51.5	18.4	47.8	16.3
Overweight (25.0–29.9)	46.4***	17.1	46.4***	17.3	46.9	16.3
Obese (≥30.0)	41.0***	15.8	41.0***	15.7	40.7***	16.2
Unknown	37.2	16.0	37.8	16.2	36.6	16.7
Daily smoker						
Never	48.0	17.9	48.5	18.2	44.5	15.7
Former	48.8	17.3	49.1	17.1	47.9	18.2
Current	45.1***	17.6	45.6***	17.9	42.4**	15.0
Unknown	47.3	20.9	48.5	22.4	41.7	12.1
Alcohol consumption (times per month)						
Never	45.1	16.4	45.6	16.6	43.5	15.7
1–4	48.3***	18.1	48.4***	18.2	47.1	16.0
≥5	51.1***	19.4	51.0***	19.0	52.3**	23.0
Unknown	46.1	18.2	47.0	18.9	44.4	15.5
Physical activity						
Inactive or very low	44.6	16.8	45.0	17.1	43.3	15.3
Low	47.7*	17.7	47.4	17.6	50.5*	18.7
Moderate	50.2***	17.8	50.3***	17.9	48.1	17.0
High	52.2***	20.9	52.7***	21.1	47.7	16.7
Unknown	45.7	16.9	45.3	17.1	43.9	16.3
Education (years)						
<10	45.6	16.8	46.3	17.1	43.7	15.9
10–12	47.6*	18.3	47.4	18.2	48.6	18.4
≥13	50.0*	18.3	50.1	18.5	48.1	16.3
Unknown	43.4	15.7	44.2	15.8	43.7	15.7
Economic difficulties						
No	48.7	17.3	48.9	17.5	46.5	14.6
Yes	46.2**	18.4	46.4**	18.0	45.0	22.0
Unknown	45.8	17.9	46.6	18.7	44.6	16.3

Multiple linear regression analysis was used to compare the mean levels of 25(OH)D with the first category (reference) for each baseline covariate.
*p<0.05; **p<0.01; ***p<0.001.
†265 subjects with missing information on chronic illness (first definition) at baseline were excluded.
25(OH)D, 25-hydroxyvitamin D.

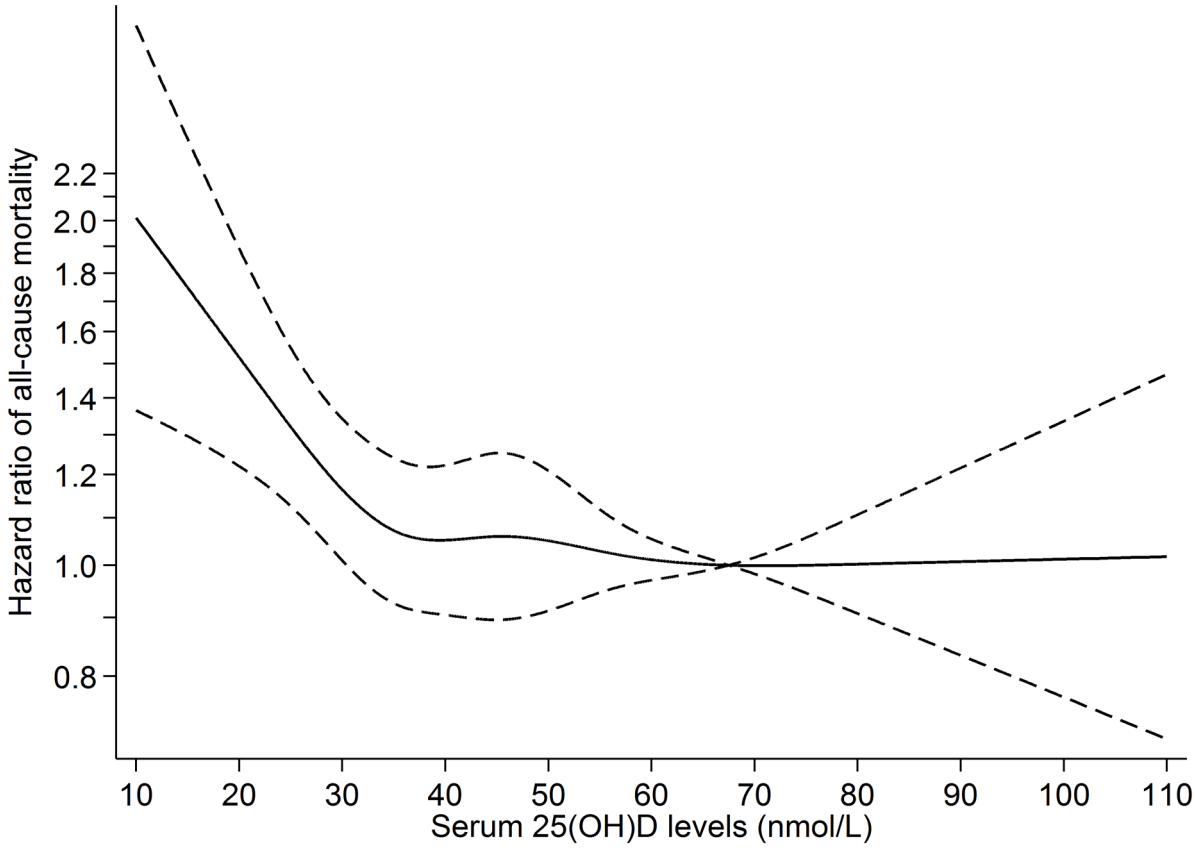

Figure 1 HR of all-cause mortality in association with continuous 25(OH)D levels by restricted cubic spline Cox regression analysis with five knots. Estimates were adjusted for season of blood draw, age, sex, body mass index, smoking, alcohol consumption, physical activity, education and economic difficulties, with 67.5 nmol/L as the reference value (median of the fourth quartile). 95% CIs are shown by dashed lines. 25(OH)D, 25-hydroxyvitamin D.

of 25(OH)D. In the subjects with chronic illness, high BMI and current smoking remained to be associated with lower 25(OH)D levels, while male sex, summer or fall season and more frequent alcohol consumption were associated with higher levels of 25(OH)D.

A non-linear association between serum 25(OH)D level and all-cause mortality was observed using restricted cubic spline Cox regression analysis (figure 1). It appeared that the mortality was minimised when the range of 25(OH)D was 60–100 nmol/L. There was a steady increase in the risk of death when 25(OH)D level was lower than 35 nmol/L (figure 1 and table 3).

Table 3 presents the HRs and 95% CIs for all-cause mortality in association with serum 25(OH)D levels by quartiles and cut-off points categories. In model 2, subjects with 25(OH)D in the first quartile (<34.5 nmol/L) showed an HR of 1.30 (95% CI 1.11 to 1.51) compared with those in the fourth quartile (≥58.1 nmol/L), while subjects with 25(OH)D <25.0 nmol/L showed an HR of 1.45 (95% CI 1.18 to 1.78) compared with those with 25(OH)D of 50.0–74.9 nmol/L. After adjustment for chronic illness (first definition) in model 3, HRs for the first quartile level and 25(OH)D <25.0 nmol/L changed to 1.27 and 1.41, respectively. After adjustment for chronic illness (second definition) in model 4, the corresponding HRs were slightly changed (1.30 and 1.46, respectively). In addition to the covariates in model 3, we further included systolic and diastolic blood pressures, and serum levels of total cholesterol, high-density lipoprotein and triglycerides, significant associations remained with HRs being 1.24 (95% CI 1.06 to 1.46) for the first quartile level and 1.38 (95% CI 1.12 to 1.71) for 25(OH)D level <25.0 nmol/L.

To address the possible effect modification by chronic diseases, we evaluated the association of serum 25(OH)D levels with all-cause mortality stratified by chronic illness at baseline (table 4). The HRs for all-cause mortality associated with the first quartile level were 1.32 in the subjects without chronic illness (first definition) and 1.35 in those with chronic illness, and the corresponding HRs associated with 25(OH)D <25.0 nmol/L were 1.24 and 1.63, respectively. Stratification by chronic illness (second definition) only showed significant associations in the group with chronic diseases. However, likelihood ratio tests did not provide evidence for any effect modification by chronic illness (p>0.28 for all).

Finally, to further address potential reverse association, we restricted our analyses to subjects without chronic illness (first definition) and with further exclusion of the

Table 3 The association of 25(OH)D level with all-cause mortality in different models (n=6377)

	Number of subjects/death	Time at risk (PY)	Rate (1000 PY)	Model 1		Model 2		Model 3		Model 4	
				HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI
25(OH)D level quartiles (nmol/L)											
First (<34.5)	1610/418	26358	15.9	1.32	(1.14 to 1.53)	1.30	(1.11 to 1.51)	1.27	(1.09 to 1.48)	1.30	(1.11 to 1.51)
Second (34.5–45.1)	1580/384	26291	14.6	1.18	(1.02 to 1.36)	0.97	(0.83 to 1.13)	0.94	(0.81 to 1.09)	0.97	(0.84 to 1.13)
Third (45.2–58.0)	1603/386	27044	14.3	1.12	(0.97 to 1.29)	1.08	(0.94 to 1.25)	1.06	(0.92 to 1.23)	1.09	(0.94 to 1.26)
Fourth (≥58.1)	1584/351	26876	13.1	1.00		1.00		1.00		1.00	
25(OH)D level (nmol/L)											
<25.0	479/122	7797	15.6	1.31	(1.07 to 1.60)	1.45	(1.18 to 1.78)	1.41	(1.14 to 1.74)	1.46	(1.18 to 1.80)
25.0–49.9	3340/845	55324	15.3	1.23	(1.09 to 1.37)	1.07	(0.95 to 1.20)	1.05	(0.93 to 1.17)	1.07	(0.96 to 1.21)
50.0–74.9	2128/472	36178	13.0	1.00		1.00		1.00		1.00	
≥75.0	430/100	7270	13.8	1.04	(0.84 to 1.29)	1.08	(0.87 to 1.35)	1.08	(0.86 to 1.34)	1.08	(0.87 to 1.34)

Model 1 adjusted for season of blood draw; model 2 adjusted for age, sex, body mass index, smoking, alcohol consumption, physical activity, education and economic difficulties in addition to model 1; model 3 adjusted for chronic illness (first definition) at baseline in addition to model 2; model 4 adjusted for chronic illness (second definition) at baseline in addition to model 2.
25(OH)D, 25-hydroxyvitamin D; PY, person-years.

first 3 years of follow-up (table 5). The first quartile level of serum 25(OH)D was associated with a 34% increased risk of all-cause mortality compared with the highest quartile level. Subjects with 25(OH)D level <25.0 nmol/L had a 29% increased risk of all-cause mortality compared with those with level of 50.0–74.9 nmol/L.

In line with the primary analyses, secondary analyses based on the imputed data for missing values of covariates including chronic illness at baseline produced similar results (see online supplementary tables 1–3).

DISCUSSION

Main findings

In this prospective study of 6377 subjects with a median follow-up period of 18.5 years, we found that the lowest 25(OH)D quartile level (<34.5 nmol/L) had a 30% increased risk of all-cause mortality compared with the fourth quartile (≥58.1 nmol/L) before adjustment for chronic illness at baseline. The subjects with 25(OH)D <25.0 nmol/L had a 45% increase in all-cause mortality compared with those with 25(OH)D of 50.0–74.9 nmol/L. The associations were not significantly confounded or modified by chronic diseases at baseline.

Comparison with other studies

Our findings of the association of 25(OH)D with all-cause mortality concurred with those of the meta-analysis studies .[5 10–15] For the non-linear association between serum 25(OH)D level and all-cause mortality (figure 1), the range of 25(OH)D for the lowest mortality in our study was similar to that in a meta-analysis study using individual participant data and standardised vitamin D levels.[13] The cut-off level of 25(OH)D for a steady increase in the risk of mortality was 35 nmol/L in our study, while it was around 40 nmol/L in this meta-analysis[13]. Compared with the 45% increased risk of all-cause mortality associated with 25(OH)D level <25 nmol/L observed in our study, a meta-analysis showed a 90% increased risk in association with 25(OH)D level <25 nmol/L after adjustment for age only.[11] Another meta-analysis study showed a 50% increased risk of all-cause mortality in subjects with 25(OH)D level <25 nmol/L.[5]

Our findings of an increased risk of all-cause mortality associated with low 25(OH)D levels were not substantially affected by having chronic diseases at baseline. This observation complies with a previous study in which no major changes in the association measures were reported after controlling for histories of chronic diseases.[21] Two meta-analysis studies including individual participant data came to the same conclusion that additional adjustment for a history of chronic diseases did not alter the association of low vitamin D with increased mortality.[12 13]Chronic diseases included in the aforementioned studies were similar to the ones in our study, namely cardiovascular disease, diabetes and cancer.[12 13 21] We also observed a similar association of low 25(OH)D levels with all-cause mortality in individuals with or without chronic illness

Table 4 The association of 25(OH)D level with all-cause mortality stratified by chronic illness in model 2

	25(OH)D nmol/L quartiles first versus fourth (reference)			25(OH)D nmol/L <25.0 vs 50.0–74.9 (reference)		
	HR	95% CI	p for interaction	HR	95% CI	p for interaction
Chronic illness (first definition)*			0.91			0.59
No (n=5256)	1.32	(1.08 to 1.62)		1.24	(0.92 to 1.67)	
Yes (n=856)	1.35	(1.04 to 1.75)		1.63	(1.17 to 2.28)	
Chronic illness (second definition)†			0.53			0.28
No (n=4007)	1.12	(0.86 to 1.46)		1.05	(0.70 to 1.56)	
Yes (n=2125)	1.36	(1.12 to 1.66)		1.58	(1.21 to 2.05)	

Model 2 adjusted for season of blood draw, age, sex, body mass index, smoking, alcohol consumption, physical activity, education and economic difficulties.
*265 subjects with missing information on chronic illness (first definition) at baseline were excluded.
†245 subjects with missing information on chronic illness (second definition) at baseline were excluded.
25(OH)D, 25-hydroxyvitamin D.

using two different definitions. A previous research reported stronger associations in subjects without cardiovascular disease, diabetes or hypertension than those with these chronic diseases.[22]

Possible mechanisms

The association between low 25(OH) levels and increased all-cause mortality can be explained by several possible mechanisms. First, vitamin D synthesis enzymes and vitamin D receptors are present in many tissues, implying a major role of vitamin D in many physiological and pathological processes.[6 7] Second, vitamin D has an important role in the regulation of proliferation, apoptosis and differentiation in many cell types, as well as functions of the immune system.[8 23] Third, epidemiological studies have suggested low vitamin D level as a risk factor for a wide range of diseases from hip fractures to cardiovascular disease and cancers.[3–5]

Recent research has attempted to study if there is a causal relationship between low vitamin D and all-cause mortality. A meta-analysis of Mendelian randomisation using four genetic variants around *DHCR7* and *CYP2R1* as instrumental variables suggested a causal effect of low levels of vitamin D on high all-cause mortality.[10] In two meta-analysis studies of randomised controlled trials of relative small sample sizes, vitamin D_3 supplementation reduced all-cause mortality by 6%–11% in elderly people.[5 24] Results from ongoing large clinical trials[25 26] are awaited to clarify the causal association of vitamin D with mortality, particularly in those with low vitamin D status prior to intervention.

Strengths and limitations

Selection bias seems not a big issue in our study since the analysis cohort is very similar to the subcohort of a random sample. However, non-participation in the later HUNT3 study was associated with lower socioeconomic status and higher mortality.[27] Non-participation in HUNT2 presented similar problems but to a less extent, which may influence the generalisability of our findings.

Table 5 The association of 25(OH)D level with all-cause mortality in subjects without chronic illness (first definition) in model 2, with further exclusion of the first 3 years of follow-up (n=5184)

	Number of subjects/death	Time at risk (PY)	Rate (1000 PY)	HR	95% CI
25(OH)D level quartiles (nmol/L)					
First (<34.5)	1280/199	18674	10.7	1.34	(1.09 to 1.66)
Second (34.5–45.1)	1261/180	18660	9.6	0.94	(0.76 to 1.16)
Third (45.2–58.0)	1310/211	19388	10.9	1.11	(0.91 to 1.36)
Fourth (≥58.1)	1333/198	19732	10.0	1.00	
25(OH)D level (nmol/L)					
<25.0	381/51	5607	9.1	1.29	(0.95 to 1.76)
25.0–49.9	2663/416	39136	10.6	1.05	(0.90 to 1.24)
50.0–74.9	1778/266	26372	10.1	1.00	
≥75.0	362/55	5339	10.3	1.04	(0.77 to 1.40)

Model 2 adjusted for season of blood draw, age, sex, body mass index, smoking, alcohol consumption, physical activity, education and economic difficulties.
25(OH)D, 25-hydroxyvitamin D; PY, person-years.

Serum 25(OH)D provides the most proper assessment of vitamin D status due to its longer half-life time and higher concentrations compared with the physiologically active metabolite 1,25-dihydroxyvitamin D.[28] One would argue that the one-time measurement of 25(OH)D level at baseline may not reflect long-term exposure of low vitamin D. Previous studies including a Norwegian study showed that 25(OH)D concentrations were rather stable up to 14 years of follow-up.[29 30] In addition, any misclassification of vitamin D due to measurement error would be non-differential as blood samples had been collected before the events occurred. Liaison immunoassay method tends to underestimate the true 25(OH)D levels.[31] Thus, caution is warranted when our results are compared with studies using other assay methods or standardised 25(OH)D levels.[13 32] Information about all-cause death of the Norwegian population is recorded and updated continuously at the Norwegian National Registry. The information is complete and accurate and therefore misclassification is unlikely.[33]

A wide spectrum of potential confounders including chronic diseases was adjusted in the current study. Chronic diseases, on one hand, may lead to both low vitamin D levels and increased mortality.[34] On the other hand, low vitamin D levels may lead to development of chronic diseases and subsequently increased mortality. Thus, chronic diseases may be either potential confounders or mediators in the causal pathway between low 25(OH)D status and all-cause mortality. No matter chronic diseases serve as a confounder or mediator, additional adjustment for this variable in model 3 and model 4 did not alter the association of 25(OH)D levels with all-cause mortality substantially. However, overadjustment may be possible if chronic diseases were in the pathway. Neither did we find significant effect modification by chronic illness defined by two definitions. Possibility of reverse association between low vitamin D and chronic diseases has been a main concern in the assessment of the vitamin D and mortality association.[16] Nevertheless, the association remained when we restricted the analysis in the subjects without chronic diseases at baseline and with further exclusion of the first 3 years of follow-up. In agreement with a previous study,[35] our results suggest that low vitamin D level is an important risk factor for all-cause mortality independent of ill health at baseline.

Apart from the limitations, our study is among the few to highlight and thoroughly investigate the potential influences of chronic diseases on the association of low vitamin D with all-cause mortality.

Conclusions

Overall, we found that low serum 25(OH)D level was associated with an increased risk of all-cause mortality in a general Norwegian population. The association was not notably influenced by existing chronic diseases. Results from the ongoing large clinical trials are being awaited to clarify a causal relationship.

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Competing interests None declared.

Patient consent All participants gave their informed consent on participation in HUNT, linkage to previous HUNT surveys and specific registries.

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