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Serum 25-hydroxyvitamin D, frailty, and mortality among the Chinese oldest old: results from the CLHLS study

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Abstract

Background and Aims: To explore whether frailty status modified the associations of serum 25(OH)D levels with all-cause mortality and cause-specific mortality in the oldest old Chinese.

Methods and Results: 1411 participants aged at least 80 were enrolled from on the Chinese Longitudinal Healthy Longevity Survey (CLHLS). Information on serum 25(OH)D level, frailty status, and covariates were examined at baseline. All-cause mortality and cause-specific mortality status were ascertained during the follow-up survey in 2017-2018 by ICD-10 codes. Cox proportional hazard models with stratified analyses were performed to evaluate potential associations. Over a median follow-up of 3.2 years, 722 (51.2%) participants deceased, including 202 deaths due to circulatory diseases and 520 deaths due to non-circulatory causes. After multivariable adjustment, both the lowest quartile of serum 25(OH)D levels (Hazard Ratios (95% Confidence Intervals), 1.85 (1.45, 2.36), 1.85 (1.45, 2.36), 1.73 (1.31, 2.29), respectively) and frailty (Odd Ratios (95% Confidence Intervals), 1.91 (1.60, 2.29), 2.67 (1.90, 3.74), 1.64 (1.31, 2.05)) were associated with significantly higher risk of all-cause mortality, circulatory mortality and non-circulatory mortality. In addition, significant interactions among 25(OH)D and frailty on all-cause mortality and cause-specific mortality were observed (all *P*-interaction < 0.001). Similar results were found in sensitivity analyses by excluding participants who died in the first year of follow-up and using clinical cut-offs of serum 25(OH)D levels.

Conclusion: Low serum 25(OH)D levels were associated with higher risk of all-cause mortality and cause-specific mortality among the Chinese oldest old, and the associations were significantly stronger in individuals with frailty.

Key words: serum 25(OH)D, frailty, mortality, oldest old

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Introduction

Vitamin D, also known as ‘sunshine vitamin’ and ‘antirachitic vitamin’, is a group of fat-soluble steroids which is the essential for life [1]. Vitamin D has a significant role in calcium homeostasis and metabolism but recent studies found the extra skeletal effects the cardiovascular system and malignancy [2-4]. Therefore, serum 25(OH)D concentration, an important compound of vitamin D in human body, has become heated discussion. vitamin D deficiency was associated with adverse outcomes including Coronary artery disease [5], hypertension [6], insulin resistance [7], cognitive impairment [8], and all-cause mortality [9].

Previous epidemiological research found that vitamin D deficiency was general in China, particular in the elderly. Lu et al. found that among the elderly aged 50 – 70 in Shanghai and Beijing, the rates of vitamin D deficiency and insufficiency were 24. 4% and 69. 2%, respectively [10]. Other studies found that vitamin D deficiency rates were over 80% in the elderly [11], indicating age was positively associated with vitamin D deficiency.

With the increasing life expectancy frailty has become a common health problem which may lead to adverse health outcomes such as falls, disabilities, depression, and the worse quality of life [12]. Previous Meta-analyses [13,14] have suggested an inverse association between serum vitamin D level and frailty, the pooled odd ratio of frailty for the lowest versus the highest level of vitamin D was 1.27 (95% confidence interval (CI)= 1.17, 1.38)), and frailty will further increased risk of all-cause mortality pooled hazard ratio (HR) = 1.35 (95% CI 1.05–1.74).

As suggested by previous studies, vitamin D deficiency significantly affects the health of the elder and further increases the risk of mortality, and the association may be more profound for people with frailty. However, few studies have evaluated the interaction between vitamin D status and frailty, especially in the oldest old Chinese, who are

susceptible to the adverse effect of vitamin D deficiency. Therefore, the purpose of this study was to determine the relationship between vitamin D status and all-cause mortality and cause-specific mortality, and whether this relationship interacted with frailty status using data from the Chinese Longitudinal Healthy Longevity Survey (CLHLS).

Method

Study design and population

CLHLS is an ongoing prospective community-based study with a multistage cluster sampling approach. More details of this study have been published elsewhere [15]. A total of 2546 elder participants have provided blood sample tests in the 7th wave (2014) of CLHLS. Then we excluded 925 participants because they were younger than 80 years (n = 818), then we excluded people with missing serum 25(OH)D values (n = 49), missing data for defining frailty (n=66) or had incorrect death date record (n = 6), leaving 1621 oldest old participants, of which follow-up status was obtained from 1411 of them (**Figure 1**). We compared the baseline characteristics of the participants who stayed or lost from the cohort, and there has been no significant difference in baseline variables (**Table S1**). All participants or their legal representatives signed written consent forms to participate in the baseline and follow-up survey.

Measurement of serum 25(OH) D

Fasting venous blood was collected after an overnight fast from participants. Procedures for the collection and shipment of blood samples were described in detail elsewhere [16]. Serum 25(OH)D was assayed by an enzyme-linked immunoassay using Immunodiagnostic Systems Limited (Bolton, UK). The 25(OH)D level was expressed as nmol/L and further divided into 4 groups by sex-specific quartiles.

Definition of frailty

Frailty was defined by the osteoporotic fractures index, which had good biological age ability among Chinese elderly [17]. Three components were included in the index: underweight (defined as body mass index < 18.5), low energy level (indicated by a positive response to the question "Over the last 6 months, have you been limited in activities because of a health problem?"), and muscle strength (inability to stand up from a chair without the assistance of arms). Participants were classified as having frailty with two or more of the three components [18].

Assessment of covariates

We further collected sociodemographic variables, health characteristics and the levels of biomarkers in the study. Sociodemographic variables included age, sex (male/female), economic income (high/medium/low), residence (rural/other), marital status (in marriage/other), education level (more than 1 year of schooling/other), frailty(yes/no), co-residence (live alone/other), vitamin supplements (almost every day/occasionally/rarely or never). Health characteristics included current smoking practice (yes/no), alcohol consumption habits (yes/no), systolic blood pressure (mmHg), diastolic blood pressure (mmHg), body mass index (kg/m^2) and self-reported hypertension, diabetes mellitus, stroke and other cerebrovascular diseases, cardiovascular disease, respiratory disease, and cancer. Levels of biomarkers included total cholesterol (TC, mmol/L), creatinine (mmol/L) and albumin (g/L). Economic income was classified as "high", "medium" and "low" by the question "Compared with other locals, how do you think about your economic position?" Participants who indicated "yes" to the questions "Do you currently smoke?" and "Do you currently drink alcohol?" were defined as current smokers and alcohol drinkers, respectively.

Outcomes

Participants' survival status (all-cause mortality, circulatory mortality and non-circulatory mortality) was ascertained during the follow-up survey in 2017-2018. Circulatory mortality was ascertained by ICD-10 (international classification of

diseases, 10th revision) codes of I00-I99.

Statistical analysis

Baseline characteristics were presented as mean (continuous variables) or on a frequency distribution (categorical variables). One-way ANOVA and chi-square tests were applied to compare the differences among serum 25(OH)D quartiles where appropriate. We used Cox proportional hazard model to investigate the relationship between serum 25(OH)D and all-cause and cause-specific mortality (highest quartile as reference or per 1 standard error decrease). Kaplan-Meier curves and log-rank tests were performed to compare the differences among quartiles of serum 25(OH)D. Three regression models were fitted, model 1 was adjusted for no confounders, model 2 was adjusted for age and gender, model 3 was adjusted for all confounders. We further evaluated the relationship between serum 25(OH)D and frailty and the relationship between frailty and all-cause and cause-specific mortality, using logistic regression and cox regression respectively.

To assess the joint associations between serum 25(OH)D, frailty and mortality, we calculated the fully adjusted hazard ratios (HRs) and 95% confident intervals (95% CI) of lower quantiles compared with highest quintiles of serum 25(OH)D, stratified by frailty status, and *P*-interaction were calculated. We also classified participants according to quartiles for serum 25(OH)D and frailty status for Cox regression analysis, using non-frail participants with the highest quartile of serum 25(OH)D as referent.

We further performed the following sensitivity analyses to assess the robustness of our results: (1) excluding participants who died in the first year; (2) including the participants who loss to follow-up (n=210); (3) using the clinically defined cutoffs of serum 25(OH)D (25, 50, 75 nmol/L) instead of quartiles. All analyses mentioned above were performed using R 4.0.0 (R Foundation for Statistical Computing). A two-tailed p-value < 0.05 was considered statistically significant in all analyses.

Results

Baseline characteristics

We included 1411 oldest old participants in the analysis. **Table 1** presented the baseline characteristics among overall participants and data categorized by quartiles of serum 25(OH)D. In brief, the mean age of study participants was 92.26 years and 62.4% of those were female. The average 25(OH)D concentration of participants was 38.29 nmol/L and 33.3% (n=470) of them were classified as having frailty. Participants with a lower level of serum 25(OH)D tended to be older, live in urban area and live alone, being classified as having frailty, less prevalent in taking vitamin supplements, have lower BMI and albumin level. The distributions of baseline serum 25(OH)D in male and female were depicted in **Figure S1**. Additionally, we provided the cut-off value of sex-specific quartiles of serum 25(OH)D, as shown in **Table S2**. The fourth quartile of 25(OH)D (Q4) was 55.95 nmol/L in men and 42.40 nmol/L in women, while the first quartiles were 30.45 and 21.70 nmol/L in men and women, respectively.

Serum 25(OH)D and frailty

In the cross-sectional analysis, we found a significant inverse association between serum 25(OH)D level and the risk of frailty. Individuals in the first (Q1) and second (Q2) quartiles had higher risk of frailty compared with the highest level (Q4). These associations remained significant after adjustment of multiple potential confounders, the Model 3 HRs were 2.91 (95%CI, 1.95-4.36) for Q1 and 1.56 (1.04-2.35) for Q2 in the final model (**Table S3**).

Serum 25(OH)D and mortality

Over a median follow-up period of 3.2 years (IQR, 1.7-3.5 years), 722 (51.2%) participants died, including 202 deaths due to circulatory diseases and 520 deaths due

to non-circulatory causes.

Figure S2 depicted the Kaplan-Meier survival curves for all-cause mortality (A), circulatory mortality (B), and non-circulatory mortality (C) by the quartiles of serum 25(OH)D. The cumulative incidence of all-cause and cause-specific mortality significantly differ by the quartiles of serum 25(OH)D as demonstrated from log-rank tests ($P < 0.001$).

Table 2 presented the association between serum 25(OH)D and mortality. In the fully adjusted model, each SD decrease in 25(OH)D was associated with 29% (95%CI, 17%-43%) increment of the risk of all-cause mortality, 53% (24%-88%) increment of the risk of circulatory mortality, and 23% (10%-39%) increased risk of non-circulatory mortality. Compared with the highest quartile (Q4) of serum 25(OH)D, the multivariable-adjusted HRs (Model 3) for all-cause mortality were 1.51 (95%CI, 1.18-1.94) for Q2 and 1.85 (1.45-2.36) for Q1. Similarly, the HRs for circulatory mortality for Q2 and Q1 were 1.64 (1.01-2.67) and 2.31 (1.44-3.71), respectively, while HRs for non-circulatory mortality for Q2 and Q1 were 1.50 (1.13-2.00) and 1.73 (1.31-2.29), respectively. The relationships between serum 25(OH)D levels and all-cause and cause-specific mortality remained consistent in a sensitivity analysis that excluding participants who died in the first years of follow-up (**Table S4**). Similarly, after including the participants who lost to follow-up and using the clinically defined cutoffs of serum 25(OH)D did not have substantial influence on these findings (**Table S5, S6**).

Association between serum 25(OH)D and mortality stratify by frailty status

Table S7 showed the associations between frailty status and all-cause mortality and cause-specific mortality. In the fully adjusted model, the presence of frailty was significantly associated with 91%, 167%, and 64% higher risk of all-cause mortality,

circulatory mortality and non- circulatory mortality, respectively.

Table 3 showed the association between serum 25(OH)D and mortality stratified by frailty status. Participants in the highest quartile of 25(OH)D were the reference group. There was a significant interaction between serum 25(OH)D and frailty with all-cause, circulatory and non-circulatory mortality (all $P < 0.001$). The lowest quartile of 25(OH)D significantly associated with a higher risk of all-cause mortality in both frail (HR: 2.01, 95% CI: 1.34, 3.01) and non-frail (HR: 1.86, 95% CI: 1.35, 2.57) participants, but the associations were more pronounced in participants with frailty (all *P*-interaction < 0.001). Similar findings were observed when using circulatory or non-circulatory mortality as outcomes.

Figure 2 displayed these data with non-frail individuals in the highest quartile of 25(OH)D as the reference group. Participants in the lowest quartile of 25(OH)D and with frailty had about three times higher risk (HR: 3.36, 95% CI: 2.49,4.52) of all-cause mortality (A), five times higher risk (HR: 5.41, 95% CI: 3.01, 9.79) of circulatory mortality (B), and three times higher risk (HR: 2.67, 95% CI: 1.88, 3.80) of non-circulatory mortality (C) comparing to the reference group.

DISCUSSION

In the present prospective community-based study among the oldest old in China, the association of serum 25(OH)D with mortality (all-cause and cause-specific) was significantly moderated by frailty. The inverse relationship between serum vitamin D and mortality was stronger in frail participants compared to those without frailty, and the results remained similar in sensitivity analysis.

Consistent with previous studies [19-22], we found an inverse relationship between serum

25(OH)D levels and all-cause mortality and circulatory mortality. The similar relationships were reported in different populations such as the Chinese elderly [19], the US residents [21,22], and the Israelis [20], which supported the conclusion of a recent meta-analysis including more than twenty-five thousand participants that the inverse relationships between serum 25(OH)D levels and all-cause mortality and cause-specific mortality for both men and women were remarkedly consistent across various countries [23]. However, different relationships, such as reverse J-shaped and U-shaped associations between serum 25(OH)D and all-cause mortality, were demonstrated in other studies [24-26]. A systematic review that included 26 cross-sectional or longitudinal observational studies has suggested an inverse association between serum 25(OH)D concentration and frailty severity among participants over 60 years old [27]. A mendelian randomization analysis revealed a reverse association between serum 25(OH)D with all-cause mortality, cancer mortality, and other mortality but not with increased cardiovascular mortality [28], indicating that these relationships may interacted by age, race and physical conditions. Further well-designed longitudinal studies were needed to clarify the relationship and the feasible mechanism between serum 25(OH)D with all-cause and cause-specific mortality.

Few studies have demonstrated the interaction between serum 25(OH)D and frailty in their associations with all-cause mortality and cause-specific mortality. In the KORA study (Cooperative Health Research in the Region of Augsburg-Age Study) including 727 participants aged 65 or older, lower 25(OH)D levels were significantly associated with increased all-cause mortality and the association (OR=3.39, 95% CI: 1.08, 10.65) , which was partly mediated by frailty status [29]. Similarly, among 4731 adult participants from the Third National Health and Nutrition Examination Survey, those who were frail and had low serum 25(OH)D levels had higher risk (OR = 1.67, 95% CI: 1.00, 2.82) of all-cause mortality compared with those who had high serum 25(OH)D levels [30]. Flicker.et al [31] found that the inverse association between vitamin D and all-cause mortality (HR = 1.20, 95% CI, 1.02, 1.42) independent of frailty among 4203 men aged

70-88 years from Western Australia. In the present study, participants were older (mean age: 92.26 years) and were from Chinese communities, which might explain the differential effect of frailty on the association between serum 25(OH)D with all-cause mortality in previous studies. In addition, the present study added evidence that frailty significantly affected the association of with serum 25(OH)D levels and circulatory mortality and non-circulatory.

Higher mortality risk in individuals with frailty combined with low serum 25(OH)D levels, and multiple mechanisms might help to explain this relationship. Firstly, vitamin D plays a crucial role in skeletal muscle function, it may exert its effects on muscles by genomic pathway, deficiency of vitamin D may decrease the gene transcription of mRNA and subsequent protein synthesis and further influence the differentiation of skeletal muscles [32]. In addition, vitamin D deficiency may lead to decrease the concentration of vitamin D receptor, which results in type II muscle fiber atrophy [33]. Individuals, in particular elders, are more likely to be frailty, which increase their risk of falling down and exacerbate their frailty status due to they have to stay in bed and be less capable to go outside for sunlight exposure, further decrease the vitamin D synthesis [34,35]. Secondly, h vitamin D deficiency may confer increased cardiovascular risk include the development of electrolyte imbalances, pancreatic b-cell dysfunction, and RAS activation [36], Moreover, disrupted adaptive immune responses with severe vitamin D deficiency result in an inflammatory milieu that promotes vascular dysfunction and insulin resistance [37], both of which lead to negative cardiovascular effects while the frailty status was more likely to increase the risk of non-cardiovascular death.

Several limitations need to be noted when interpreting these findings. First, vitamin D and frailty were both examined at baseline, the possibility of reverse causality cannot be fully excluded in our study. Second, some factors (e.g. calcium and parathyroid

hormone) that might influence vitamin D metabolism were not collected in the CLHLS. Furthermore, data on the types of vitamin D supplementation of participants were not available in the study. However, the rate of habitual intake of vitamin D supplements was very low in the Chinese older adults and it might have little influence on the estimation of vitamin D levels in the present study [38]. Third, the follow-up time was relatively short (3.2 years). However, our study included the oldest old population which had a median age of 92.26 years with a high mortality rate (51.2%). Therefore, the study had sufficient statistical power to examine the relationship between vitamin D levels and mortality risk, and to determine the interaction effect of frailty status. Fourth, status of serum vitamin D and frailty was obtained only once at baseline, therefore the changes across in cohort were not clear. However, we argue if that is a major flaw of this study given the short follow-up time. Finally, although participants of the CLHLS were the national representative of Chinese populations, extrapolating the findings of our study to geographically or ethnically different populations should be cautious.

Conclusion

In conclusion, our study suggested that the associations between low serum vitamin 25(OH)D and mortality were more pronounced in frail participants among a community of oldest old in China. Our study highlighted the importance of frailty screening among the oldest old, as it might improve the ability to identify individuals who could benefit most from improved vitamin D status.

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Conflicts of interest

The authors declared no potential conflicts of interest.

Author contributions

Y.F., Y.H., L.L., C.C., and J.H. designed the research study. L.L. and C.C. analyzed the data and performed statistical analysis. L.L., C.C., J.H., K.L., and Y.Y. wrote the paper. Y.F. and Y.H. had primary responsibility for the final content. All authors read and approved the final manuscript.

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Table 1. Baseline characteristics of study participants by quartiles of sex-specific serum 25(OH)D concentrations

	Overall	Q1	Q2	Q3	Q4	*P*-value
N	1411	353	350	354	354	
Age, years	92.26 ± 7.77	94.31 ± 7.56	92.46 ± 7.48	91.53 ± 7.76	90.76 ± 7.85	<0.001
Female	881 (62.4)	220 (62.3)	218 (62.3)	222 (62.7)	221 (62.4)	0.999
Current smoking practice	140 (10.0)	40 (11.4)	33 (9.5)	28 (8.0)	39 (11.1)	0.407
Alcohol consumption habits	155 (11.1)	49 (14.0)	37 (10.6)	39 (11.1)	30 (8.6)	0.164
Economic income						0.165
High	179 (12.8)	51 (14.5)	54 (15.6)	41 (11.6)	33 (9.4)	
Medium	1080 (77.0)	264 (75.2)	255 (73.5)	274 (77.6)	287 (81.8)	
Low	143 (10.2)	36 (10.3)	38 (11.0)	38 (10.8)	31 (8.8)	
Rural residents	1116 (79.1)	264 (74.8)	252 (72.0)	279 (78.8)	321 (90.7)	<0.001
In marriage	333 (23.9)	80 (23.1)	79 (22.7)	90 (25.6)	84 (24.3)	0.801
Regular exercise	161 (11.7)	32 (9.3)	46 (13.5)	37 (10.7)	46 (13.3)	0.250
Education, >1 year	299 (21.7)	78 (22.4)	72 (21.4)	78 (22.5)	71 (20.6)	0.913
Frailty (%)	470 (33.3)	183 (51.8)	115 (32.9)	94 (26.6)	78 (22.0)	<0.001
Live alone (%)	354 (25.4)	58 (16.6)	71 (20.5)	90 (25.9)	135 (38.7)	<0.001
Vitamin supplements						0.010
Almost everyday	36 (2.6)	5 (1.4)	6 (1.7)	10 (2.8)	15 (4.2)	
Occasionally	181 (12.9)	30 (8.5)	51 (14.7)	45 (12.7)	55 (15.5)	
Rarely or never	1190 (84.6)	317 (90.1)	291 (83.6)	298 (84.4)	284 (80.2)	
Systolic blood pressure, mmHg	144.24 ± 25.78	142.38 ± 30.61	145.71 ± 25.37	144.91 ± 23.07	143.97 ± 23.36	0.356
Diastolic blood pressure, mmHg	79.77 ± 14.66	79.51 ± 14.68	78.35 ± 12.43	79.82 ± 13.54	81.39 ± 17.39	0.052
Body mass index, kg/m²	19.82 ± 5.72	18.76 ± 6.24	20.27 ± 5.26	20.08 ± 5.56	20.18 ± 5.66	<0.001
Serum 25(OH)D, nmol/L	38.29 ± 20.23	18.03 ± 6.46	29.89 ± 5.74	40.86 ± 7.27	64.24 ± 18.57	<0.001

Total cholesterol, mmol/L	4.71 ± 1.03	4.76 ± 1.12	4.70 ± 1.08	4.65 ± 1.03	4.71 ± 0.90	0.571
Creatinine, mmol/L	84.80 ± 31.76	85.82 ± 40.05	87.86 ± 35.02	83.98 ± 26.91	81.59 ± 21.70	0.058
Albumin, g/L	41.60 ± 4.61	40.16 ± 4.23	41.48 ± 6.55	42.36 ± 3.27	42.39 ± 3.23	<0.001
Hypertension	386 (27.5)	89 (25.2)	86 (24.7)	96 (27.4)	115 (32.8)	0.067
Diabetes mellitus	29 (2.1)	8 (2.3)	8 (2.3)	8 (2.3)	5 (1.4)	0.811
Cardiovascular disease	127 (9.0)	33 (9.3)	34 (9.7)	27 (7.6)	33 (9.4)	0.770
Stroke and cerebrovascular disease	83 (5.9)	26 (7.4)	18 (5.2)	14 (4.0)	25 (7.1)	0.173
Respiratory disease	111 (7.9)	27 (7.6)	31 (8.9)	29 (8.2)	24 (6.8)	0.769
Cancer	6 (0.4)	0 (0.0)	0 (0.0)	4 (1.1)	2 (0.6)	0.062

Data are represented as mean (standard deviation) for continuous variables and n (%) for categorical variables. Q, quartiles.

Table 2. Cox-proportional hazard models for the association of serum 25(OH)D levels with all-cause and cause-specific mortality

	Case/total	Model 1	Model 2	Model 3
All-cause mortality				
Per SD decrease		1.5 (1.41, 1.69)	1.47 (1.34, 1.62)	1.29 (1.17, 1.43)
Quartiles				
Q4	252/353	Ref	ref	ref
Q3	193/350	1.23 (0.97, 1.56)	1.16 (0.92, 1.48)	1.08 (0.83, 1.39)
Q2	152/354	1.83 (1.46, 2.30)	1.73 (1.38, 2.17)	1.51 (1.18, 1.94)
Q1	125/354	2.77 (2.23, 3.44)	2.37 (1.90, 2.94)	1.85 (1.45, 2.36)
P-trend		< 0.001	< 0.001	< 0.001
Circulatory mortality				
Per SD decrease		1.76 (1.47, 2.11)	1.81 (1.5, 2.19)	1.53 (1.24, 1.88)
Quartiles				
Q4	79/353	ref	ref	ref
Q3	53/350	1.43 (0.88, 2.30)	1.37 (0.85, 2.21)	1.28 (0.77, 2.11)
Q2	41/354	2.15 (1.36, 3.38)	2.05 (1.30, 3.28)	1.64 (1.01, 2.67)
Q1	29/354	3.66 (2.39, 5.61)	3.26 (2.12, 5.01)	2.31 (1.44, 3.71)
P-trend		< 0.001	< 0.001	< 0.001
Non-circulatory mortality				
Per SD decrease		1.48 (1.33, 1.64)	1.37 (1.23, 1.53)	1.23 (1.10, 1.39)
Quartiles				
Q4	173/353	ref	ref	ref
Q3	140/350	1.17 (0.89, 1.54)	1.11 (0.84, 1.45)	1.02 (0.76, 1.38)
Q2	111/354	1.74 (1.34, 2.26)	1.64 (1.27, 2.13)	1.50 (1.13, 2.00)
Q1	96/354	2.50 (1.95, 3.21)	2.10 (1.63, 2.70)	1.73 (1.31, 2.29)
P-trend		< 0.001	< 0.001	< 0.001

Data presented hazard ratio (95% confident interval). SD, standard deviation; Q, quartiles.

Model 1 adjust for none.

Model 2 adjust for age and gender.

Model 3 adjust for age, gender, smoke, alcohol, economic income, rural residents, in marriage, regular exercise, education, live alone, systolic blood pressure, diastolic blood pressure, body mass index, hypertension, diabetes mellitus, cardiovascular disease, stroke and cerebrovascular disease, cancer, vitamin supplements, total cholesterol, creatinine, albumin and frailty.

Table 3. Cox-proportional hazard models for the associations of serum 25(OH)D levels with all-cause and cause-specific mortality by frailty status

	Case/total	Quartiles of serum 25(OH)D concentrations				Per SD decrease	*P*-trend	*P*-interaction
		Q4 (highest)	Q3	Q2	Q1 (lowest)			
All-cause mortality								
Frailty	350/470	ref	1.43 (0.92, 2.21)	1.62 (1.05, 2.49)	2.01 (1.34, 3.01)	1.32 (1.12, 1.55)	< 0.001	< 0.001
Non-frailty	372/941	ref	0.85 (0.61, 1.10)	1.38 (1.01, 1.89)	1.86 (1.35, 2.57)	1.28 (1.11, 1.47)	< 0.001	
Circulatory mortality								
Frailty	106/470	ref	2.06 (0.84, 5.05)	2.10 (0.87, 5.05)	2.83 (1.22, 6.56)	1.64 (1.18, 2.28)	< 0.001	< 0.001
Non-frailty	96/941	ref	0.90 (0.47, 1.71)	1.32 (0.70, 2.49)	2.58 (1.41, 4.75)	1.55 (1.16, 2.05)	< 0.001	
Non-circulatory mortality								
Frailty	244/470	ref	1.28 (0.77, 2.13)	1.58 (0.96, 2.60)	1.89 (1.19, 3.00)	1.25 (1.03, 1.52)	< 0.001	<0.001
Non-frailty	276/941	ref	0.85 (0.59, 1.24)	1.43 (0.99, 2.06)	1.68 (1.15, 2.45)	1.22 (1.04, 1.42)	< 0.001	

Data are presented as hazard ratio (95% confident interval). Q, quartiles; SD, standard deviation.

Analyses were adjusted for age, gender, smoke, alcohol, economic income, rural residents, in marriage, regular exercise, education, live alone, systolic blood pressure, diastolic blood pressure, body mass index, hypertension, diabetes mellitus, cardiovascular disease, stroke and cerebrovascular disease, cancer, vitamin supplements, total cholesterol, creatinine, and albumin.

Figure legends

Figure 1. Flow chart of study participants.

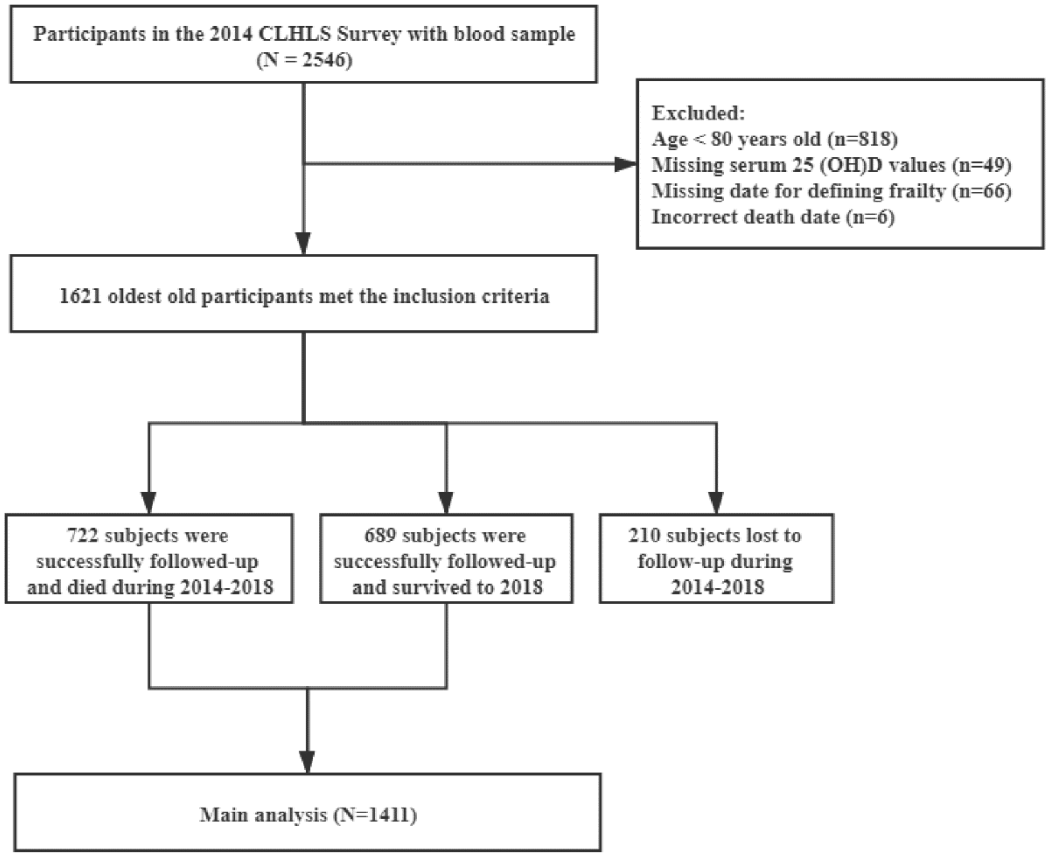
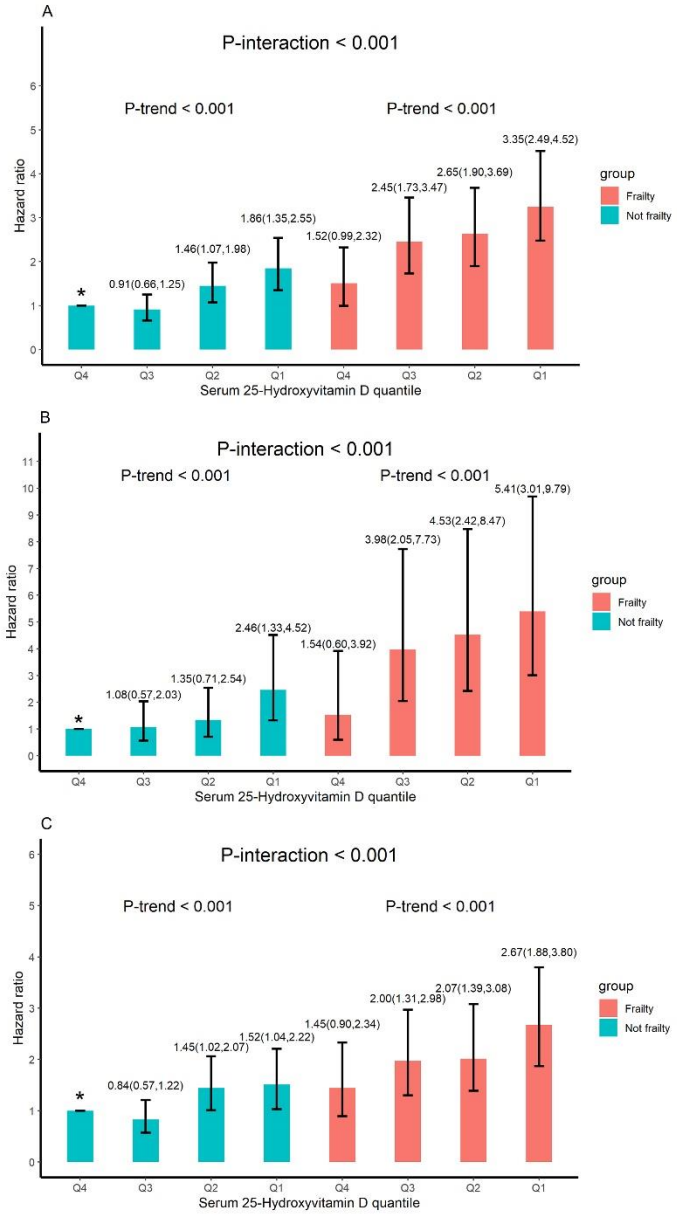


Figure 2. Association of serum 25(OH)D with all-cause mortality (A), circulatory mortality (B) and non - circulatory mortality (C) within frailty strata.



Data are presented as hazard with 95% confident interval, adjusting for age, gender, smoke, alcohol, economic income, rural residents, in marriage, regular exercise, education, live alone, systolic blood pressure, diastolic blood pressure, body mass index, hypertension, diabetes mellitus, cardiovascular disease, stroke and cerebrovascular disease, cancer, vitamin supplements, total cholesterol, creatinine, and albumin. Q4 represents individuals with the highest serum 25(OH)D level and Q1 represents individuals with the lowest. Individuals in the highest quartile of serum 25(OH)D and without frailty were used as the reference group (*). P-interaction describes the interaction between serum 25(OH)D levels and frailty status with all-cause and cause-specific mortality. P-trend describes the association of serum 25(OH)D levels with all-cause and cause-specific mortality within frailty strata.

Supplement material

Table S1. Comparison of the baseline Characteristics between participants who were enrolled or lost to follow up

	Enrolled	Lost to follow up	P-value
N	1411	210	
Age, years	92.26 ± 7.77	91.44 ± 7.36	0.15
Female	881 (62.4)	141 (67.1)	0.215
Current smoking practice	140 (10.0)	25 (12.0)	0.445
Alcohol consumption habits	155 (11.1)	20 (9.7)	0.616
Economic income			0.027
High	179 (12.8)	28 (13.4)	
Medium	1080 (77.0)	147 (70.3)	
Low	143 (10.2)	34 (16.3)	
Rural residents	1116 (79.1)	157 (74.8)	0.182
In marriage	333 (23.9)	39 (18.8)	0.126
Regular exercise	161 (11.7)	25 (12.1)	0.948
Education, >1 year	299 (21.7)	50 (25.9)	0.225
Frailty (%)	470 (33.3)	84 (40.0)	0.067
Live alone (%)	354 (25.4)	46 (22.1)	0.348
Vitamin supplements			0.965
Almost everyday	36 (2.6)	5 (2.4)	
Occasionally	181 (12.9)	28 (13.5)	
Rarely or never	1190 (84.6)	175 (84.1)	
Systolic blood pressure, mmHg	144.24 ± 25.78	145.05 ± 23.73	0.669
Diastolic blood pressure, mmHg	79.77 ± 14.66	80.17 ± 32.45	0.767
Body mass index, kg/m²	19.82 ± 5.72	18.77 ± 7.20	0.016
25(OH)D, nmol/L	38.29 ± 20.23	38.32 ± 17.56	0.984
Total cholesterol, mmol/L	4.71 ± 1.03	4.78 ± 1.02	0.327
Creatinine, mmol/L	84.80 ± 31.76	86.23 ± 33.75	0.548
Albumin, g/L	41.60 ± 4.61	40.77 ± 4.25	0.014
Hypertension	386 (27.5)	70 (34.0)	0.066
Diabetes mellitus	29 (2.1)	7 (3.3)	0.355
Cardiovascular disease	127 (9.0)	17 (8.1)	0.77
Stroke and cerebrovascular disease	83 (5.9)	14 (6.7)	0.768
Respiratory disease	111 (7.9)	16 (7.7)	1.000
Cancer	6 (0.4)	2 (1.0)	0.625

Data are represented as mean (standard deviation) for continuous variables and n (%) for categorical variables. Q, quartiles.

Table S2. Cut-off point for sex-specific serum 25(OH)D quintiles

Gender	serum 25(OH)D, nmol/L			
	Q4 (Highest)	Q3	Q2	Q1 (Lowest)
Men	≥ 55.95	42.45 - 55.95	30.45 - 42.45	< 30.45
Women	≥ 42.40	30.70 - 42.40	21.70 - 30.70	< 21.70

Q, quartiles.

Table S3. The association between serum 25(OH)D levels and frailty

	Odds ratio (95% confident interval)				
	Q4	Q3	Q2	Q1	*P*-trend
Model 1	ref	1.27 (0.90, 1.80)	1.73 (1.24, 2.42)	3.80 (2.75, 5.30)	<0.001
Model 2	ref	1.25 (0.87, 1.80)	1.61 (1.13, 2.31)	3.40 (2.40, 4.84)	<0.001
Model 3	ref	1.24 (0.83, 1.86)	1.56 (1.04, 2.35)	2.91 (1.95, 4.36)	<0.001

Model 1, adjusted for none.

model 2, adjusted for age and gender.

model 3, adjusted for age, gender, smoke, alcohol, economic income, rural residents, in marriage, regular exercise, education, live alone, systolic blood pressure, diastolic blood pressure, body mass index, hypertension, diabetes mellitus, cardiovascular disease, stroke and cerebrovascular disease, cancer, vitamin supplements, total cholesterol, creatinine, and albumin.

Table S4. Sensitivity analysis on association between serum 25(OH)D levels and mortality by excluding participants who died in the first year

	Case/total	HR (95% CI) *
All-cause mortality		
Per SD decrease		1.47 (1.32, 1.64)
Quartiles		
Q4	85/300	ref
Q3	113/305	1.20 (0.89,1.62)
Q2	130/294	1.47 (1.08, 1.99)
Q1	180/298	2.07 (1.55, 2.76)
P-trend		< 0.001
Circulatory mortality		
Per SD decrease		1.52 (1.23, 1.88)
Q4	20/300	ref
Q3	30/305	1.39 (0.77, 2.53)
Q2	36/294	1.67 (0.92, 3.04)
Q1	45/298	2.18 (1.21, 3.91)
P-trend		< 0.001
Non-circulatory mortality		
Per SD decrease		1.28 (1.12, 1.48)
Q4	65/300	ref
Q3	83/305	1.16 (0.82, 1.65)
Q2	94/294	1.40 (0.98, 1.99)
Q1	135/298	2.07 (1.48, 2.89)
P-trend		< 0.001

*Data are presented as HR (hazard ratio) with 95% CI (confident interval) after adjusting for age, gender, smoke, alcohol, economic income, rural residents, in marriage, regular exercise, education, live alone, systolic blood pressure, diastolic blood pressure, body mass index, hypertension, diabetes mellitus, cardiovascular disease, stroke and cerebrovascular disease, cancer, vitamin supplements, total cholesterol, creatinine, albumin and frailty.

Table S5. Sensitivity analysis on association between serum 25(OH)D levels and mortality by comprising the loss of follow-up participants (n=210)

	Case/total	HR (95%CI) *
All-cause mortality		
Per SD decrease		1.53 (1.40, 1.68)
Quartiles		
Q4	124/407	ref
Q3	148/404	1.10 (0.85, 1.42)
Q2	188/406	1.45 (1.13, 1.86)
Q1	262/404	1.89 (1.49, 2.41)
P-trend		< 0.001
Circulatory mortality		
Per SD decrease		1.74 (1.46, 2.08)
Quartiles		
Q4	29/407	ref
Q3	40/404	1.30 (0.79, 2.15)
Q2	52/406	1.57 (0.96, 2.56)
Q1	81/404	2.30 (1.44, 3.69)
P-trend		< 0.001
Non-circulatory mortality		
Per SD decrease		1.23 (1.09, 1.38)
Quartiles		
Q4	95/407	ref
Q3	108/404	1.05 (0.78, 1.41)
Q2	136/406	1.44 (1.08, 1.92)
Q1	181/404	1.79 (1.35, 2.37)
P-trend		< 0.001

*Data are presented as HR (hazard ratio) with 95% CI (confident interval) after adjusting for age, gender, smoke, alcohol, economic income, rural residents, in marriage, regular exercise, education, live alone, systolic blood pressure, diastolic blood pressure, body mass index, hypertension, diabetes mellitus, cardiovascular disease, stroke and cerebrovascular disease, cancer, vitamin supplements, total cholesterol, creatinine, albumin and frailty.

Table S6. Sensitivity analysis on association between serum 25(OH)D levels and mortality by using the clinically defined cutoffs

	All-cause mortality		Circulatory mortality		Non-circulatory mortality	
Serum 25(OH)D	Case/total	HR (95%CI) *	Case/total	HR (95%CI) *	Case/total	HR (95%CI) *
≥ 50 nmol/L	114/334	ref	29/334	ref	85/334	ref
< 50 nmol/L	608/1077	1.40 (1.12, 1.74)	173/1077	1.52 (1.00, 2.33)	435/1077	1.36 (1.05, 1.77)
≥ 50 nmol/L	114/334	ref	29/334	ref	85/334	ref
25-50 nmol/L	343/709	1.25 (1.00, 1.57)	94/709	1.31 (0.84, 2.04)	249/709	1.23 (0.94, 1.62)
< 25 nmol/L	265/368	1.89 (1.46, 2.44)	79/368	2.24 (1.38, 3.64)	166/368	1.78 (1.31, 2.41)
≥ 75 nmol/L	18/65	ref	4/65	ref	14/65	ref
50-75 nmol/L	96/269	1.29 (0.75, 2.21)	25/269	1.91 (0.57, 6.36)	71/269	1.15 (0.63, 2.11)
25-50 nmol/L	343/709	1.55 (0.93, 2.59)	94/709	2.29 (0.71, 7.32)	249/709	1.39 (0.78, 2.46)
< 25 nmol/L	265/368	2.35 (1.39, 3.97)	79/368	3.91 (1.2, 12.76)	186/368	2.00 (1.11, 3.61)

*Data are presented as HR (hazard ratio) with 95% CI (confident interval) after adjusting for age, gender, smoke, alcohol, economic income, rural residents, in marriage, regular exercise, education, live alone, systolic blood pressure, diastolic blood pressure, body mass index, hypertension, diabetes mellitus, cardiovascular disease, stroke and cerebrovascular disease, cancer, vitamin supplements, total cholesterol, creatinine, albumin and frailty.

Table S7. The associations between frailty and all-cause and cause-specific mortality

	Model 1	Model 2	Model 3
All-cause mortality			
Non-frailty	ref	ref	ref
Frailty	2.83 (2.44, 3.28)	2.28 (1.94, 2.67)	1.91 (1.60, 2.29)
Circulatory mortality			
Non-frailty	ref	ref	ref
Frailty	3.25 (2.46, 4.30)	3.13 (2.31, 4.24)	2.67 (1.90, 3.74)
Non-circulatory mortality			
Non-frailty	ref	ref	ref
Frailty	2.69 (2.26, 3.20)	2.02 (1.68, 2.44)	1.64 (1.31, 2.05)

Data are presented as hazard ratio (95% confident interval).

Model 1, adjusted for none.

Model 2, adjusted for age and gender.

Model 3, adjusted for age, gender, smoke, alcohol, economic income, rural residents, in marriage, regular exercise, education, live alone, systolic blood pressure, diastolic blood pressure, body mass index, hypertension, diabetes mellitus, cardiovascular disease, stroke and cerebrovascular disease, cancer, vitamin supplements, total cholesterol, creatinine, albumin, and serum 25(OH)D.

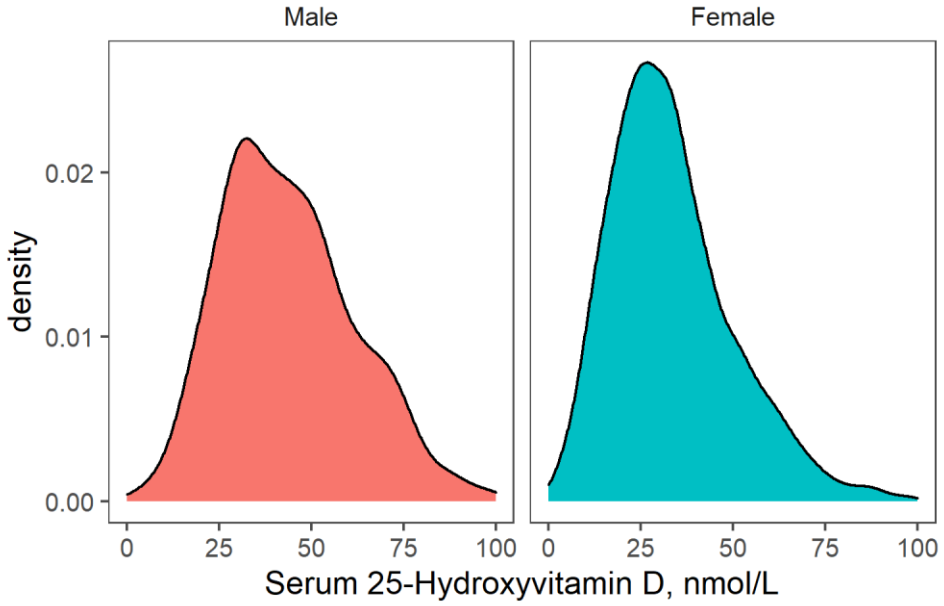


Figure S1. Distribution of Serum 25(OH) D of the participants at baseline.

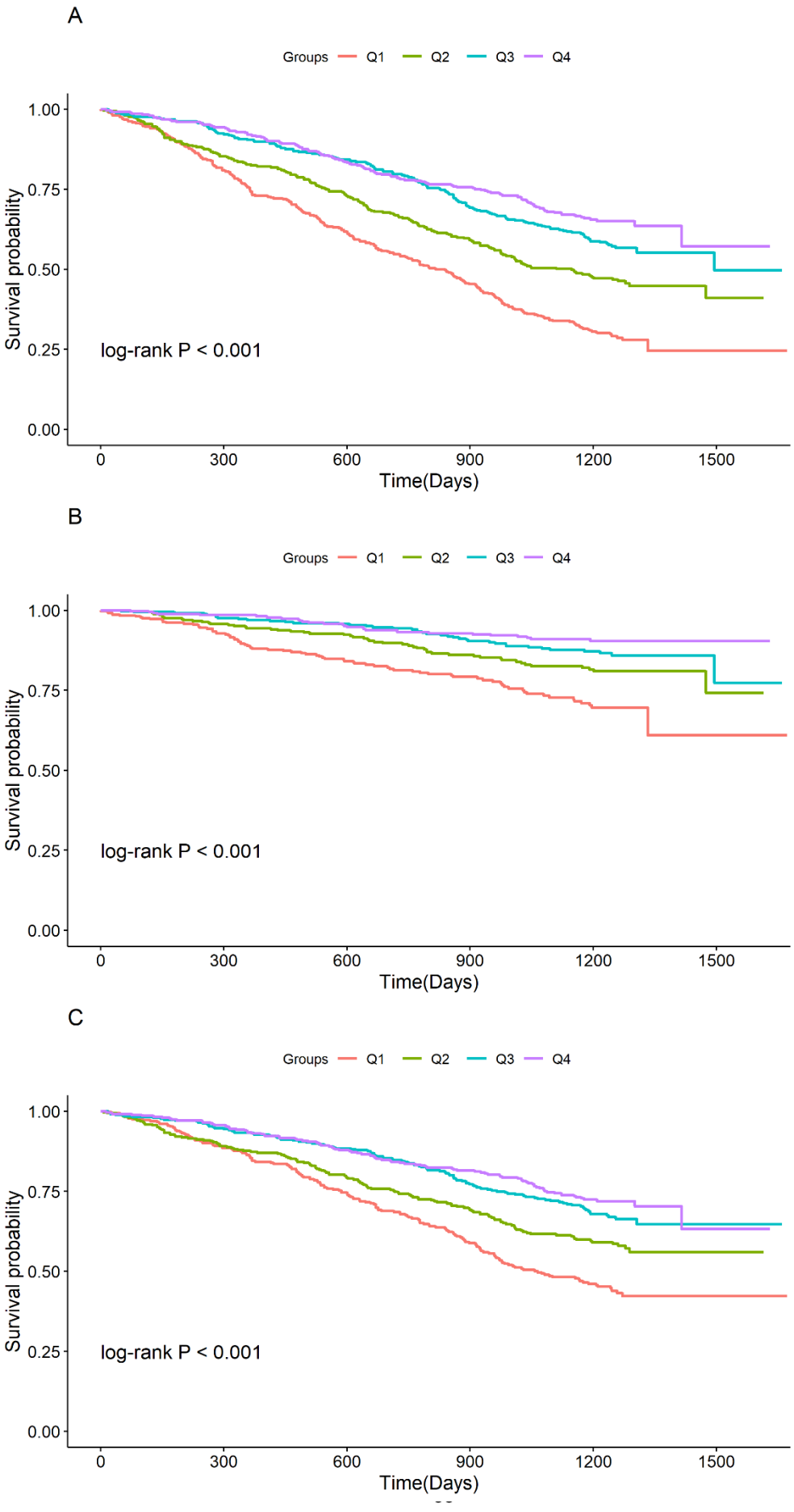

A
Groups Q1 Q2 Q3 Q4
Survival probability
1.00
0.75
0.50
0.25
0.00
log-rank P < 0.001
0
300
600
900
1200
1500
Time(Days)
B
Groups Q1 Q2 Q3 Q4
Survival probability
1.00
0.75
0.50
0.25
0.00
log-rank P < 0.001
0
300
600
900
1200
1500
Time(Days)
C
Groups Q1 Q2 Q3 Q4
Survival probability
1.00
0.75
0.50
0.25
0.00
log-rank P < 0.001
0
300
600
900
1200
1500
Time(Days)

Figure S2. Kaplan-Meier curves for all- cause mortality (A), circulatory mortality (B) and Non- circulatory mortality (C) according to quartiles of serum 25(OH)D.

Q, quartiles.